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Modification of Chitosan and Polymethyl methacrylate with 5-Phenyl-1,3,4-oxadiazole Derivatives and their Antifungal Activity

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Abstract

Functionalization of chitosan (Cs) and modified polymethylmethacrylate (PMMA) with 5-phenyl-1,3,4-oxadiazole derivatives was achieved for biological applications. Reaction of 5-phenyl-1,3,4-oxadiazole-2-thiol (1) with both Cs and functionalized PMMA with 1,2-ethylenenediamine afforded the grafted polymers 2 and 3, respectively. Additionally, reaction of compound 1 with ethylchloroformate furnished O-ethyl S-(5-phenyl-1,3,4-oxadiazol-2-yl) carbonothioate (4). Similarly, grafting of compound 4 with both Cs and functionalized PMMA yielded polymers 5 and 6, respectively. Some of the grafted polymers were characterized using Fourier transform infrared (FT-IR). The thermal properties of these polymers were investigated using thermo gravimetric analysis (TGA) which displayed the first degradation of water and 5-phenyl-1,3,4-oxadiazole derivatives followed by the degradation of the main Cs and PMMA chains. X-ray diffraction (XRD) explained the crystal structure of the functionalized polymers with the appearance of Cs and PMMA peaks and the new peaks that referred to oxadiazole moieties. The biological activity of these polymers was investigated as antifungal agents where compound 1 showed the highest antifungal activity.

Keywords: 5-phenyl-1,3,4-Oxadiazole, Chitosan, Polymethylmethacrylate, Antifungal Activity

Introduction

The emergence of pesticidal resistance to existing pesticides has posed a serious concern for pesticide professionals during the last decade due to the harmful insect pest in agriculture has brought about actual losses in productivity of crops. Number of researches showed that 1,3,4-Oxadiazole scaffold had been reported as potential insecticide[1], fungicide [2], herbicide [3, 4], antibacterial [5-7], antimicrobial[8, 9], anticancer[10-12].
Sulphides and sulphones derived from 5-phenyl-1,3,4-oxadiazole-2-thione showed pesticidal activity[13]. In addition, different substituted 1,3,4-oxadiazole rings exhibit antifungal, pesticidal, antibacterial, antiinflammatory, and hypoglycemic activities

[14]. Extensive research showed that 1,3,4-oxadiazole scaffold had been reported as potential insecticide[7], fungicide and herbicide[3].Some high active compounds were treated as pesticide candidates for further commercialization[15]. Recently, anthranilicdiamide with an 1,3,4-oxadiazole pharmacophore was reported as an insecticidal agent.

Materials and methods

Synthesis of 5-phenyl-1,3,4-oxadiazole-2-thiol 1 :

A solution of benzohydrazide (20g, 0.147 mol)[15] dissolved in ethanol containing (8.232 g, 0.147 mol) of potassium hydroxide, was cooled to 0 ^{o}C to which carbon disulphide (8.85 ml, 0.147 mol) was added dropwise. The reaction mixture was refluxed for 24 h (tlc). The solvent was evaporated and the resulted solid was dissolved in distilled water and neutralized by dil. HCl. The precipitate formed was filtered off and crystallized from ethanol/water, m.p. 217 – 219 ^{O}C, yield 95%.

Reaction of compound 1 with Cs :

Cs (0.5 g, 0.029 mol) was dissolved in 1% aqueous solution of acetic acid, then a solution of compound 1(0.5 g, 0.0017 mol) dissolved in DMF (5 ml) was added. The reaction mixture was refluxed for 48 h. The solvent was evaporated till dryness and the precipitate was collected and dried to give polymer (2), yield 55 %. [16] **FT-IR** Fig.(1):ν (cm^{-1}) 3466 (st. OH and st. NH), 3142 (ar. CH), 2948,2760 (alph. CH), 1605 (C=C), 1566 (C=N).

Functionalization of PMMA by 1,2-ethylenediamine:

PMMA was prepared by polymerization of methylmethacrylate[17]. A mixture of PMMA (9 g, 0.09 mol), 1,2-ethylenediamine (6.11 ml, 0.09 mol), n-BuLi (1 ml) and n-hexane (50 ml) was stirred for 4 days [18]. The reaction mixture was poured onto distilled water. The precipitate formed was filtered off, washed by ethanol and dried on oven at 40^{0} C for 48 h to furnish PMMA-NH$(CH_2)_2NH_2$, yield 91%.

Reaction of compound 1 with PMMA-NH$(CH_2)_2NH_2$ formation of compound 3:

A solution of PMMANH$(CH_2)_2NH_2$ (0.5 g, 0.0039 mol) in 1,4-dioxane (20 ml) and compound 1 (1.03 g ,0.0039 mol) was refluxed for 48 h. The solvent was evaporated till dryness and the precipitate formed was dried under vacuum to give polymer 3, yield 65%. **FT-IR** Fig.(2):ν (cm^{-1}) 3547 (N-H), 3148 (st.ar.C-H), 2948, 2760 (aliph. C-H), 1729 (C=O), 1609 (C=C), 1490 (C=N). **^{1}H-NMR** ($CDCl_3$) Fig. (3): δ (ppm) = 0.619-2.06 (br CH_2 of PMMA), 2.41 (s, 3H,CH_3C), 3.32 (br. 2CH_2N), 3.5-3.752 (1H, 2NH), 7.1-8.2 (m, CH ar)..

Reaction of compound 1 with ethylchloro-formate. Formation of compound 4:

A mixture of compound 1 (5 g ,0.0281mol) and (2.3 g, 0.0281mol) anhydrous sodium acetate in ethanol (30ml) was refluxed for 1 h. to which ethylchloroformate (3 ml, 0.0281mol) was added. The reaction mixture was stirred for 2 h (tlc), concentrated and poured onto ice. The precipitate formed was filtered off and crystallized from ethanol/water to give compound 4, m.p. 78 – 80^{o}C, yield 89% [19]. **FT-IR** Fig.(1):ν (cm^{-1}) 3057 (st.ar.C-H), 2976, 2932 (aliph. C-H), 1778 (C=O), 1620(C=C), 1576(C=N).

Reaction of 4 with Cs:

Cs (0.5 g, 0.0031 mol) was dissolved in 1% aqueous acetic acid, then a solution of 4 (0.5 g, 0.0019 mol) in DMF (5ml) was added. The reaction mixture was refluxed for 48 h. The solvent was evaporated and the residual solid was collected to give polymer 5, yield 40% . **FT-IR** Fig.(1):ν (cm^{-1}) 3466 (O-H), 3138 (N-H), 2946, 2758 (aliph. C-H), 1745 (C=O), 1605 (C=N), 1492 (C-N).

Reaction of 4 with PMMA-NH$(CH_2)_2NH_2$:

A mixture of PMMANH$(CH_2)_2NH_2$ (0.5 g ,0.0039 mol), 1,4-dioxane (20 ml) and compound 4 (1.03 g ,0.0039 mol) was refluxed for 48 h. The solvent was evaporated till dryness. The precipitate formed was dried

under vacuum to give polymer 6, yield 75%. **FT-IR** Fig.(2):*v* (cm^{-1}) 3436(N-H), 3045 (st.ar.C-H), 2997, 2945 (aliph. C-H), 1729 (2C=O), 1611 (C=C), 1530 (C=N).

Antifungal assay:

A solution of 1% of compounds 1,4 and the polymers 2,3,5 and 6 was prepared in DMSO. The two fungi *Fusarium spp.* and *Alternaria alternate* were collected from infected cotton bolls in the field the Gimmeza Agriculture Research Center Station. Preliminary in vitro antifungal assays were performed with the treatments on Potato Dextrouse Agar (PDA) using the radial growth method against the aggressive isolates of *Fusarium spp.* and *Alternaria* as described by [20] to assist in the selection of the effective treatments for this study. PDA medium was prepared by autoclaving at 121°C and cooled to 45°C. Afterwards, appropriate volume of each compounds and polymers was added to PDA medium to get several concentrations, while sterilized distilled water was used as negative control. PDA medium were thoroughly mixed with treatments and 15 ml of the mixture were poured into sterilized 9 cm-Petri plates and allowed to solidify. Three plates were used for each concentration as replicates. The plates were then incubated with 5 mm-disks of 7-day old culture of grown on PDA and incubated at (27ºC±2) until mycelial growth of pathogenic fungi covered the surface of medium in control treatment (after 7 days). The percent inhibition of radial growth of *Fusarium* and *Alternaria*were calculated using the following equation.[21]

$$\% \text{ Inhibition} = \frac{C - T}{C} \times 100$$

Where C is the diameter of hyphal extension (cm) of controls and T is the corresponding diameter of treatments.

Characterization:

FT-IR spectra were recorded on Bruker, Tensor 27 FT-IR spectrophotometer with frequency range 4000 cm^{-1} to 400 cm^{-1} with KBr pellets(Faculty of Science, Tanta University, Egypt)..

TGA data were obtained by using shimadzu thermal analyzer system at a heating rate of 10 ºC/min, sample weight of 5-6 mg under nitrogen (20 mL/min) flow. The range investigated from 30-800 ºC(The Central Lab, Tanta University, Egypt).

XRD measurements were carried out using GRN, APD 2000 PRO X-Ray diffraction, equipped with Ni-filtered Cu-Kα radiation (λ=1.54 Å) at a scanning rate of 0.05°/s and divergent slit 0.3(The Central Lab, Tanta University, Egypt).

Results and Discussion

The grafting of Cs and PMMA with some 5-phenyl-1,3,4-oxadiazole derivatives are shown in **scheme 1**. Modification Cs with compound 1 afforded the corresponding polymer 2. FT-IR spectrum of compound 4 showed stretching carbonyl group as mentioned in the experimental part. Additionally, FT-IR spectra of polymers 2,3,5 and 6 displayed the aliphatic stretching CH of both Cs and PMMA, the stretching NH, C=O and C=N groups exist in the polymer chains as shown in Figs. 1 and 2.^{1}H NMR of polymer 3 showed peaks of the methylene group and methyl group of PMMA, the two NH groups and the aromatic protons for the hetero moiety.

Thermogravimeteric analysis (TGA):

The thermal degradation of Cs and PMMA functionalized with 5-phenyl-1,3,4-oxadiazole derivatives was screened through TGA as shown in Fig. 3.

TGA of Cs shows a weight loss in two stages. The first stage ranges between 10 and 100 °C that was corresponding to the loss of adsorbed and bound water. The second stage of weight loss starts at 210 °C and continues up to 360 °C which due to the degradation of Cs chain [22].The thermal degradation of PMMA occurred in two-steps. The first one is attributed to the decomposition of PMMA unsaturated chain ends and the second

degradation is related to random scission of the polymer chains [23].

TGA showed that the thermal degradation of PMMA functionalized with 1,2-ethylenediamine occurred in two steps, the first step is around 180 ºC that referred to the loss of absorbed water and ethylenediamine moieties and the second step is in the range 180-800 ºC that referred to the random scission of PMMA chains. The thermal degradation of polymer 2 occurred in one-step degradation that is referred to the random degradation of both 5-phenyl-1,3,4-oxadiazole moieties and Cs chains. Additionally, the thermal degradation of polymer 3 occurred in two-step degradation. The first one at T≤320 ºC is due to the loss of water, ammonia and degradation of 5-phenyl-1,3,4-oxadiazole moieties from the polymer chains. The second one is attributed to the random degradation of PMMA chains. Similarly, the thermal degradation of polymer 6 occurred in two-steps. The first one at T≤300 ºC is due to the loss of water and degradation of 5-phenyl-1,3,4-oxadiazole moieties from the polymer chains and the second one is attributed to the random degradation of Cs polymer at T ≥300 ºC. Additionally, polymer 7 showed two-degradation steps, the first one at T≤320 ºC is due to loss of water molecules and the degradation of 5-phenyl-1,3,4-oxadiazole moieties and the second one is for the scission of PMMA at T ≥320 ºC.

X-Ray Diffraction (XRD):

The XRD spectra of Cs, PMMA, and their grafting with 5-phenyl-1,3,4-oxadiazole derivatives are shown in Fig. 4. The XRD pattern of pure Cs exhibits a strong characteristic peak at about 2Ɵ = 10 and 20º [24]**.** The broad characteristic peaks of pure PMMA are observed at angles of 2θ =13.28° and 29° [25]**.** XRD pattern of PMMA modified with 1,2-ethylenediamine showed the same broad and amorphous structures as PMMA only.

XRD spectrum of polymer 2 showed its crystalline structure compared with Cs with the formation of new peaks at 2Ɵ equal to 16, 16.65, 17.65, 18.35, 23.95, 25.35, 27.55, 35.35 and 38.05º. These peaks referred to compound 1 grafted with Cs. In addition, the crystal structures of polymer 3 were enhanced and increased according to the amorphous structure of PMMA due to the grafting by compound 1 with the appearance of new characteristic peaks around 2Ɵ equal 16.05, 16.75, 18.4, 24, 25.45 and 27.25º. Similarly, XRD spectra showed the crystalline structures of polymer 5 (2Ɵ equal 16, 24.65 and 26.65 º) compared to Cs structure. This increase in crystallinity is referred to the presence of 5-phenyl-1,3,4-oxadiazole derivatives on Cs backbone structure.

XRD study showed that polymer 6 yielded from the grafting of modified PMMA (treated with ethylenediamine) with 5-phenyl-1,3,4-oxadiazole derivative (have a notable crystalline structure as compared to PMMA structure. The crystalline structure of polymer 6 is referred to the appearance of new peaks at 2Ɵ equal 10.15, 11.6, 13.3, 16.15, 18.5, 22.5, 25.45 and 17.55º where these peaks is referred to 5-phenyl-1,3,4-oxadiazole derivative.

Antifungal activity:

The antifungal activity showed that compound 1 have the highest antifungal effect on the *Fusarium spp.* even at the low concentration 100 ppm, and the inhibition effect increases with increasing concentration till 500 ppm. The polymer 2 comes in the second place which have also high inhibition effect on *Fusarium spp.* followed by polymer 5, compound 4, polymer 3 and finally polymer 6 which have the lowest inhibition effect even at the concentration 500 ppm (Table 1).

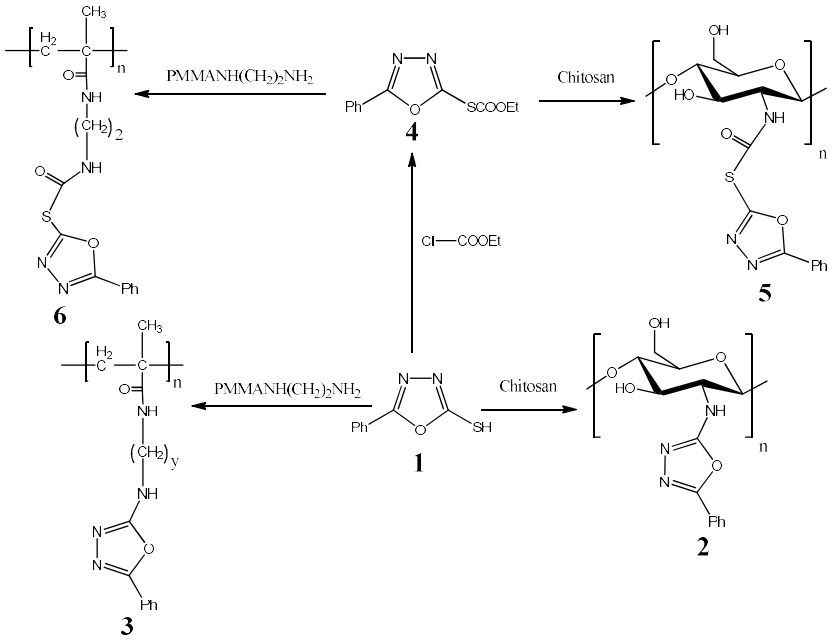
Compound 1 showed the highest antifungal activity against *Alternaria allternata*, the inhibition effect increase with increasing the concentration till reach to 500 ppm. Polymer 5 comes in the second place followed by compound 4 , polymer 3 , polymer 6, finally polymer 2 has the lowest inhibition effect. It is noticed that polymers 3 and 5 that contain Cs polymer displayed high antifungal activity as

compared to polymers 2 and 6 that contain PMMA against the two tested fungi. This may be due to the high biological activity of Cs itself [26].

Conclusion

The grafting of Cs and PMMA with 5-phenyl-1,3,4-oxadiazole derivatives was successfully achieved in this study. Some of the resulted polymers were characterized using FT-IR, TGA and XRD techniques. The thermal degradation of most of the grafted polymers occurred in two step degradation where the first step is attributed to the loss of water and the degradation of oxadiazole moieties and the second step is for the random scission of the main chains of both Cs and PMMA. XRD showed the crystal structure of the grafted polymers as compared to Cs and PMMA patterns.

Compound 1 showed the highest antifungal activity against *Fusarium spp.* and *Alternaria alternata* followed by the polymers 3 and 5 that contain the biologically active Cs.



Scheme : Synthetic pathway of grafting of Cs and PMMA with 5-phenyl-1,3,4-oxadiazole derivatives.

Table 1: Antifungal inhibition zones of 1–6 and their LSD against *Fusarium spp.*

Code No.	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
1	45.18	70.0	100	100	100
2	34.44	34.44	86.66	100	100
3	7.40	20.0	41.11	71.48	100
4	7.77	50.0	66.66	70.74	100
5	11.11	20.37	27.77	77.77	100
6	0.00	0.00	22.22	40.0	79.25
LSD 5%	2.40	2.68	0.37	1.40	0.42

Table 2: Antifungal inhibition zones of 1–6 and their LSD against *Alternaria alternata*.

Code No.	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
1	87.03	90.0	92.22	94.44	100
2	45.55	77.77	81.11	93.33	96.66
3	53.33	79.25	81.11	85.55	100
4	62.59	77.77	88.14	88.88	92.22
5	64.44	81.11	83.33	94.44	100
6	52.22	58.88	77.77	82.22	100
LSD 5%	1.89	0.42	0.42	1.99	2.67

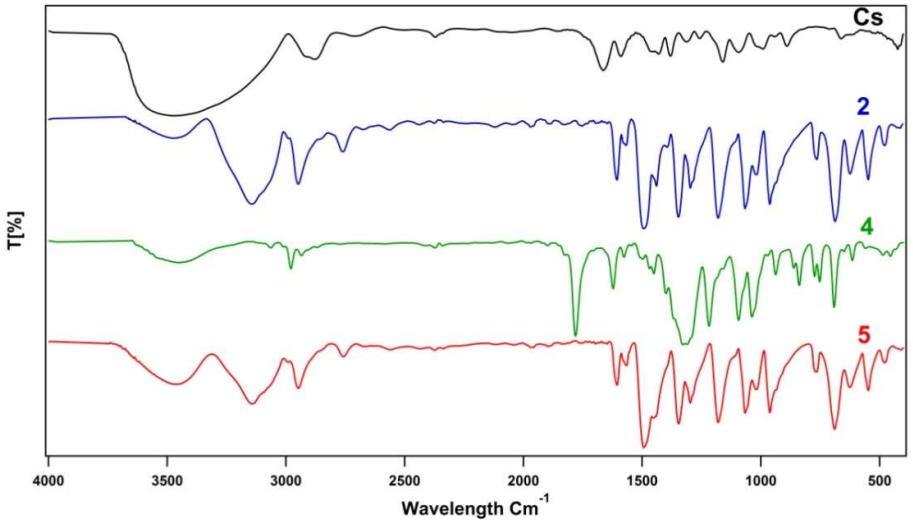


Fig.1: FT-IR of Cs, 2, 4 and 5.

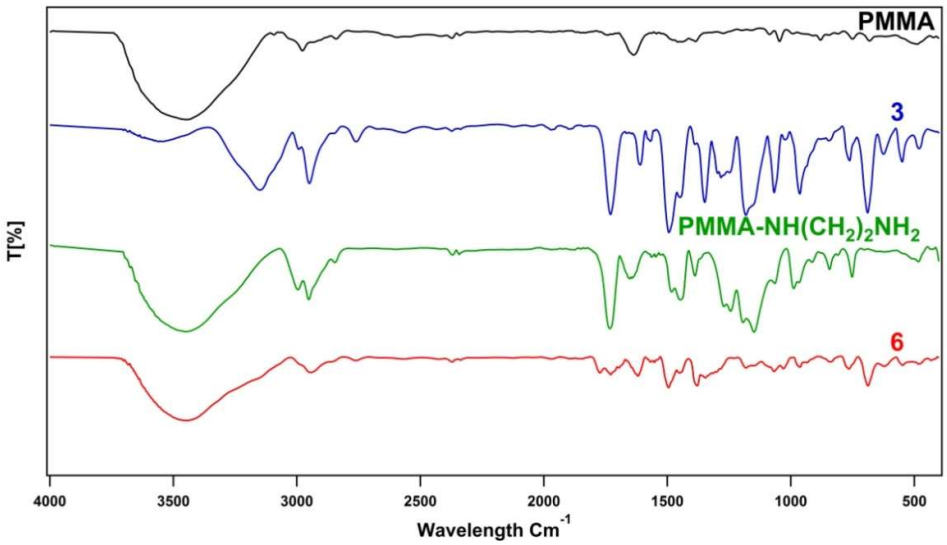


Fig.2: FT-IR of PMMA, 3, PMMA-NH(CH_2)NH_2 and 6.

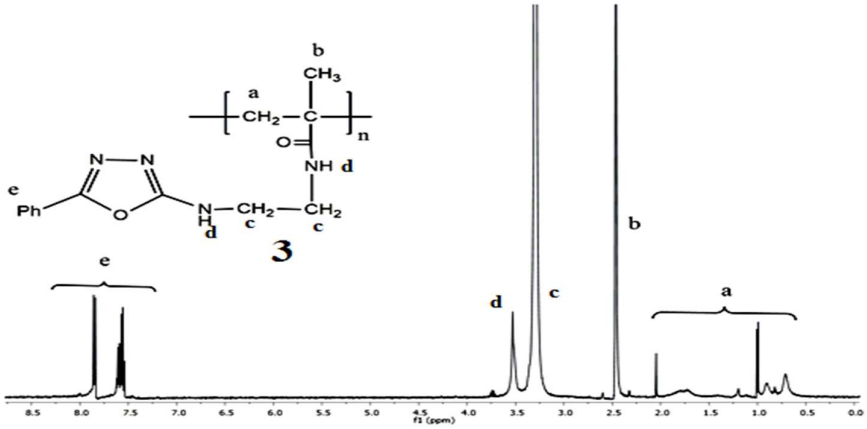


Fig.3: ^{1}HNMR of 3

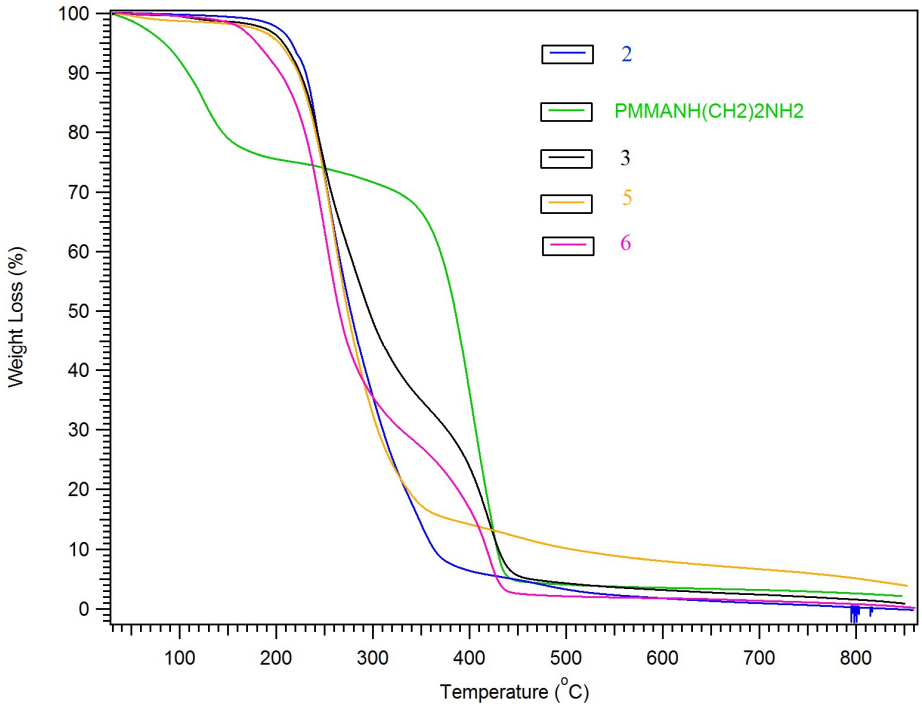


Fig.3: TGA of PMMANH(CH_2)NH_2, 2, 3, 5 and 6.

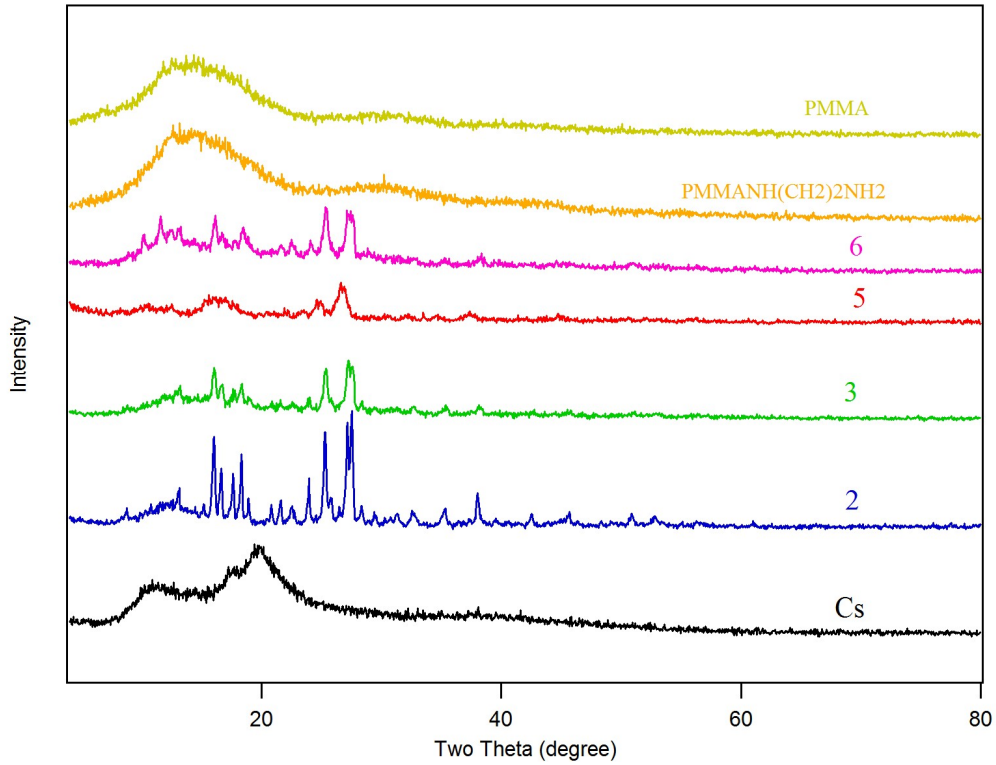


Fig.4: XRD of Cs, PMMA, PMMANH(CH_2)NH_2, 2, 3, 5 and 6.

References

1. Xu, F.; Wang, Y.; Luo, D.; Yu, G.; Wu, Y.; Dai, A.; Zhao, Y.; Wu, J., Novel Trifluoromethyl Pyridine Derivatives Bearing a 1,3,4-Oxadiazole Moiety as Potential Insecticide. *ChemistrySelect* **2018,** 3, (10), 2795-2799.
2. Zhang, Z.-J.; Jiang, Z.; Zhu, Q.; Zhong, G.-H., Discovery of β-Carboline Oxadiazole Derivatives as Fungicidal Agents Against Rice Sheath Blight. *J. Agric. and Food Chem.* **2018**.
3. Kalhor, M.; Dadras, A., Synthesis, Characterization, and Herbicidal Activities of New 1, 3, 4-Oxadiazoles, 1, 3, 4-Thiadiazoles, and 1, 2, 4-Triazoles Derivatives Bearing (R)-5-Chloro-3-fluoro-2-phenoxypyridine. *J. of Heterocyclic Chemistry* **2013,** 50, (2), 220-224.
4. Duan, W.-G.; Li, X.-R.; Mo, Q.-J.; Huang, J.-X.; Cen, B.; Xu, X.-T.; Lei, F.-H., Synthesis and herbicidal activity of 5-dehydroabietyl-1, 3, 4-oxadiazole derivatives. *Holzforschung* **2011,** 65, (2), 191-197.
5. Song, X.; Li, P.; Li, M.; Yang, A.; Yu, L.; Luo, L.; Hu, D.; Song, B., Synthesis and investigation of the antibacterial activity and action mechanism of 1, 3, 4-oxadiazole thioether derivatives. *Pesticide Biochemistry and Physiology* **2018,** 147, 11-19.
6. Siddiqa, A.; Abbasi, M. A.; Siddiqui, S. Z.; Khan, S. G.; Rasool, S.; Shah, S. A. A., Synthesis, spectral analysis and antibacterial activity of some novel 5-substituted-2-((6-bromo-3, 4-methylenedioxybenzyl) thio)-1, 3, 4-oxadiazole derivatives. *Pakistan J. Pharm. Sci.* **2018,** 31, (5).
7. Tian, K.; Li, X.-Q.; Zhang, L.; Gan, Y.-Y.; Meng, J.; Wu, S.-Q.; Wan, J.-L.; Xu, Y.; Cai, C.-T.; Ouyang, G.-P., Synthesis of novel indole derivatives containing double 1, 3, 4-oxadiazole moiety as efficient bactericides against phytopathogenic bacterium Xanthomonas oryzae. *Chemical Papers* **2018**, 1-9.
8. Sekhar, M. M.; Nagarjuna, U.; Padmavathi, V.; Padmaja, A.; Reddy, N. V.; Vijaya, T., Synthesis and antimicrobial activity of pyrimidinyl 1, 3, 4-oxadiazoles, 1, 3, 4-thiadiazoles and 1, 2, 4-triazoles. *J. Euro. med. chem.* **2018,** 145, 1-10.
9. Zheng, Z.; Liu, Q.; Kim, W.; Tharmalingam, N.; Fuchs, B. B.; Mylonakis, E., Antimicrobial activity of 1, 3, 4-oxadiazole derivatives against planktonic cells and biofilm of Staphylococcus aureus. *Future medicinal chemistry* **2018,** 10, (3), 283-296.
10. Madhavilatha, B.; Bhattacharjee, D.; Sabitha, G.; Reddy, B. S.; Yadav, J.; Jain, N.; Reddy, B. J. M., Synthesis and In Vitro Anticancer Activity of Novel 1, 3, 4-Oxadiazole-Linked 1, 2, 3-Triazole/Isoxazole Hybrids. *J. of Heterocyclic Chemistry* **2018,** 55, (4), 863-870.
11. He, X.; Li, X.-y.; Liang, J.-w.; Cao, C.; Li, S.; Zhang, T.-j.; Meng, F.-h., Design, synthesis and anticancer activities evaluation of novel 5H-dibenzo [b, e] azepine-6, 11-dione derivatives containing 1, 3, 4-oxadiazole units. *Bioorganic & medicinal chemistry letters* **2018,** 28, (5), 847-852.
12. Mohan, C. D.; Anilkumar, N. C.; Rangappa, S.; Shanmugam, M. K.; Mishra, S.; Chinnathambi, A.; Alharbi, S. A.; Bhattacharjee, A.; Sethi, G.; Kumar, A. P., Novel 1, 3, 4-Oxadiazole Induces Anticancer Activity by Targeting NF-κB in Hepatocellular Carcinoma Cells. *Frontiers in oncology* **2018,** 8, 42.
13. Siddiqui, S. Z.; Aziz-ur-Rehman, M. A. A.; Abbas, N.; Khan, K. M.; Ashraf, M.; Ejaz, S. A., Synthesis, characterization and biological screening of N-substituted derivatives of 5-benzyl-1, 3, 4-oxadiazole-2yl-2′′-sulfanyl acetamide. *Pak. J. Pharm. Sci* **2013,** 26, (3), 455-463.
14. Fatima, A.; Abbasi, M. A.; Rasool, S.; Malik, A.; Ashraf, M.; Ahmad, I.; Ejaz, S. A., Synthesis of new N-(5-Chloro-2-methoxyphenyl)-4-(5-substituted-1, 3, 4-Oxadiazol-2-ylthio) butanamide derivatives as suitable lipoxygenase

inhibitors. *Journal of Saudi Chemical Society* **2016,** 20, S488-S494.
15. Xu, W.; He, J.; He, M.; Han, F.; Chen, X.; Pan, Z.; Wang, J.; Tong, M., Synthesis and antifungal activity of novel sulfone derivatives containing 1, 3, 4-oxadiazole moieties. *Molecules* **2011,** 16, (11), 9129-9141.
16. Salahuddin, N.; Elbarbary, A.; Allam, N. G.; Hashim, A. F., Chitosan modified with 1, 3, 4-oxa (thia) diazole derivatives with high efficacy to heal burn infection by Staphylococcus aureus. *J. of Bioactive and Compatible Polym* **2018,** 33, (3), 254-268.
17. Vondrák, J.; Reiter, J.; Velická, J.; Sedlaříková, M., PMMA-based aprotic gel electrolytes. *Solid State Ionics* **2004,** 170, (1-2), 79-82.
18. Henry, A. C.; Tutt, T. J.; Galloway, M.; Davidson, Y. Y.; McWhorter, C. S.; Soper, S. A.; McCarley, R. L., Surface modification of poly (methyl methacrylate) used in the fabrication of microanalytical devices. *Analytical chemistry* **2000,** 72, (21), 5331-5337.
19. Salahuddin, N.; Elbarbary, A.; Allam, N. G.; Hashim, A. F., Polyamide-montmorillonite nanocomposites as a drug delivery system: Preparation, release of 1, 3, 4-oxa (thia) diazoles, and antimicrobial activity. *J. of Appl. Polym Sci.* **2014,** 131, (23).
20. Latha, P.; Anand, T.; Ragupathi, N.; Prakasam, V.; Samiyappan, R., Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against Alternaria solani. *Biological Control* **2009,** 50, (2), 85-93.
21. Bobbarala, V.; Katikala, P. K.; Naidu, K. C.; Penumajji, S., Antifungal activity of selected plant extracts against phytopathogenic fungi Aspergillus niger F2723. *Ind. J. Sci. and Tech.* **2009,** 2, (4), 87-90.
22. Huacai, G.; Wan, P.; Dengke, L., Graft copolymerization of chitosan with acrylic acid under microwave irradiation and its water absorbency. *Carbohydrate polymers* **2006,** 66, (3), 372-378.
23. Valandro, S. R.; Lombardo, P. C.; Poli, A. L.; Horn Jr, M. A.; Neumann, M. G.; Cavalheiro, C. C. S., Thermal properties of poly (methyl methacrylate)/organomodified montmorillonite nanocomposites obtained by in situ photopolymerization. *Materials Research* **2014,** 17, (1), 265-270.
24. Wang, S.; Shen, L.; Tong, Y.; Chen, L.; Phang, I.; Lim, P.; Liu, T., Biopolymer chitosan/montmorillonite nanocomposites: preparation and characterization. *Polymer Degradation and Stability* **2005,** 90, (1), 123-131.
25. Zhang, H.; Zhang, P.; Li, Z.; Sun, M.; Wu, Y.; Wu, H., A novel sandwiched membrane as polymer electrolyte for lithium ion battery. *Electrochemistry communications* **2007,** 9, (7), 1700-1703.
26. Long, L. T.; Tan, L. V.; Boi, V. N.; Trung, T. S., Antifungal activity of water-soluble chitosan against Colletotrichum capsici in postharvest chili pepper. *J. of Food Processing and Preservation* **2018,** 42, (1), e13339.