

Accepted Manuscript

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PII: S0141-3910(18)30243-X

DOI: [10.1016/j.polymdegradstab.2018.07.021](https://doi.org/10.1016/j.polymdegradstab.2018.07.021)

Reference: PDST 8605

To appear in: *Polymer Degradation and Stability*

Received Date: 25 January 2018

Revised Date: 20 July 2018

Accepted Date: 21 July 2018

Please cite this article as: Nayanathara U, Kottegoda N, Perera I, Mudiyansele TK, Synthesis, photodegradable and antibacterial properties of polystyrene-cinnamaldehyde copolymer film, *Polymer Degradation and Stability* (2018), doi: 10.1016/j.polymdegradstab.2018.07.021.

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Synthesis, photodegradable and antibacterial properties of**Polystyrene-cinnamaldehyde copolymer film**

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ABSTRACT

Copolymerization of styrene (St) and different ratios of Cinnamaldehyde (Cin) from cinnamon oil and synthetic cinnamaldehyde were carried out by free radical polymerization, and were characterized by Fourier Transform Infrared (FTIR) spectroscopy, Thermo Gravimetric Analysis (TGA), Differential Scanning Calorimetric (DSC) analysis, and Gel Permeation Chromatography (GPC). Photodegradation of cinnamaldehyde incorporated copolymer (Poly (St-co-Cin)) films have been studied at outdoor weathering and under direct ultraviolet (UV) radiation. The extent of photodegradation was assessed by FTIR spectroscopy, percentage weight loss, surface analysis, average molecular weights and polydispersity indices. FTIR analysis confirms the photodegradable mechanisms of the copolymer films as Norrish Type I, Norrish Type II and photo-oxidation. Extreme weight loss was observed at outdoor weathering with 36.76% weight loss for 75% (w/w) cinnamaldehyde (natural-from cinnamon oil) incorporated copolymer (Poly(St-co-N-Cin)), and 45.58% weight loss for 75% (w/w) cinnamaldehyde (synthetic) incorporated copolymer (Poly(St-co-S-Cin)) with respect to 2.5% weight loss for polystyrene (PS) homopolymer for the period of 100 days. The average molecular weights of Poly(St-co-Cin) copolymers after outdoor weathering have drastically reduced compared to polystyrene. Scanning Electron Microscope (SEM) images clearly depict the fracture generation on Poly(St-co-Cin) films upon degradation. Embrittlement and color developments of the copolymer films with aging were noticeably evident. Furthermore, copolymers were tested positive for antibacterial activity against both gram negative and gram positive bacteria.

Keywords: Polystyrene; Photodegradation; Cinnamaldehyde; Photosensitizer; Antibacterial activity

1. INTRODUCTION

Due to the extraordinary properties and relative low cost, polymers have become imperative to mankind [1]. Albeit their attractiveness, due to the deficiency of degradability, polymeric materials have impeded environmental sustainability [2]. A grand challenge facing today is to develop environmentally friendly polymeric products having similar particularity as traditional materials. On that note, degradable polymers could be considered as an emerging resolution.

Different degradation mechanisms have been highlighted recently as photo degradation [3], thermal degradation [4,5], ozone-induced degradation [6], mechanochemical degradation [7,8], catalytic degradation [9] and biodegradation [10,11]. In the view of the facts concerning practical aspects, photodegradation is a very attractive approach although not much attention had been paid [12].

Incorporation of a photosensitizer is the key approach in this regard [13]. Two methods have been practiced in literature for such development of photodegradable products. The first approach is centered on the impregnation of photosensitizer into the polymer material, while the second advancement is based on introducing the photosensitizer into the polymer chain itself through co-polymerization or grafting [13]. Degradation is facilitated by both methods through the generation of oxidizable polymeric radicals. The main advantages of the latter over the former is that there is no leachability of the sensitizer from the polymer with time, and thus the higher degradable rate [14].

The direct incorporation of carbonyl group sensitizers into the polymer backbone is gaining more attention as the best solution for the above drawback, also due to its performance on accelerated degradation. Polymers containing carbonyl groups undergo photodegradation via Norrish type I and Norrish type II degradation mechanisms [12].

During the past few years, researchers were able to find photodegradable polymers by directly incorporating the carbonyl functional group in to the polymer chain. Cernia et al. has synthesized ethylene vinyl alcohol copolymer grafted with diazoacetophenones to study the photodegradable behavior [15]. Tanaka et al. have synthesized atactic and isotactic polymers of tert-butyl vinyl ketone (t-BVK) as photodegradable polymers. As those poly (t-BVK) homopolymers were shown as highly photodegradable, they have synthesized the copolymers of t-BVK with styrene and α -methyl styrene (α -MSt) by bulk polymerization, and found that copolymers were highly photodegradable with styrene than α -MSt [16]. Li and Gulliet have studied the photochemistry of ethylene copolymers with ketone photosensitizers such as carbon monoxide, methyl vinyl ketone, and methyl isopropenyl ketone [17]. Andrady et al. has studied the degradation behavior of ethylene carbon monoxide (ECO) copolymer in terms of changes in carbonyl index, molecular weight and tensile test and found to degrade than normal polyethylene films [18]. Hanner et al. have analyzed the degradation of polystyrene blends and copolymers containing the same amount of vinyl ketone content (methyl vinyl ketone or methyl iso-propenyl ketone) confirming that copolymers degrade more efficiently than that of the blend, because copolymer can degrade from both Norrish I and II mechanisms while the Norrish I mechanism is dominant in the blend [14]. In 1998, Choi et al. has synthesized photodegradable polystyrene by copolymerizing with methyl vinyl ketone (MVK) and phenyl vinyl ketone (PVK) [19]. Han et al. also have synthesized a photodegradable terpolymer by graft polymerization of MVK and styrene with ethylene-propylene-diene terpolymer (EPDM) [20].

Unfortunately, common synthetic photosensitizers are toxic and hostile to be applied in food and medicinal related polymer applications. Cinnamaldehyde, as it is a natural sensitizer which is most abundantly found in *Cinnamomum zeylanicum* bark oil [21] is a perfect candidate to introduce into a polymer architecture to develop a photodegradable copolymer.

Its profound antibacterial activity would be an added advantage of its utilization [22,23]. Thus, this investigation focuses on the development of styrene-co-cinnamaldehyde polymer (Poly (St-co-Cin)) films for food and medicinal applications. The main objective of this work is to evaluate the photodegradable and antibacterial capability of the subjected copolymer. The cinnamaldehyde has been copolymerized with polystyrene to develop photodegradable PS film. Synthesized copolymer was characterized by FT-IR spectroscopy, TGA, GPC and DSC. Both outdoor weathering test and direct UV radiation were performed. The extent of photodegradation was assessed by FT-IR spectroscopic data, percentage weight loss, molecular weight, polydispersity indices and morphological changes. Antibacterial property of copolymers was further investigated qualitatively by an overlay diffusion method [24] respectively with *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus cereus* (ATCC 11778) bacteria cultures.

2. EXPERIMENTAL

2.1 Materials

Styrene monomer was purchased from Daejung chemicals. Benzoyl peroxide and 2, 2-Diphenyl-1-picrylhydrazyl were purchased from Sigma Aldrich. Cinnamaldehyde with 98% purity was purchased from Loba Chemie Suppliers. Toluene and butan-2-one were purchased from Research-Lab Fine Chem Industries. All solvents and chemicals used were in analytical grade. Cinnamon bark oil was obtained from Rathna Cinnamon Pvt. Ltd., Sri Lanka.

2.2 Characterization of commercially available cinnamon oil

2.2.1 FTIR analysis

FTIR spectra of commercially available cinnamon oil and analytical grade cinnamaldehyde were compared to confirm the presence of cinnamaldehyde in the commercially available

cinnamon oil. The analysis was done by Thermo Fisher SCIENTIFIC NICOLET iS10 IR spectrophotometer in ATR mode. The samples were analyzed in the range from 40 cm^{-1} to 4000 cm^{-1} with 32 number of background and sample scans.

2.2.2 Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

GC-MS analysis was performed to determine the purity and composition of the commercially available cinnamon oil. Oven temperature was increased from 40°C to 230°C at a constant heating rate of $5^\circ\text{C}/\text{min}$ with a flow rate of $1\text{ ml}/\text{min}$. Injector temperature was 250°C [25].

2.3 Synthesis of styrene-co-cinnamaldehyde copolymer.

Poly(St-co-Cin) copolymer was synthesized exploiting cinnamaldehyde from both synthetic and natural cinnamon oil. The selected amount of styrene and cinnamaldehyde were dissolved in 50 ml of toluene to develop polymers having compositions of 75:25 and 25:75 of styrene: cinnamaldehyde. The solution was stirred in the dark for about 1 h and 1% (w/w) of benzoyl peroxide was added. Flasks were sealed after charging with dried nitrogen gas. Polymerization was carried out at 60°C for 4 h . The resultant viscous solution was dissolved in toluene and allowed to precipitate in 200 ml of methanol under vigorous stirring. Re-precipitation was repeated twice to remove any un-polymerized components. The purified polymer was dried in an oven at 50°C until it reached a constant weight [19]. Polymer films of synthesized poly(St-co-Cin) were prepared by casting the polymer on glass slides using toluene as the solvent [26].

2.4 Characterization of Poly(St-co-Cin) copolymers

2.4.1 Infrared spectroscopy

Poly(St-co-Cin) copolymer was analyzed by Thermo Fisher SCIENTIFIC NICOLET iS10 IR spectrophotometer in ATR mode. The samples were analyzed in the range from 40 cm^{-1} to 4000 cm^{-1} with 32 numbers of background and sample scans.

2.4.2 Differential Scanning Calorimetry (DSC)

DSC measurements were performed using a TA DSC Q20 V24.4 Build 116 instrument. Powdered samples of Poly(St-co-N-Cin) copolymer was loaded to an aluminium pan which was then sealed. Once loaded on the DSC, samples were heated from 50°C to 250°C at a heating rate of $10^{\circ}\text{C}/\text{min}$ in nitrogen gas. The analysis was repeated for polystyrene [27,28].

2.4.3 Thermo Gravimetric Analysis (TGA)

The TGA analysis on Poly(St-co-N-Cin) copolymer was conducted on SDTQ600 Thermo Gravimetric Analyzer at a heating rate of $10^{\circ}\text{C}/\text{min}$ from 40°C to 500°C under nitrogen atmosphere [28].

2.5 Photodegradation studies of Poly(St-co-Cin) copolymers

Direct UV radiation and outdoor weathering tests were performed to determine the extent of photodegradation of Poly(St-co-Cin) copolymer with respect to the control polystyrene homopolymer.

OSRAM XBO 5000 W/H OFR xenon arc lamp with interference filters for 254 nm and 365 nm (Omega 280nm W3288 $254 \leq 1\%$ and 380 SP W236 70% avg 365 nm) respectively were used where samples were held 5 cm away from the lamp [13]. Water cooling system was used to maintain the temperature at 60°C . Samples were exposed to a total of 100 h under the lamp (6 h UV exposure followed by 6 h relaxation).

Outdoor weathering tests were performed by displaying the polymer films outside where the samples were exposed to day to day weathering conditions. The location was selected according to exposure of ample sunlight throughout the day [29]. Weather information at the site of exposure during the studied period are given in Table 1 on the supplementary documents. Samples were displayed horizontally for 6 months (October 2016 to April 2017, at the University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka).

2.5.1 Fourier Transform Infrared (FTIR) measurements

FTIR spectra of each of aged polymer films were obtained periodically at different time intervals.

2.5.2 Percentage weight loss

The Percentage weight loss of each polymer films was calculated according to the equation 1 at different time intervals.

$$\text{Equation 1: Weight percentage loss} = \frac{W_i - W_t}{W_i} \times 100\%$$

W_i = Weight of polymer film before irradiation

W_t = Weight of polymer film after irradiated for time, t

2.5.3 Gel Permeation Chromatography (GPC)

The average molecular weights of Poly(St-co-Cin) copolymers were determined by gel permeation chromatography (GPC) (Shimadzu LC-10AT Liquid Chromatography). The eluent was used as THF [19,20].

2.5.4 Morphological changes

Visual observations of Poly(St-co-Cin) copolymer were recorded periodically in terms of their color and appearance with aging.

Scanning Electron Microscopy (SEM) analysis was performed using Hitachi SU6600 SEM in the secondary electron mode before and after the aging of Poly(St-co-N-Cin) copolymer [20]. The Polymer films were coated with a thin coating of gold prior to observations.

2.6 Study the antibacterial activity of the Poly(St-co-Cin) copolymer films

2.6.1 Agar-disc diffusion assay

The antibacterial activity of Poly(St-co-Cin) copolymer films was qualitatively investigated against four bacterial strains: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus cereus* (ATCC 11778) using agar-disc diffusion method [30]. Briefly, bacterial strains were grown in Muller Hinton broth and diluted in 0.9% sterile saline solution to obtain a turbidity comparable to that of 0.5 McFarland standard ($\sim 1 \times 10^8$ CFU/ml). Then, 100 μ l of this suspension was spread on Muller Hinton Agar plates and distributed homogeneously. Poly(St-co-Cin) copolymer films and polystyrene polymer films (control) were cut into 6 mm diameter discs and were placed on inoculated Muller Hinton Agar surfaces and incubated at 37 °C for 24 h and the antibacterial activity was evaluated. The test was performed in triplicate for each bacterial strain.

3. RESULTS AND DISCUSSION

3.1 Characterization of commercially available cinnamon oil

3.1.1 FTIR Spectroscopy

The FTIR spectra of commercially available cinnamon oil and synthetic cinnamaldehyde displayed clear peaks at 1624 cm^{-1} (C=C bond; stretching vibration), 1668 cm^{-1} (C=O bond;

stretching vibration), 2854 cm^{-1} and 2748 cm^{-1} (aldehyde C-H bond; stretching vibration) and 3028 cm^{-1} (aromatic C-H; stretching vibration) [22]. Thus, the comparison of the FTIR spectra of cinnamon oil and synthetic cinnamaldehyde evidently displayed the resemblance of all the characteristic peaks of the two spectra which confirms the presence of cinnamaldehyde in commercially available cinnamon oil consumed in the study (Figure 3.1).

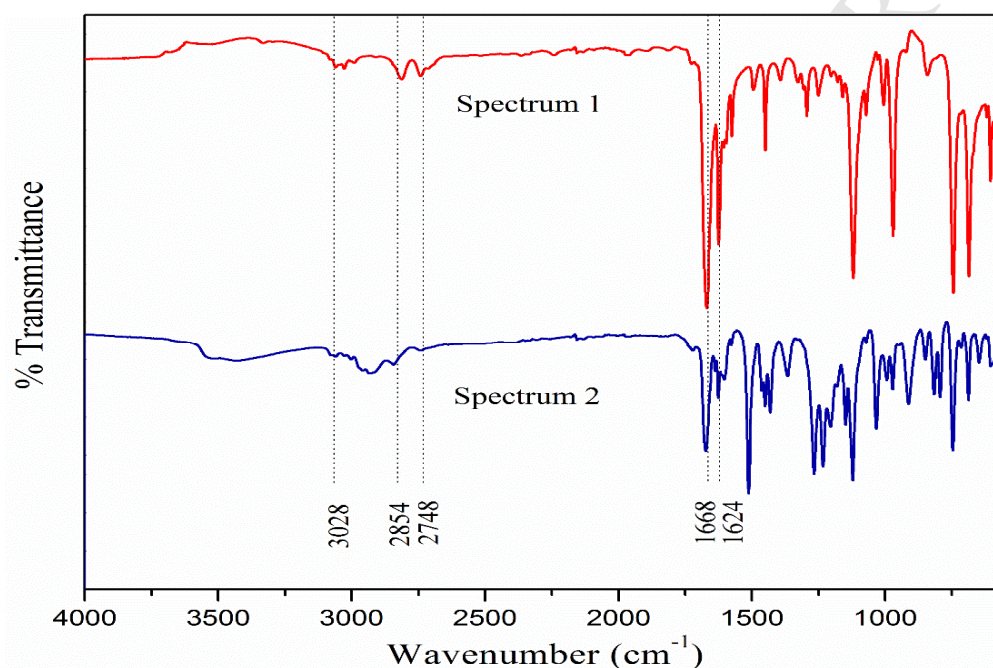


Figure 3.1 The FTIR spectra of (spectrum 1) analytical grade synthetic cinnamaldehyde, and (spectrum 2) commercially available cinnamon oil

3.1.2 GC-MS analysis

The Gas Chromatogram of cinnamon oil (Figure 3.2) displayed the peak for cinnamaldehyde at a retention time of 17.234 with a clear MS fragmentation pattern for cinnamaldehyde (Figure 3.3). Cinnamaldehyde is the second major component of cinnamon oil with the area percentage of 22.409%. No further purification was conducted on this commercially available cinnamon oil to separate cinnamaldehyde since none of the components in the oil neither can affect negatively on the polymerization nor are harmful in food/medicinal application of the

final product. Further any un-polymerized components could be removed during the purification stages. Major components identified by GC analysis of cinnamon oil has been tabulated in Table 2 in supplementary documents.

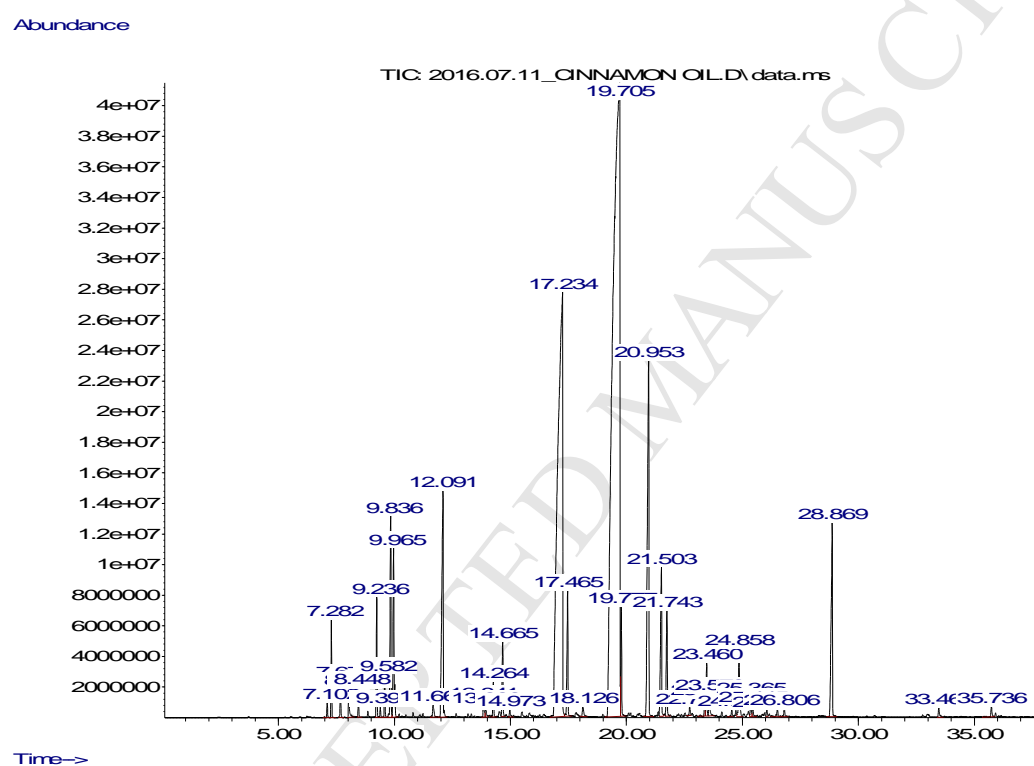


Figure 3.2 The GC chromatogram of commercially available cinnamon oil

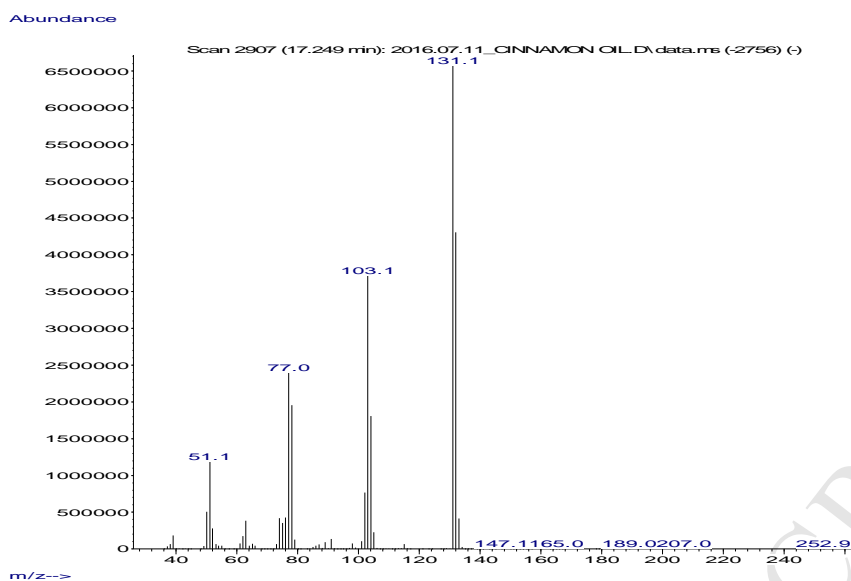


Figure 3.3 The mass spectrum of cinnamaldehyde with retention time of 17.234

3.2 Characterization of synthesized copolymer

3.2.1 FTIR – ATR

The structure of Poly(St-co-Cin) copolymers were characterized with respect to homopolymer polystyrene (PS) by FTIR analysis (Figure 3.4). General representative peaks of polystyrene can clearly be seen in styrene-cinnamaldehyde copolymers. Poly(St-co-Cin) copolymers depicted characteristic peaks at 695 cm^{-1} and 752 cm^{-1} (aromatic C-H bond; out-of-plane vibration), $1480\text{-}1600\text{ cm}^{-1}$ (aromatic C=C bond; stretching vibration), $2850\text{-}3000\text{ cm}^{-1}$ (aliphatic C-H bond; stretching vibration) and $3000\text{-}3100\text{ cm}^{-1}$ (aromatic C-H bond; stretching vibration) respectively due to the styrene component. In addition, the peak appeared at 1678 cm^{-1} on the copolymers could be assigned to C=O stretching vibrations of cinnamaldehyde moiety. Abundance of the functional groups are proportional to its corresponding peak intensities according to the Beer Lambert law. It was observed clearly

that the intensity of the peak assigned to C=O group increased with the increasing cinnamaldehyde composition denoting that the number of carbonyl groups within the copolymer increases accordingly from 25% (w/w) and 75% (w/w) respectively.

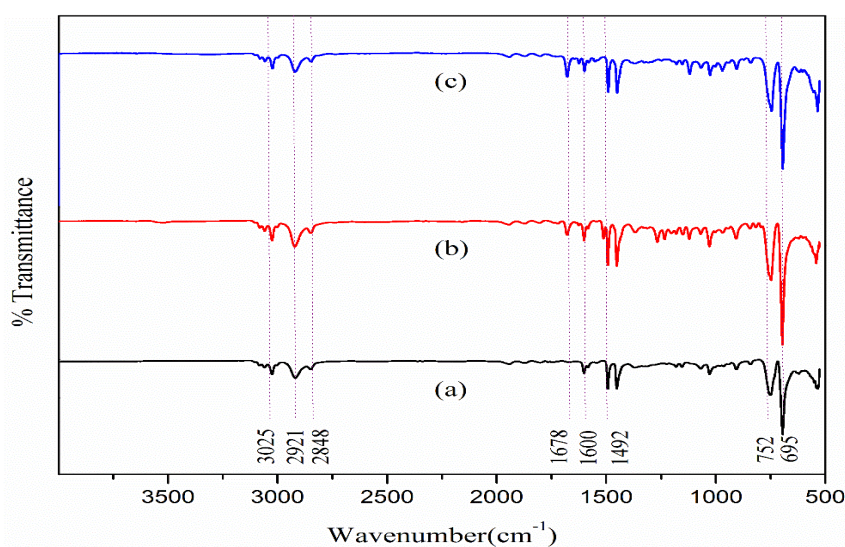


Figure 3.4 FTIR spectra of (a) Polystyrene (b) 75% (w/w) Poly(St-co-N-Cin) copolymer (c) 75% (w/w) Poly(St-co-S-Cin) copolymer

It would be reasonable to doubt on confirming Poly(St-co-Cin) copolymer through FTIR spectra as similar peaks would have appeared even if it produces a polymer blend with cinnamaldehyde instead of copolymerization. The C=C bond stretching vibration peak of cinnamaldehyde which appeared at 1624 cm^{-1} was not noticeable in the copolymer spectra which could indicate the consumption of double bonds through polymerization. Nevertheless, synthesized polymer has triply re-precipitated in methanol to obtain the final polymer. During the re-precipitation unreacted, trapped or loosely bound styrene and cinnamaldehyde can be easily removed from the polymer as they are completely miscible in

methanol. Thus, the carbonyl band observed from the FTIR spectra is due to the polymerized cinnamaldehyde.

3.2.2 DSC analysis

DSC analysis was performed on polystyrene and 75% (w/w) Poly(St-co-N-Cin) copolymer and the results are summarized in Table 3.1. (Detailed DSC curves are displayed in supplementary documents (Figure 2))

Table 3.1 DSC data of Polystyrene and 75% (w/w) Poly(St-co-N-Cin) copolymer

Sample name	Glass transition temperature (°C)	Melting point (°C)	Degree of crystallinity (%)
Polystyrene	100.43	164.37	0.1220
75% (w/w) Poly(St-co-N-Cin) copolymer	100.31	165.24	0.2118

The glass transition temperature (T_g) of polystyrene and 75% (w/w) Poly(St-co-N-Cin) copolymer were almost the same. Closely related T_g values suggest that the amorphous nature of polystyrene remained quite the same even after copolymerizing with cinnamaldehyde.

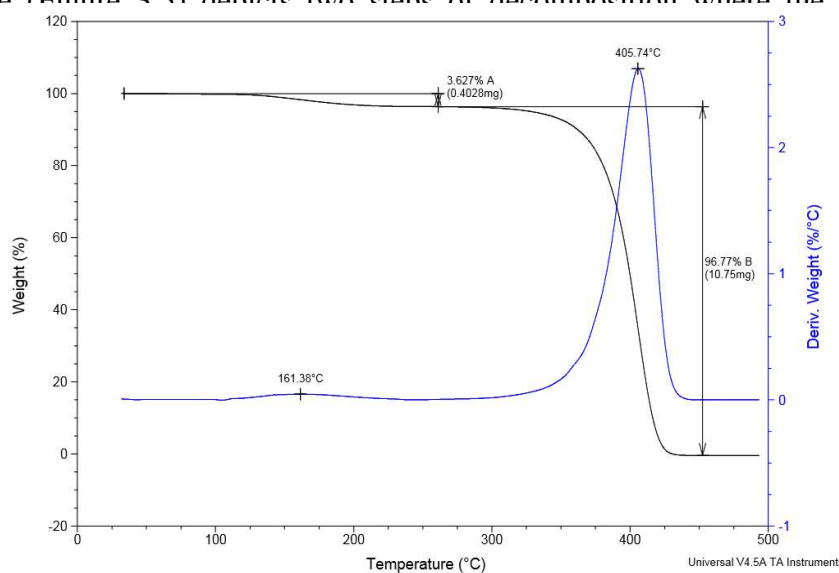
The melting point of a polymer is related to its crystalline nature. The melting point of the copolymer was about one degree higher than that of polystyrene which is nearly negligible. The crystallinity percentage of the copolymer was 0.2118% which is a minute amount higher

than that of polystyrene, 0.1220%. The percent crystallinity refers to the relationship with the crystalline regions to its amorphous regions. The minute increment of percent crystallinity of the copolymer could be due to the arrangement of copolymerized polymer chains in a more ordered manner due to higher intermolecular interactions through carbonyl functional groups.

3.2.3 TGA Analysis

TGA analysis was conducted to compare the thermal behavior of polystyrene and 75% (w/w) Poly(St-co-N-Cin) copolymer in an inert environment. The TGA curve of homopolymer polystyrene (Figure 3.5) depicts two steps of decomposition where the decomposition at

161°C with
which ma



s loss of 96.77%

Figure 3.5 TGA and DTGA curves of polystyrene

The TGA curve of copolymer had two main weight losses at 177°C and 413°C corresponding to cinamaldehyde decomposition, and degradation of the styrene segment respectively (Figure 3.6). Degradation of styrene segments took place around 320°C-420°C with a weight loss of 86.34%.

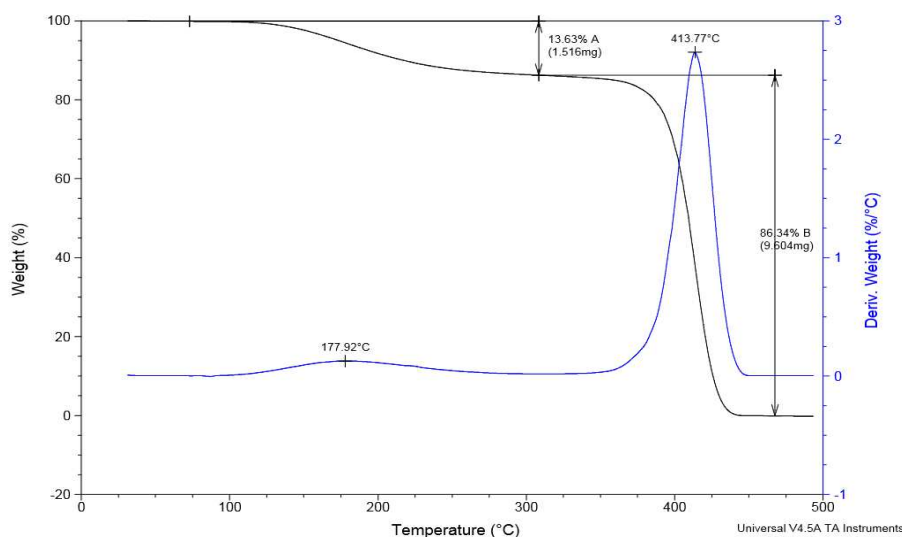


Figure 3.6 TGA and DTGA curves of 75% (w/w) Poly(St-co-N-Cin) copolymer

3.3 Photodegradation studies of synthesized copolymers

3.3.1 FTIR spectroscopic data

The incorporation of the carbonyl group into a polymer would accelerate photodegradation because the carbonyl group functionalizes as a good photosensitizer. Synthesized Poly(St-co-Cin) copolymers are expected to undergo Norrish I or II degradation reactions through the carbonyl functional group. Radicals produced by these processes would continue degradation by means of autoxidation. FTIR spectroscopy is a major tool to determine the structural changes occurred during the polymer degradation [29].

Direct UV radiation:

Figure 3.7 and Figure 3.8 represent the comparison of carbonyl and hydroxyl absorption bands of 25% (w/w) and 75% (w/w) Poly(St-co-N-Cin) copolymer films in different time intervals of UV exposure (8 h, 16 h and 24 h). The FTIR spectra of 25% (w/w) and 75% (w/w) Poly(St-co-N-Cin) film for 8 h UV exposure clearly indicate generation of two additional peaks in the range of $1716\text{--}1721\text{ cm}^{-1}$ and $3400\text{--}3500\text{ cm}^{-1}$ which are attributed to

different C=O stretching and OH stretching frequencies respectively [31]. Existing carbonyl absorption peak at 1672 cm^{-1} decreases its intensity upon irradiation, while a new peak appears at 1721 cm^{-1} . The intensity of those new peaks subsequently increased with irradiation time.

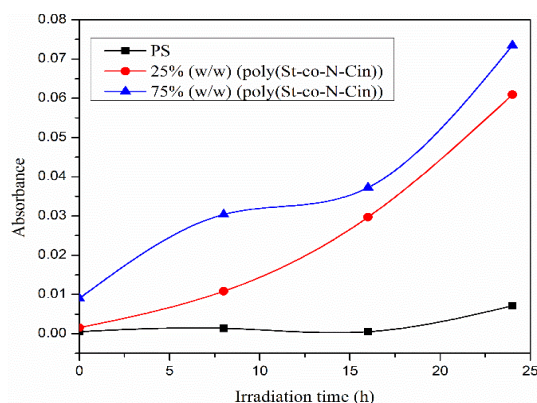


Figure 3.7 The changes of the intensity of carbonyl peak at 1721 cm^{-1} of Poly(St-co-N-Cin) copolymer vs. irradiation time

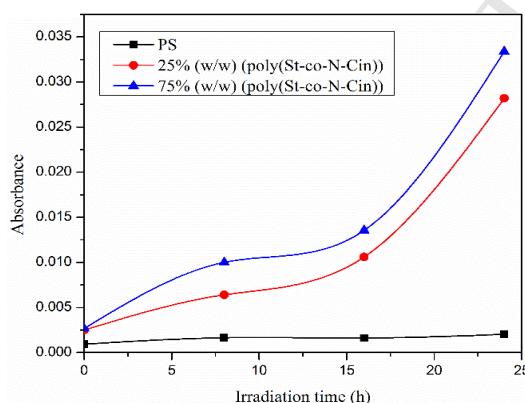


Figure 3.8 The changes of the intensity of hydroxyl peak at 3431 cm^{-1} of Poly(St-co-N-Cin) copolymer vs. irradiation time

The hydroxyl peak that began to appear at 3431 cm^{-1} with increasing irradiation time suggests the formation of unstable hydroperoxide intermediates. The hydroperoxide intermediates transpire due to oxidation of polymer radicals in the presence of air [29]. Those which are intermediate within the main chain decompose to form a new carbonyl groups through chain scission. The generation of the new carbonyl group upon irradiation indicates that both copolymers had undergone photodegradation either by Norrish I or II mechanisms. Appearance of C=C peak (1672 cm^{-1}) together with the carbonyl peak indicates that copolymer had undergone Norrish II mechanism. There was no significant change observed for polystyrene films with increasing UV irradiation time. (Detailed FT-IR spectrums are displayed in supplementary document (Figure 3).)

Outdoor weathering test:

The FTIR spectroscopic data of outdoor weathered polymer films were obtained for the period of 6 months. Similar IR peaks were observed as with the direct UV radiation tests with minute differences. FTIR spectra of 25% (w/w) Poly(St-co-N-Cin) copolymer showed generation of new C=O peak (1721 cm^{-1}) and OH stretching peak (3464 cm^{-1}) after 40 days of outdoor weathering while the 75% (w/w) Poly(St-co-N-Cin) copolymer peak generation was observed after 20 days. On the other hand, no significant change was observed on FTIR spectra of polystyrene even after 6 months of outdoor weathering (Figure 3.9 & Figure 3.10). (Detailed FT-IR spectra are mentioned in supplementary document (Figure 4).)

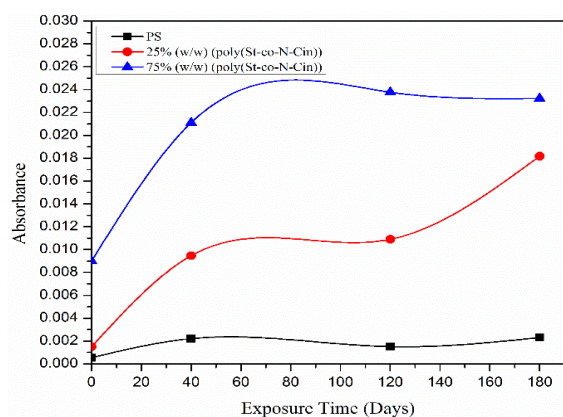


Figure 3.9 The changes of the intensity of carbonyl peak at 1721 cm^{-1} of Poly(St-co-N-Cin) copolymer vs. weathering time

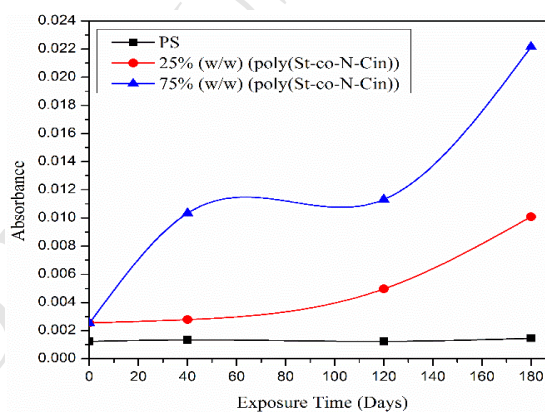


Figure 3.10 The changes of the intensity of hydroxyl peak at 3464 cm^{-1} of Poly(St-co-N-Cin) copolymer vs. weathering time

Similarly, C=O and OH stretching peaks generation were observed in both 25% (w/w) and 75%(w/w) Poly(St-co-S-Cin) copolymer films after 10 days of weathering respectively (Figure 3.11 and 3.12). The intensity of the above peaks was subsequently increased with the weathering time period. (Detailed FT-IR spectra are mentioned in supplementary document (Figure 5).)

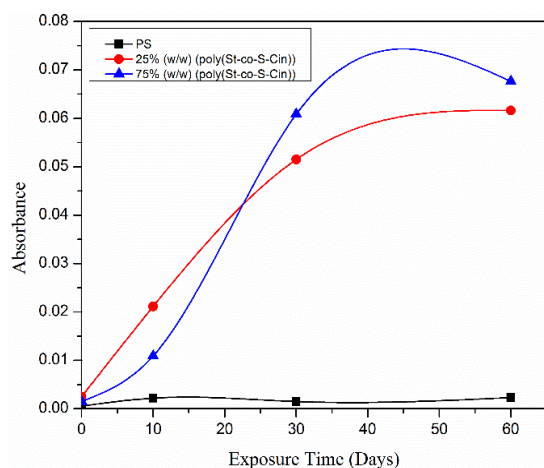


Figure 3.11 The changes of the intensity of carbonyl peak at 1721 cm^{-1} of Poly(St-co-S-Cin) copolymer vs. weathering time

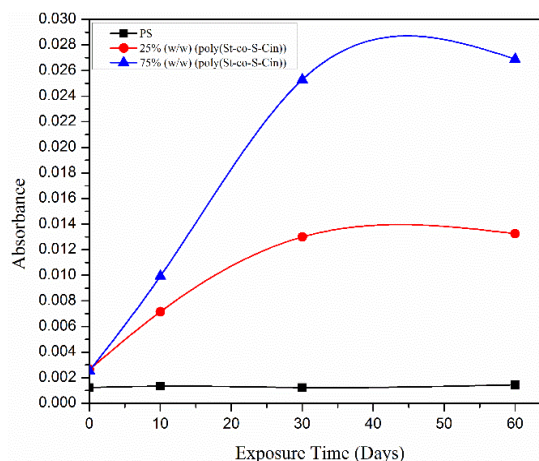
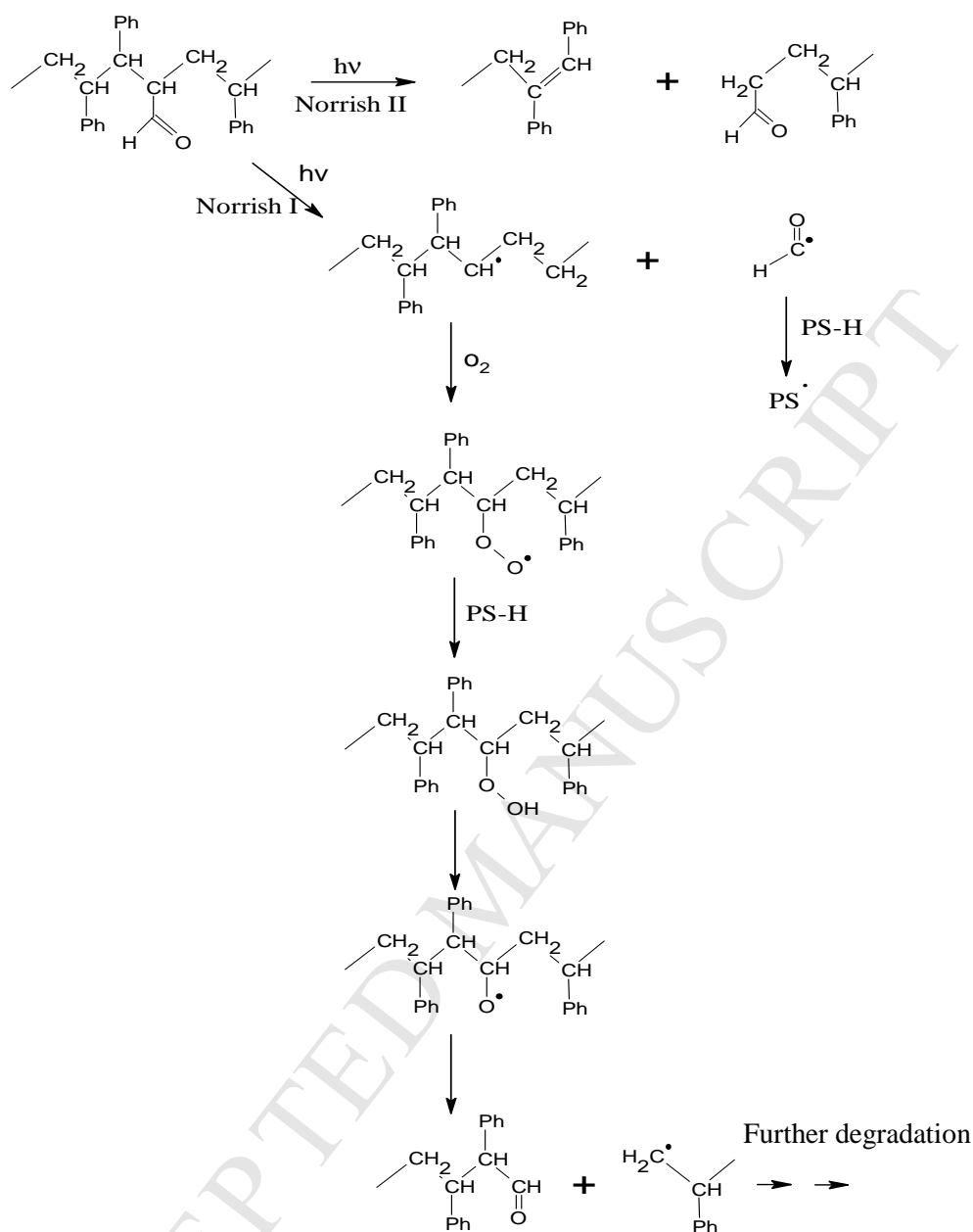


Figure 3.12 The changes of the intensity of hydroxyl peak at 3464 cm^{-1} of Poly(St-co-S-Cin) copolymer vs. weathering time

Conclusively, it can be resolved that both copolymers degrade under outdoor weather conditions through Norrish I and II mechanisms and autoxidation mechanism while polystyrene remained un-degraded. The proposed degradation mechanism is displayed in Scheme 3.3. Radicals produced by these processes will undergo autoxidation to cause further degradation of the polymer [14].



(PS: Polymer radical, PS-H: Polymer molecule)

Scheme 3.3 Proposed degradation mechanism for styrene-co-cinnamaldehyde copolymer

3.3.2 The percentage of the weight loss

The percentage of the weight loss was taken in order to determine the extent of degradation of each polymer sample. Figure 3.13 represents the percentage weight loss of direct UV radiation test results of 25% (w/w) Poly(St-co-N-Cin) films, 75% (w/w) Poly(St-co-N-Cin) films and polystyrene films with increasing irradiation time.

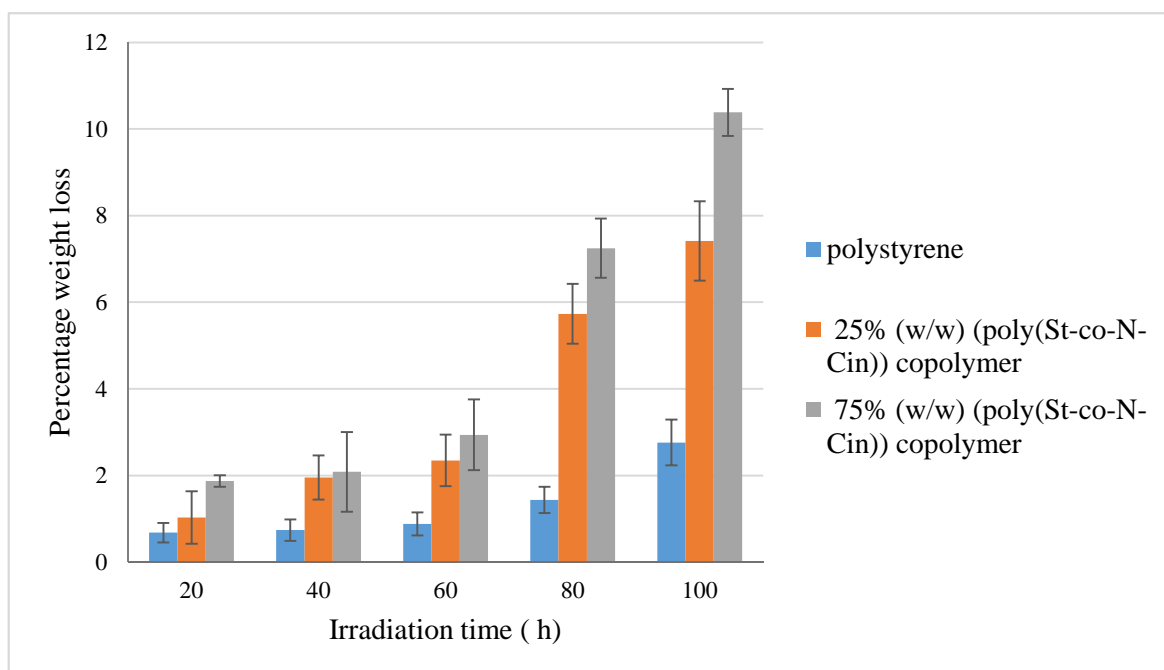


Figure 3.13 The percentage of the weight loss vs. increasing irradiation time in direct UV radiation test (n = 3)

The percentage of the weight losses of copolymers were much higher than that of polystyrene indicating the efficiency of the photo-sensitizer. It increased slowly during the first 60 h of irradiation and became rapid subsequently. The percentage of the weight loss of 75% (w/w) Poly(St-co-N-Cin) was higher than that of 25% (w/w) Poly(St-co-N-Cin) as expected.

The percentage of the weight loss of outdoor weathering tests (Figure 3.14) clearly illustrates that the cinnamon oil incorporated copolymers degraded rapidly over the homopolymer polystyrene. 75% (w/w) Poly(St-co-N-Cin) films have shown higher percentage of the weight loss than 25% (w/w) Poly(St-co-N-Cin) copolymer films, as well as the film embrittlement as expected.

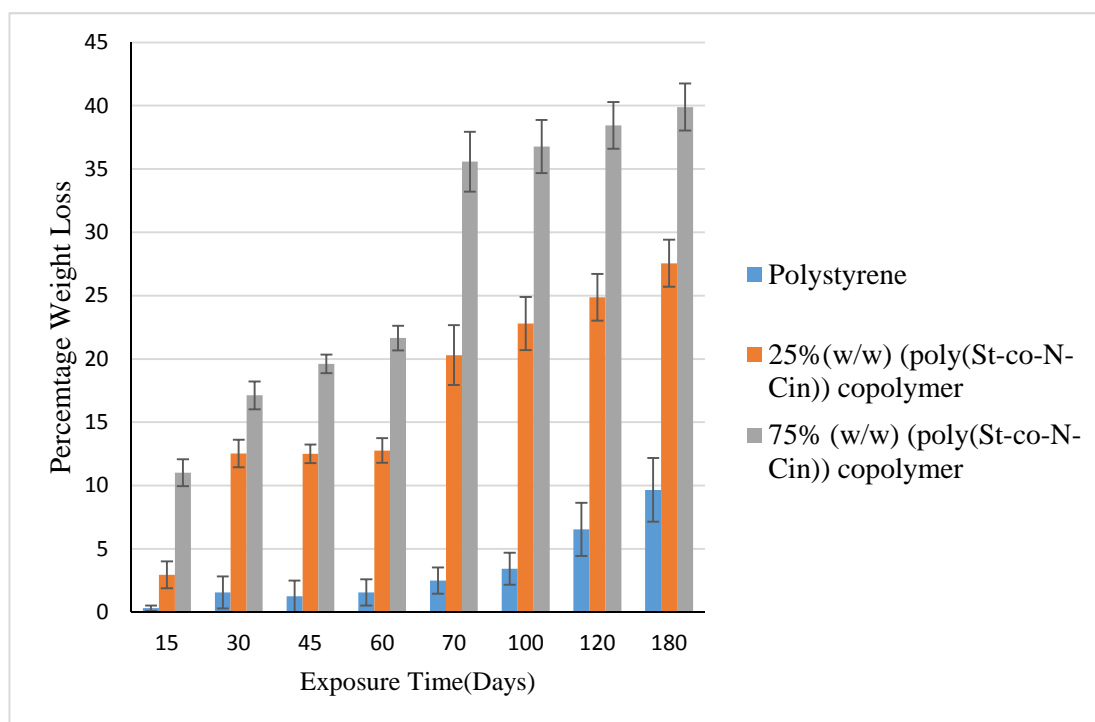


Figure 3.14 Percentage weight loss of cinnamon oil incorporated copolymer films vs. increasing exposure time in outdoor weathering test (n=3)

Furthermore, outdoor weathering tests were performed on Poly(St-co-S-Cin) copolymers up to 100 days of exposure time (Figure 3.15). Poly(St-co-S-Cin) copolymers have shown higher percentage weight loss compared to that of Poly(St-co-N-Cin) copolymers in each time intervals.

Results further confirm that cinnamaldehyde act as an efficient photosensitizer in instigating the degradation of polymer. The higher the sensitizer concentration in the polymer, the higher the rate of degradation, as then the number of generated radicals would be high. The rate of degradation of the Poly(St-co-S-Cin) was observed as greater than that of the Poly(St-co-N-Cin)) and could explain considering the amount of pure sensitizer percentage in each of the initial sources. The outdoor weathering tests were more effective than accelerated tests. Even though the initiation of the degradation mechanism could happen through the photosensitizer, many other channels of degradation could involve in proceeding the degradation. Outdoor

whether opening up many more degradation pathways could be the reason of higher efficiency of polymer degradation.

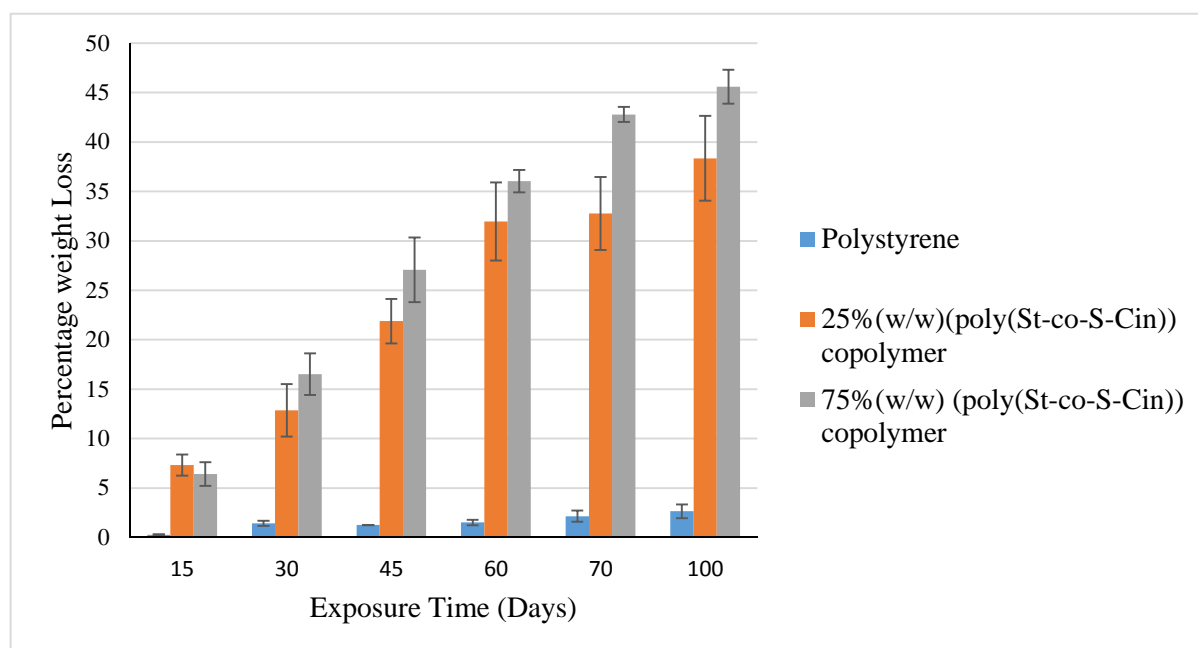


Figure 3.15 The Percentage of the weight loss of cinnamaldehyde incorporated copolymer (Poly(St-co-S-Cin)) films with increasing exposure time in outdoor weathering test. (n=3)

3.3.3 GPC analysis

The average molecular weights and polydispersity indices of Poly(St-co-Cin) copolymers (before and after outdoor weathering) are shown in Table 3.2 together with polystyrene homopolymer. The number average molecular weight of 75% (w/w) Poly(St-co-N-Cin) after 180 days of outdoor weathering was 34795 compared with 1063653 before outdoor weathering. In 75% (w/w) Poly(St-co-S-Cin) of the number average molecular weight after 100 days of weathering was 41974 compared with 2342985 before outdoor weathering. The polydispersity indices of 75% (w/w) Poly(St-co-N-Cin) has deviated from 1.8 to 3.7 after outdoor weathering, whereas in 75% (w/w) Poly(St-co-S-Cin) polydispersity indices has deviated from 1.4 to 3.0. Therefore, it has clearly shown that Poly(St-co-Cin) copolymers

after outdoor weathering have low molecular weights and broad molecular weight distribution compared to that of before weathering.

In homopolymer polystyrene, the number average molecular weights after 180 days of weathering slightly differed from that of before weathering in which polydispersity indices deviate from 3.2 to 3.16. Thus, it can be concluded that copolymers have undergone main chain scission reactions due to the presence of the carbonyl group which can absorb UV radiations in order to cause photodegradation.

Table 3.2 The Changes of average molecular weights according to outdoor weathering time

Sample	Weathering time	M_n	M_w	M_w/M_n
75% (w/w)	0 days	1063653	1924646	1.80
Poly(St-co-N-Cin)	180 days	34795	130612	3.7
75% (w/w)	0 days	2342985	3368270	1.4
Poly(St-co-S-Cin)	100 days	41974	126182	3.0
Polystyrene	0 days	455018	1459589	3.2
	180 days	461846	1461428	3.16

The number average molecular weight of homopolymer polystyrene has slightly increased after outdoor weathering from 455018 to 461846 and polydispersity index has slightly decreased from 3.2 to 3.16. This might be due to the crosslinking reactions. (The GPC chromatograph of each copolymer before and after degradation is displayed in supplementary documents (Figure 6)).

3.3 Morphological changes

3.3.3.1 Visual changes

Direct UV radiation test

Films of 25% (w/w) Poly(St-co-N-Cin) started to show significant increase in stiffness and brittleness while decreasing the flexibility after 80 h of UV light exposure, while 75% (w/w) Poly(St-co-N-Cin) films showed the same after 60 h. However, polystyrene films remained flexible throughout the time of experiment. Discolorations, from white to yellow, were observed in 25% (w/w) and 75% (w/w) Poly(St-co-N-Cin) copolymer films with increasing irradiation time (Figure 3.16). (More pictures are displayed in the supplementary documents under Figure 7).

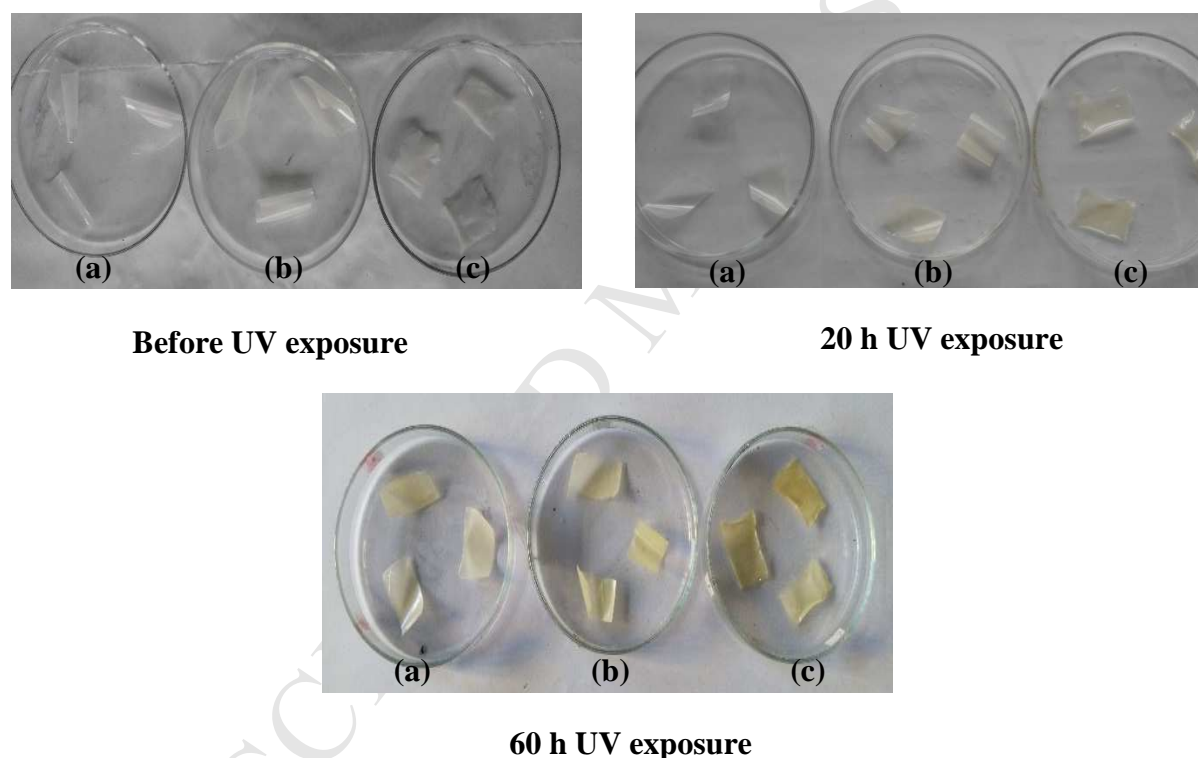


Figure 3.16 Appearance of (a) Polystyrene (b) 25% (w/w) Poly(St-co-N-Cin) (c) 75% (w/w) Poly (St-co-N-Cin) with respect to UV irradiation time

Outdoor weathering test:

All films remained flexible nearly for 30 days of outdoor weathering. 75% (w/w) Poly(St-co-N-Cin) showed brittleness after 30 days and started to undergo serious fragmentation, while 25% (w/w) Poly(St-co-N-Cin) films showed brittleness only after 45 days of weathering.

Moreover, color changes were observed in both copolymer films with increasing exposure time from white to yellow. Nevertheless, polystyrene films did not show any color change or embrittlement throughout the tested weathering period (Figure 3.17).

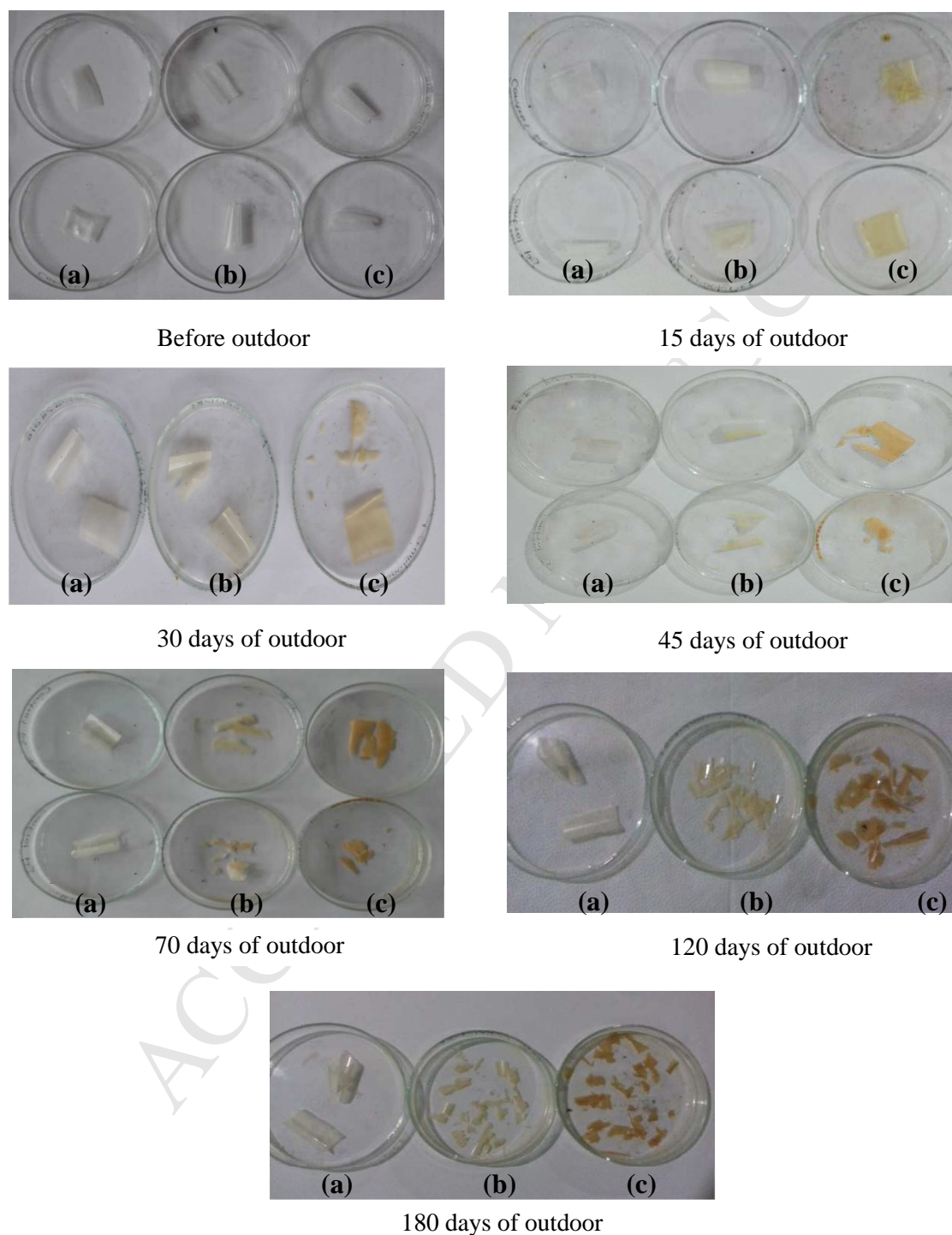


Figure 3.17 Visual appearance of polymer films with weathering time period (a) polystyrene, (b) 25% (w/w) Poly(St-co-N-Cin)) copolymer, (c) 75% (w/w) Poly(St-co-N-Cin)) copolymer

Furthermore, outdoor weathering test was performed for 25% (w/w) and 75 % (w/w) Poly (St-co-S-Cin) copolymer films. Both copolymers started to show color change within 10 days of weathering while there was no any change in homopolymer polystyrene film (Figure 3.18). After 20 days of weathering both copolymers had started to undergo fragmentation and within 100 days a remarkable amount of fragmentation was observed for 75 % (w/w) Poly(St-co-S-Cin) copolymer.

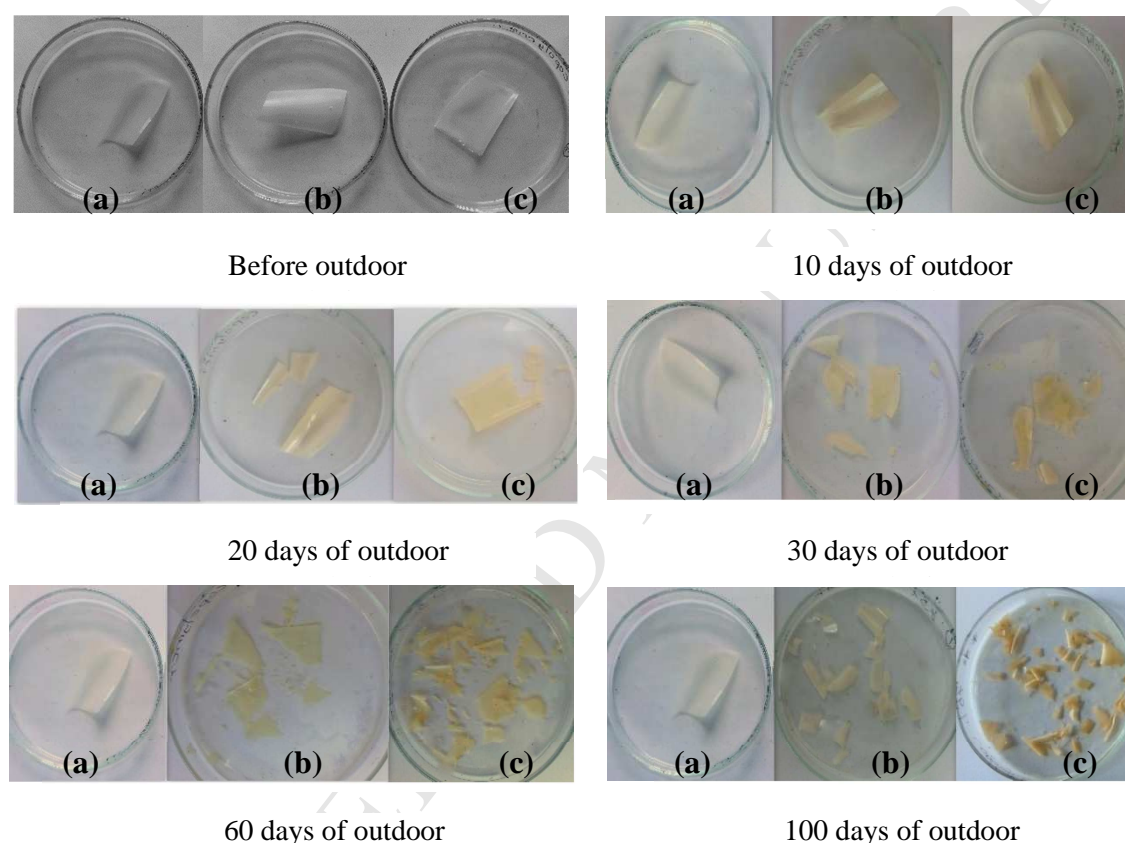


Figure 3.18 Appearance of polymer films with weathering time period (a) polystyrene, (b) 25% (w/w) Poly(St-co-S-Cin) copolymer, (c) 75% (w/w) Poly(St-co-S-Cin) copolymer

3.3.3.2 SEM analysis

SEM micrographs of 75% (w/w) Poly(St-co-N-Cin)) copolymer film before outdoor weathering (Figure 3.19(a)) showed clear smooth surface while that of after 80 days of outdoor weathering showed extensive surface fractures (Figure 3.19(b)). Meanwhile, SEM

micrographs of homopolymer polystyrene film after 80 days of outdoor weathering had not indicated any fractures at all (Figure 3.19(c)).



Figure 3.20 SEM micrograph of 75% (w/w) Poly(St-co-N-Cin) copolymer film (a) before outdoor weathering ($\times 150$) (b) after 80 days of outdoor weathering ($\times 150$) (c) Polystyrene film after 80 days of outdoor weathering ($\times 150$).

Therefore, it could clearly be concluded that fracture formation leads to embrittlement of 75% (w/w) Poly(St-co-N-Cin) copolymer films after 80 days exposure to outdoor weathering, while polystyrene remained unchanged without any indication of brittleness. This indicates that copolymerization of cinnamaldehyde with polystyrene initiate photochemical reaction, which results in physical disintegration of copolymer films faster than control polystyrene films.

3.4 Antibacterial Study

Cinnamaldehyde has antibacterial activity against the gram positive and gram negative bacterial strains tested. It was reported that carbonyl group of cinnamaldehyde plays a major role for its antibacterial activity because it can be bound with protein to inhibit the production of essential enzymes in the bacteria and at the end could damage the cell wall of bacteria [22]. Also cinnamaldehyde inhibit the growth of bacteria by inhibiting energy generation and glucose uptake [32].

The Agar-disc diffusion assay was used to evaluate the antibacterial activity of synthesized copolymers against gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* and gram-positive *Staphylococcus aureus* and *Bacillus cereus* bacterial cultures. Poly(St-co-Cin) copolymer films did not demonstrate clear inhibitory zones. The diffusion ability of cinnamaldehyde is reduced when cinnamaldehyde is copolymerized. Hence inhibition zones of synthesized copolymers were not sharp and clear. However, copolymerized cinnamaldehyde can successfully destroy bacteria at the contact surface of the polymer films. Thus, a positive inhibitory effect was observed directly underneath the contact area of the film disc [33](Figure 3.20).

Table 3.3 Inhibition at the contact surface of Poly (St-co-cin) copolymers (+ symbolizes an inhibitory effect and – symbolizes no inhibitory effect)

Microorganisms	Contact area			
	Control	25% (w/w)	75% (w/w)	75% (w/w)
	Polystyrene	Poly(St-co-N-Cin)	Poly(St-co-N-Cin)	Poly(St-co-S-Cin)
Gram – positive bacteria				
<i>S. aureus</i>	-	+	+	+
<i>B. cereus</i>	-	+	+	+
Gram – negative bacteria				
<i>E. coli</i>	-	+	+	+
<i>P. aeruginosa</i>	-	+	+	+

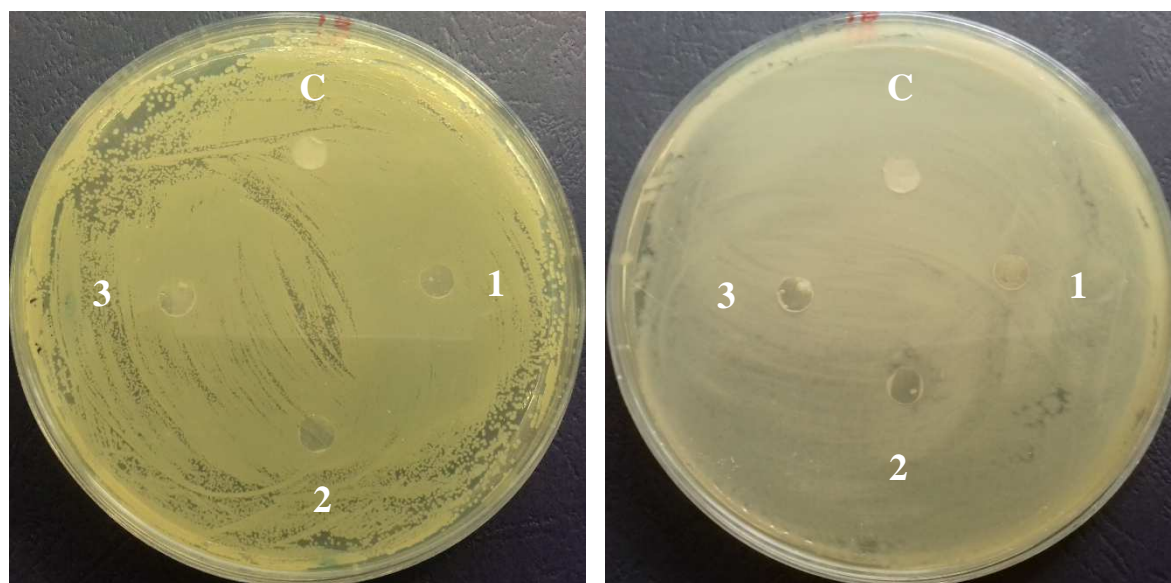
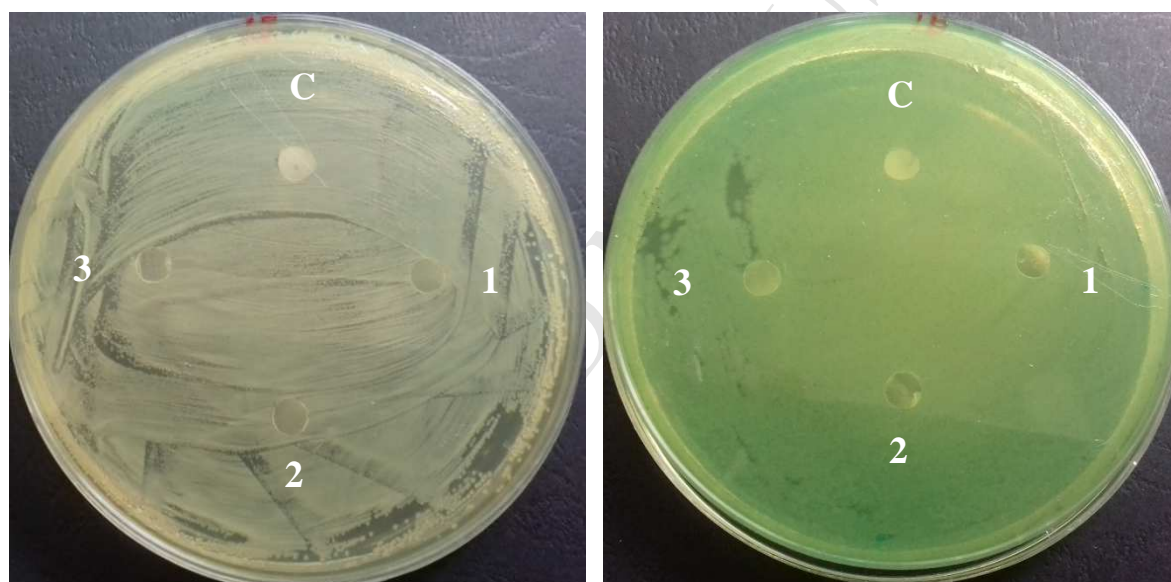
(a) *Staphylococcus aureus*(b) *Bacillus cereus*(c) *Escherichia coli*(d) *Pseudomonas aeruginosa*

Figure 3.20 Representative pictures of overlay diffusion test respectively. (C- control, 1-25% (w/w) Poly(St-co-N-Cin), 2 - 75% (w/w) Poly(St-co-N-Cin), 3 - 75 % (w/w) Poly(St-co-S-Cin))

Polystyrene is a hydrocarbon polymer which does not have antibacterial activity. But each styrene-co-cinnamaldehyde copolymer has shown inhibitory activity against tested bacteria cultures: gram-positive and gram-negative bacteria (Table 3.3). Therefore it is clear that

copolymerized cinnamaldehyde has had positive antibacterial effects, and antibacterial activity of Poly (St-co-N-Cin) occurs due to cinnamaldehyde. In addition, 25% (w/w) Poly(St-co-N-Cin) also depicts a positive inhibitory effect compared to 75% (w/w) Poly(St-co-N-Cin) copolymer film.

4. CONCLUSION

Styrene-co-cinnamaldehyde copolymers have proven to undergo Norrish I and II reactions as well as autoxidation degradation mechanisms upon UV exposure. The percentage weight loss of 75% (w/w) Poly(St-co-N-Cin) copolymer was higher than 25% (w/w) Poly(St-co-N-Cin) copolymer, in both outdoor weathering and accelerated aging tests as expected. In outdoor weathering tests, average molecular weights of both copolymers after weathering were reduced compared to homopolymer polystyrene, due to main chain scission reactions occurred due to absorption of UV light by the carbonyl group. Further, Poly(St-co-S-Cin) has shown fast degradable properties compared to Poly(St-co-N-Cin). Clear fragmentation was observed in SEM micrograph of 75% (w/w) Poly(St-co-N-Cin) copolymer after weathering for 80 days. Poly(St-co- Cin) copolymers undergo remarkable fragmentation after 2, 3 months of outdoor weathering compared to polystyrene homopolymer films. Moreover, Poly(St-co-Cin) copolymer has antibacterial activity at its contact surface against gram positive and negative bacteria.

Thus, the developed copolymer will have potential applications in high end medicinal submissions such as medicinal packaging and equipment as well as in the food packaging industry.

ACKNOWLEDGEMENT

Financial support was provided by the University Research Grants ASP/06/RE/SCI/2013/10 and ASP/06/RE/SCI/2014/06 at University of Sri Jayewardenepura, Sri Lanka. The

Advanced Material Research Center and The Central Instrumentation Facility of University of Sri Jayewardenepura have facilitated the instrumental analysis for this study. Dr Chayanika Padumadasa at the Department of Chemistry, University of Sri Jayewardenepura is acknowledged for the supplement of synthetic cinnamaldehyde. We are also grateful to Prof. Frank D. Blum and Dr. Ugo Aura at Department of Chemistry, Oklahoma State University, USA and Dr. Madhubhashini Maddumaarachchi at the Department of Chemistry, University of Sri Jayewardenepura for their support in GPC analysis.

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**Synthesis, photodegradable and antibacterial properties of
Polystyrene-cinnamaldehyde copolymer film**

Highlights:

- Photodegradable Styrene-co-cinnamaldehyde (Poly(St-co-Cin)) copolymer was developed using synthetic cinnamaldehyde or cinnamaldehyde from cinnamon oil.
- Poly(St-co-Cin) polymers have followed Norrish Type I, Norrish Type II and photo-oxidation mechanisms.
- 75% (w/w) Poly(St-co-Cin) showed fast degradation compared to that of 25% (w/w) Poly(St-co-Cin).
- Synthetic cinnamaldehyde incorporated copolymer (Poly(St-co-S-Cin)) showed fast degradation compared to natural cinnamaldehyde incorporated copolymer (Poly(St-co-N-Cin)).
- Poly(St-co-Cin) copolymers have antibacterial activity.