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Chemical Modification of Radiation Copolymerized of [N-Vinyl-2-Pyrrolidone/2-(4-Methoxy-Benzylidine)-Malononitrile] with Some Organic Compounds and Their Biological Activity

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ABSTRACT

N-Vinyl-2-Pyrrolidone (NVP) was copolymerized with 2-(4-methoxy-benzylidine)- malononitrile (MBM) monomers to form a new copolymer, P(MBM-co-NVP), containing nitrile groups. The characterization of copolymer and their modified with organic compounds such as thiourea, thiosemicarbazide, 2-aminothiazole, N-Glycyl glycine, 4-amino-N-[5-methyl-3-isoxazolyl]benzene sulfonamide and 4-amino-N-[4-methyl-2-pyrimidnyl]benzene sulfonamide was carried out by Fourier transform infrared spectroscopy (FTIR), Elemental analysis (EA), Gel Permeation Chromatograph (GPC), Differential Scanning Calorimetry (DSC) and Thermal Gravimetric analysis (TGA). The changes in surface morphology of the prepared copolymer and its modified copolymers were observed by scanning electron microscopy (SEM). The biological activity of the modified copolymers with some organic compounds containing with or without sulfur was investigated. The results revealed that the biological activity of the modified copolymers was higher than that of the copolymer ones, under the same conditions. An improvement of prepared copolymers by modification with various organic compounds showed great promise in some practical applications in the field of antibacterial activity.

Keywords, Chemical modification; Radiation; Surface morphology; Biological activity

1. INTRODUCTION

The preparation of a copolymer can be obtained by radiation polymerization of monomers or various vinyl monomers onto different polymeric materials (1-4). The use of N-vinyl-2-pyrrolidone (NVP) as a

hydrophilic monomer is well-known in radiation grafting on various polymers and was described for the preparation of biocompatible polymer surfaces (5). Radiation-induced copolymers with polyfunctionally substituted heterocyclic ring derivatives comprise a very interesting class of polymers because of their significant biological and pharmaceutical activity (6). Antimicrobial activity based on polymers with polyfunctionally substituted functions have attracted considerable (7,8). Our interest in the biological activity using some biological active compounds, for example α-cyano-β-(pyridyl) crotonitrile; α-cyano-β-(thienyl) crotonitrile and α-cyano-β-(phenyl) crotonitrile (9). Consequently, the modified graft copolymerization of NVP onto various monomers or polymers is widely used in the biomedical applications because of its excellent biocompatibility and high water permeability (10). Therefore, radiation polymerization is a convenient method for the modification of physical and chemical properties of polymeric materials. The surface structures and morphology or modified copolymes such as grafted polypropylene (PP) with NVP or with 2-hydroxyethylmethacrylate (HEMA) were also investigated (10,11).

In the present study, the direct radiation induced polymerization of NVP with 2-(4-methoxy-benzylidine)- malononitrile (MBM) was studied and then modified with some organic compounds. The analysis by Fourier transform infrared (FTIR), elemental analysis (EA), gel permeation chromatograph (GPC), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and scanning electron microscopy (SEM) have been investigated. The biological activity of the modified copolymer powder has been determined.

2. EXPERIMENTAL

2.1. Materials

N-vinyl-2-pyrrolidone (NVP), Merck, Germany, of purity 99% was used without purification. Thiourea, thiosemicarbazide, 2-aminothiazole, N-Glycyl glycine, 4-amino-N-[5-methyl-3-isoxazolyl] benzene sulphonamide and 4-amino-N-[4-methyl-2-pyrimidnyl] benzene sulphonamide, compounds [**3-8**], were prepared. Other chemicals were reagent grade and used without purification.

2.2. Preparation of 2-(4-methoxy-benzylidine)- malononitrile (MBM)

Malononitrile (1.32 ml, 20.8 mmol) dissolved in ethanol (14ml) was added onto p-methoxy benzaldehyde (20 mmol) followed by 3 drops of

piperidine. The reaction mixture was then refluxed for an hour and allowed to cool to room temperature upon which a precipitate was formed. The crude product was collected by filtration and without any further purification. Off white solid, 683% yield. ^{1}H-NMR S($CDCl_3$): 7.96-7.91 (m, 3H, phenyl- H+ CH), 7.04 (d, 2H, J= 8.76 Hz, phenyl-H), 3.9 (S, 3H, CH_3). m.p. = 82^{o}C, molecular formula $C_{11}H_8N_2O$ and has a molecular weight of 184 g/mol (12).

2.3. Radiation Copolymerization

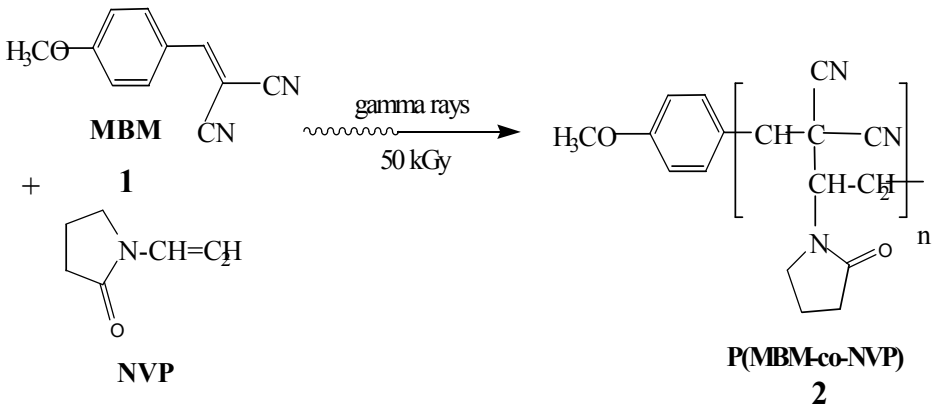
Copolymer was obtained by radiation polymerization of NVP with MBM monomer using γ-rays, in which monomer powder, MBN, was dissolved in DMF and then added to NVP monomer, then deareated by N_2 gas for 5 min, sealed and subjected to γ-radiation from ^{60}Co source to 50 kGy at dose rate of 3.7 kGy/h. The ampoule content was poured into an excess of ethanol and the polymeric substrate of p(MBM-co-NVP) was filtered off and re-precipitated from DMF solution into ethanol. Finally, it was dried in vacuum oven until a constant weight and analyzed. The copolymerization reaction can be represented as shown in Scheme 1.

Elemental analysis:

Found: C=69.23%, H= 5.95% and N= 15.13%

Calc.: C=69.15%, H= 5.76% and N = 14.29%

The melting point of the prepared copolymer = 126^{o}C (cf. Table 1).



Sch.1. Schematic representation for radiation copolymerization of NVP with MBM monomer

2.4. Chemical Modification of Copolymers [3-8]

The development of the copolymer structure was carried out in two sequential steps: reaction of copolymer with aldehyde such as glutraldehyde or glyoxal followed by condensation of aldehyde with the immobilized different amines.

2.4.1. Reaction of Copolymer with Aldehyde

Into a 100 ml round-bottomed flask, a mixture of 0.5g of copolymer in 20ml DMF with 5ml glutraldehyde (25%) or glyoxal was stirred for 2h at room temperature. The precipitate was filtered off and dried under vacuum to give yellow crystals.

2.4.2. Condensation of Compound [2] with the Immobilized of Different Amines

To a solution of 1.5 g of compound **2** in 30ml EtOH/$EtO^{-}Na^{+}$ as a catalyst with drops of DMF and an appropriate amount of thiourea, thiosemicarbazide, 2-aminothiazole, N-Glycyl glycine, 4-amino-N-[5-methyl-3-isoxazolyl]benzene sulphonamide and 4-amino-N-[4-methyl-2-pyrimidnyl]benzene sulphonamide. The reaction mixture was refluxed for 3h at 80^{o}C.The solid separated out was filtered off, washed with ethanol and crystallized from ethanol to give compounds **3-8** as shown in Scheme 2.

2.5. Infrared spectroscopy (FTIR)

Analysis by infrared spectrophotometer was carried out in the form of KBr pellets by using FTIR 6300, Jasco, Japan, in the range of 400 - 4000 cm^{-1}.

2.6. Gel Permeation Chromatography (GPC)

The molecular weight of the prepared copolymers was measured by gel permeation chromatography (GPC) 1100 Agilant instrument equipped with organic GPC-SEC start up kits with a flow rate of 1ml/min, pressure 150-5 bar, injection volume 50µL and column temperature thermostat 25°C. The molecular weights were determined from a calibration curve using polystyrene standards (13).

2.7. Thermal Gravimetric Analysis (TGA)

A Shimadzu TGA system of type TGA-50 (Japan) was used under nitrogen atmosphere to examine the thermal stability of the samples at heating rate ≈ 20^{o}C/min.

2.8. Differential Scanning Calorimetry (DSC)

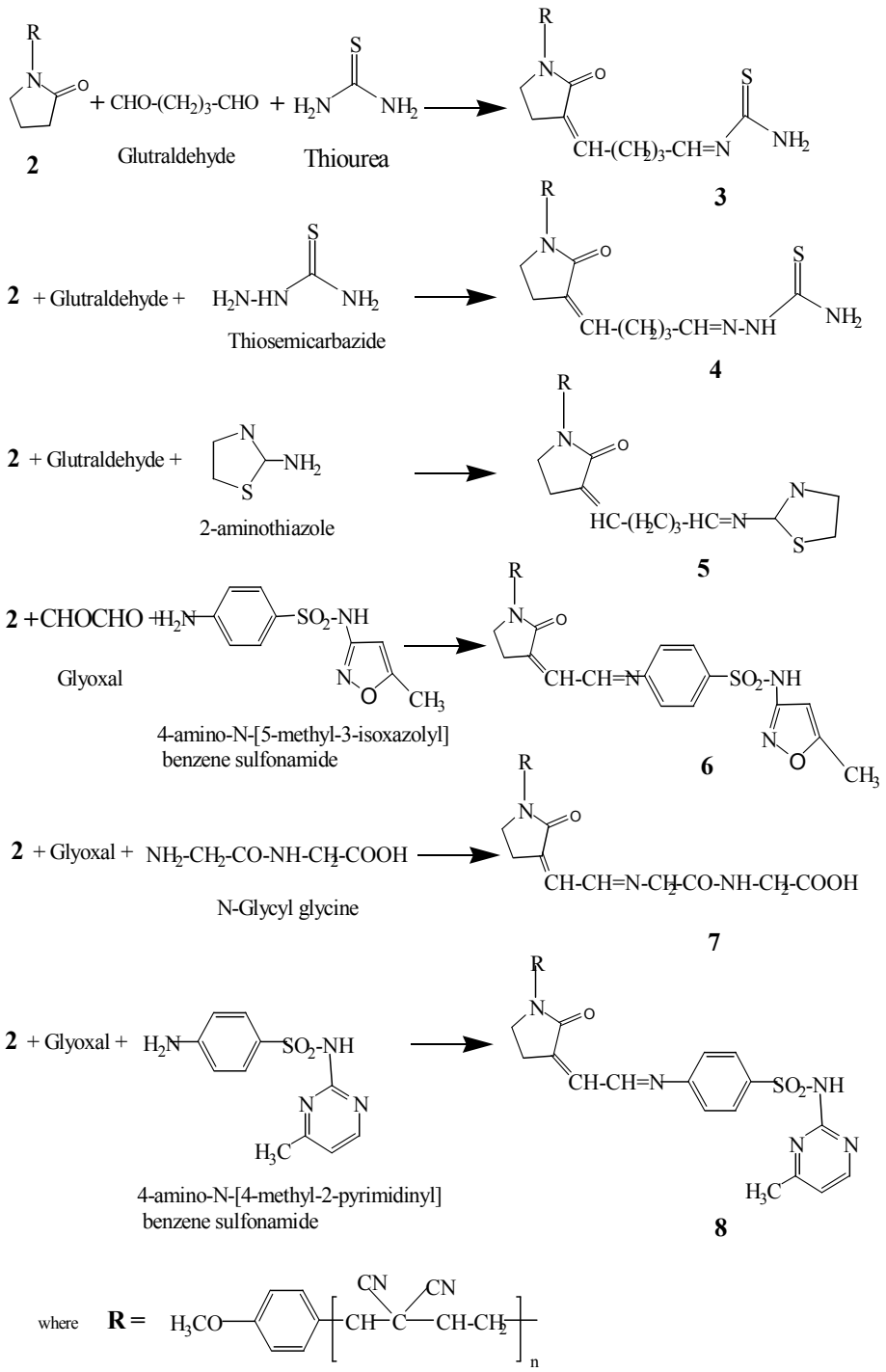
The melting temperature (T_m) was determined using Shimadzu DSC system, Japan. The measurements were carried out in N_2 atmosphere at a heating rate of $10^oC\ min^{-1}$ using specimen weight (≈5 mg).

2.9. Scanning Electron Microscopy (SEM)

The surface morphology of the prepared copolymer and its modified copolymers was measured with JEOL JSM-5400 (Japan) at 20 kV. The surfaces of the samples were sputter-coated with gold for 3 min.

2.10. Procedure of Biological Activity Tests

The biological activity of copolymers under study has been carried out as antibacterial activity. Four strains of bacteria were used i.e. Staphytococcus *aureus, Pseudomonas aeruginosa, Escherichia coli* and *Strepococcus*. The microorganisms under study were inoculated each in 5 ml sterile Nutrient broth and incubated at 32°C over night with shaking, 1ml from each culture was transferred to 5 ml sterile nutrient broth and incubated at 32°C for 2h, the OD_{600} for each was measured to give 0.2 (i.e. 10^7 CFU). Aliquots of 30 ml Nutrient broth agar medium was left to cool to 55°C, 100 µL of the prepared culture were added and poured in sterile Petri dish, the plates were left to solidify and 8 holes were made aseptically with cork borer having 6mm diameter. In each hole 100 µL of polymer was added. Plates were kept for prediffusion for 30 min. After it normalized to room temperature, the plates were incubated at 32°C for 24h. In the same time aliquots of 100µL of the prepared culture and 100µL of each polymer were added to 5ml Nutrient broth incubated at 32°C over night. The growing culture was centrifuged; the precipitate was lyophilized and examined under scanning electron microscopy (14).



Sch. (2): Schematic representation for condensation of compound [2] with the immobilized of different amines.

3. RESULTS AND DISCUSSION

3.1. Characterization of the Monomer and Copolymer

Figure (1) shows the IR spectroscopy of MBM monomer (curve a) and copolymer of NVP with it, p(MBM-co-NVP), (curve b). It can be seen from the monomer (curve a) that the absorption band at 2260-2200 cm^{-1} was characteristic for nitrile groups. The band at 1725 cm^{-1} was characteristic for C=O and that at 1570-1560 cm^{-1} is assigned to C=C in plan vibration of the phenyl ring of the monomer. However, in the case of copolymer, it can be seen (curve b), p(MBM-co-NVP), had absorption band at 3300-3600 cm^{-1} were characterized of structure of NVP after polymerization process. Also, the bands appeared at 1600, 880 and 860 cm^{-1} are characteristic for p-disubstituted benzene ring (15,16).

Figure (2) shows the GPC elution curve of the prepared copolymer by γ-rays at 50 kGy. The molecular weight (Mw) was $2.04x10^4$ at retention time of 6.741 min. Also, all the molecular weights of mpdified copolymers were measured and listed in Table (1). Meanwhile, the DSC was performed and it has T_g between 35-40°C, probably depending on molecular weight (17). The melting point of the prepared copolymer **2** appeared at 126°C. While, the melting point of the modified copolymer **3, 4** and **5** appeared at 176, 182 and 166°C, respectively as shown in Figure (3). However, the melting point of the modified copolymer **6, 7** and **8** appeared at 250, 130 and 260°C, respectively. The results suggest that the the higher molecular weight, the higher melting point was obtained and may be efficient towards strains of bacteria used.

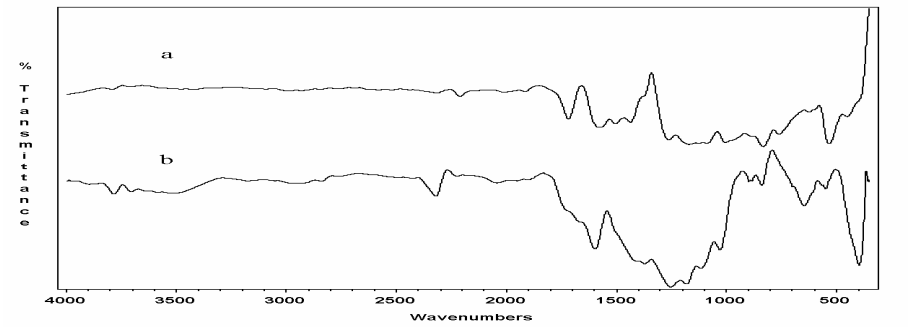


Figure (1: FTIR spectra of 2-(4-methoxy-benzylidene)-malononitrile (MBM) and P(MBM-co-NVP).

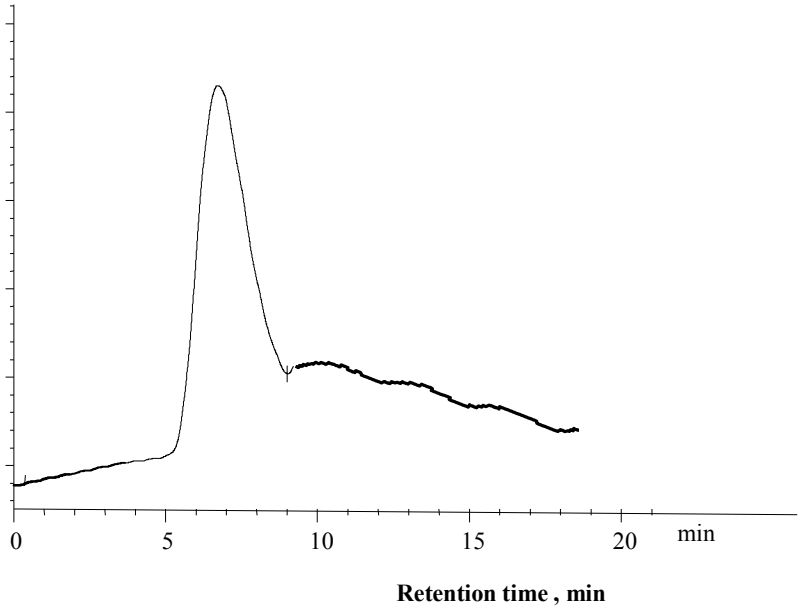


Figure (2): GPC elution curve of P(MBM-co-NVP) as a function of retention time at 50 kGy.

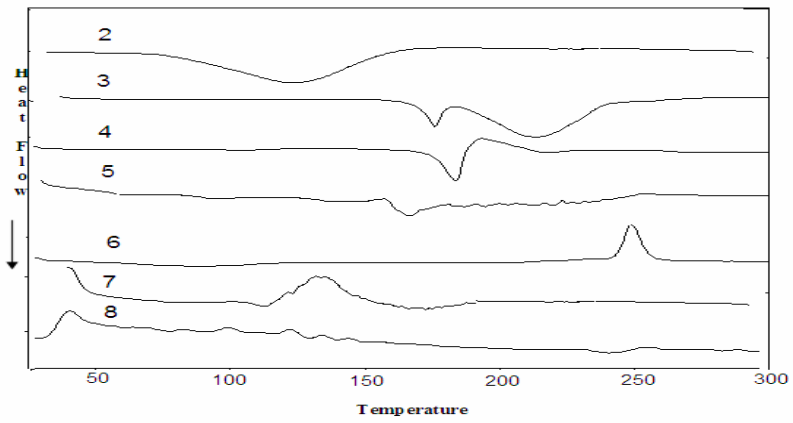


Figure (3): DSC curves of P(MBM-co-NVP) [2] and modified copolymers at 50 kGy.

3.2. FTIR spectroscopy of Modified Copolymers

NVP can be viewed as a species containing three active sites: vinyl group, carbonyl group and active methylene group. These sites were reduced to two sites only after copolymerization process by radiation. The other two sits, carbonyl group and methylene function group show a variety in reactivity. The amide carbonyl group shows no reactivity toward the nucleophilic reagent and this may be due to the chain steric effect. This result prompted us to investigate the reactivity of α- methylene function. Compounds **[3, 4 and 5]** (cf. Sch. 2) established not only by IR spectra but also on basis of the elemental analysis results for the non-modified and modified copolymer reactions (cf Table 1). The presence of sulfur and nitrogen amounts and it can be seen by comparing the data with the prepared copolymer **2** in Table 1. The modified copolymer [**3**] (Figure 4, curve a), **4** (Figure 4, curve b) and **5** (Figure 4, curve c) reveal the presence of cyano groups at 2195-2191 cm^{-1}, which were present in both monomer **[1]** and their copolymer **[2]**. The characteristic absorption of carbonyl group and –C=N– appeared at 1650 cm^{-1} and phenyl ring at 1550-1460 cm^{-1} . Also, the band at 1200-1050 cm^{-1} is characteristic for C=S. The absorption band at 2850-2810 cm^{-1} exhibit $–OCH_3$ in addition to the absorption band appears at 3500-3300 cm^{-1} for $–NH_2$ and NH stretching.

Table (1): Elemental analysis of compounds (2-8)

Compound	Mwt of modified copolymer	*	C%	H%	N%	S%
2	٢,٠٤x10^4	Calc. Exp.	69.15 69.23	5.76 5.95	14.24 15.13	- -
3	٢,٠٨x10^4	Calc. Exp.	63.44 64.12	5.74 6.2	16.1 16.81	7.36 6.99
4	٢,١٦x10^4	Calc. Exp.	61.33 62.24	5.8 6.44	18.7 19.22	7.11 8.5
5	٢,٢١x10^4	Calc. Exp.	64.93 65.46	6.06 6.92	15.15 16.22	6.92 7.54
6	٢,٢٢x10^4	Calc. Exp.	62.7 63.43	4.86 5.43	15.12 15.54	5.76 6.26
7	٢,١٩x10^4	Calc. Exp.	61.46 62.22	5.12 6.11	15.59 16.18	- -
8	٢,٢٤x10^4	Calc. Exp.	63.71 64.51	4.77 5.53	17.34 16.81	5.66 6.13

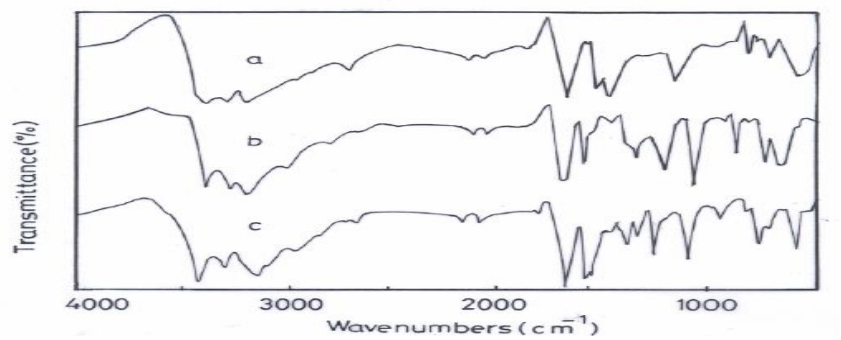


Figure (4): IR spectra of P(MBM-co-NVP) modified with (a) thiourea, (b) *thiosemicarbazide and (c) aminothiazole.*

3.3. Thermal Stability

Figure (5) illustrates the TG curves of the copolymer, P(MBM-co-NVP), (curve a), modified copolymer with thiourea (curve b) and the modified copolymer with thiosemicarbazide (curve c). It can be seen from Figure 5 that the prepared copolymer (curve a) is stable up to 150°C. Afterwards, the remaining weight (%) was decreased rapidly with elevating temperature. However, the thermal stability of both copolymers with thiourea or thiosemicarbazide was stable up to 220°C, the degradation for both are sharply occurred. It can be observed that, the thermal stability of modified copolymer with thiosemicarbazide is higher than that of the modified with thiourea. Therefore, the formation of long chains may be improved the thermal stability of polymers. The results showed that the increase on thermal stability depend on the increase in the molecular weight due to the addition of some organic compound by chemical reactions.

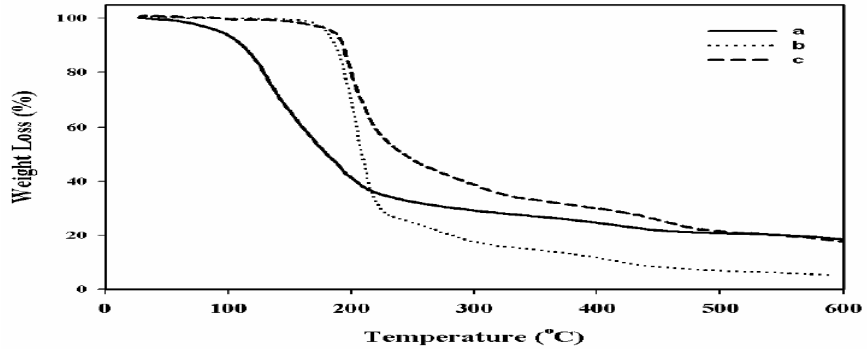


Figure (5): TG curve for (a) P(MBM-co-NVP), (b) P(MBM-co-NVP) modified with thiourea and (C) P(MBM-co-NVP) modified with thiosemicarbazide.

3.4. Biological Activity

Organic compounds containing sulfur show a reasonable biological activity (18,19The various organic compounds reacted with the prepared copolymer and it has been produced slightly, moderate and severe effects toward the studied microorganisms i.e. it has antibacterial potentialties against the bacterial strained as shown in Table 2.

Table (2): Antibacterial activity of copolymer and modified copolymers.

Compound	Antibacterial Activity			
	Microorganism			
	S.aureus	***Ps.aerogenosa***	***E.coli***	***Streptococcus***
1	-	-	-	-
2	-	-	-	-
3	+	+	+	+
4	++	++	+	+
5	+++	++	++	+
6	++	+++	+++	++
7	++++	++	++	+
8	++++	++++	+++	+++

Table 2 shows the biological activity of the copolymer and their modified ones by different organic moiety against *S.aureus, Ps.aerogensa, E. coli* and *Streptococcus*. The activity could be classified as follows: both compounds **[1]** and **[2]** show no effects. Compound **[3]** has a slight effect; compounds **[4**, **5** and **7]** have moderate effects. The previous classification indicates that the biological activity increases with the organic compounds containing sulfur. In other words, as the amount of modified moiety increase, the biological activity increases. After incubation of the bacterial strains and the copolymer under study, it was observed that the bacterial cells precipitated after 24 h incubation time. This may be due to the deproteinization the bacterial cells. The scanning electron micrographs of precipitated bacterial cells was depicted as shown in Figure (6) for compounds **[6** and **8**], respectively. The change of surface morphology due to deproteinization of the bacterial cells was depicted as (gram +ve *Staphaurous*, Fig. 6a), (gram +ve, *E. coli*, Fig. 6b), (gram –ve *Ps. aeruginosa*, Fig. 6c) and (gram –ve *Streptococcus*, Fig. 6d).

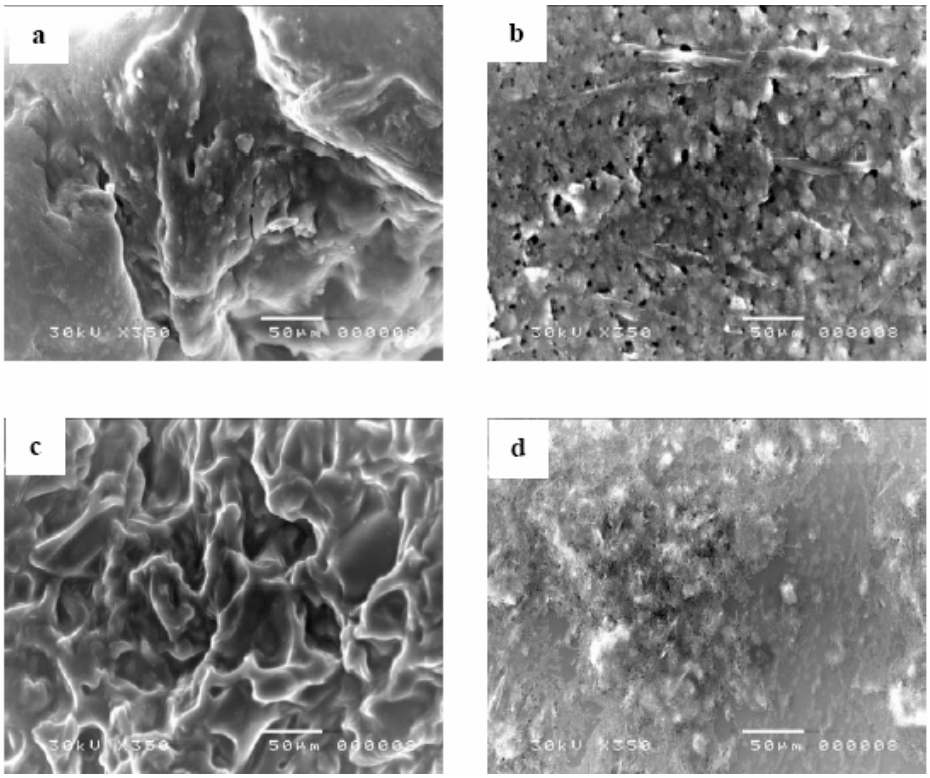


Figure (6): Scanning electron micrographs of compound 6 with gram +ve (a and b) bacteria and compound 8 with gram –ve (c) and gram +ve (d) bacteria.

3.5. Morphology

The scanning electron micrographs depicted in Figure (7) shows that the monomer **1** (Fig. 7a), copolymer **[2]** (Fig. 7b), modified copolymer **[3]** (Fig. 7c), **[4]** (Fig. 7d), **[5]** (Fig. 7e), **[6]** (Fig. 7f), **[7]** (Fig. 7g) and **[8]** (Fig. 7h), respectively. It can be seen that the monomer MBM **[1**] (Fig. 7a) appears to have a shape like stone. Meanwhile, the copolymer **2,** p(MBM-co-NVP), appears as similar mountain due to the embedding of NVP chains with MBM after polymerization process as in (Fig. 7b). However, the modified copolymer **3** (Fig. 7c) appears to be stalk or straw due to the presence of thiourea in the structure of formed product. While modified copolymer **[4]** (Fig. 7d) appears as micropores distributed on the surface, which may be due to the presence of thiosemicarbazide chains. Also, the modified copolymer **[5]** (Fig. 7e) the pores were somewhat enlarged. Meanwhile, the modified copolymer exhibits assume a crystalline appearance, which may be due to the presence of SO_2 in 4-amino-N-[4-methyl-2-pyrimidinyl] benzene sulphonamide structures. However, it can

be seen that the morphologies of modified copolymer **[8]** (Fig. 7h) has unconspicuous shape. But the modified copolymer **[7]** (Fig. 7g) appears more crystalline due to the presence of N-Glycyl glycine which containing a carboxylic group which enhanced the hydrophilicity with more distribution of NVP structure in the formed product.

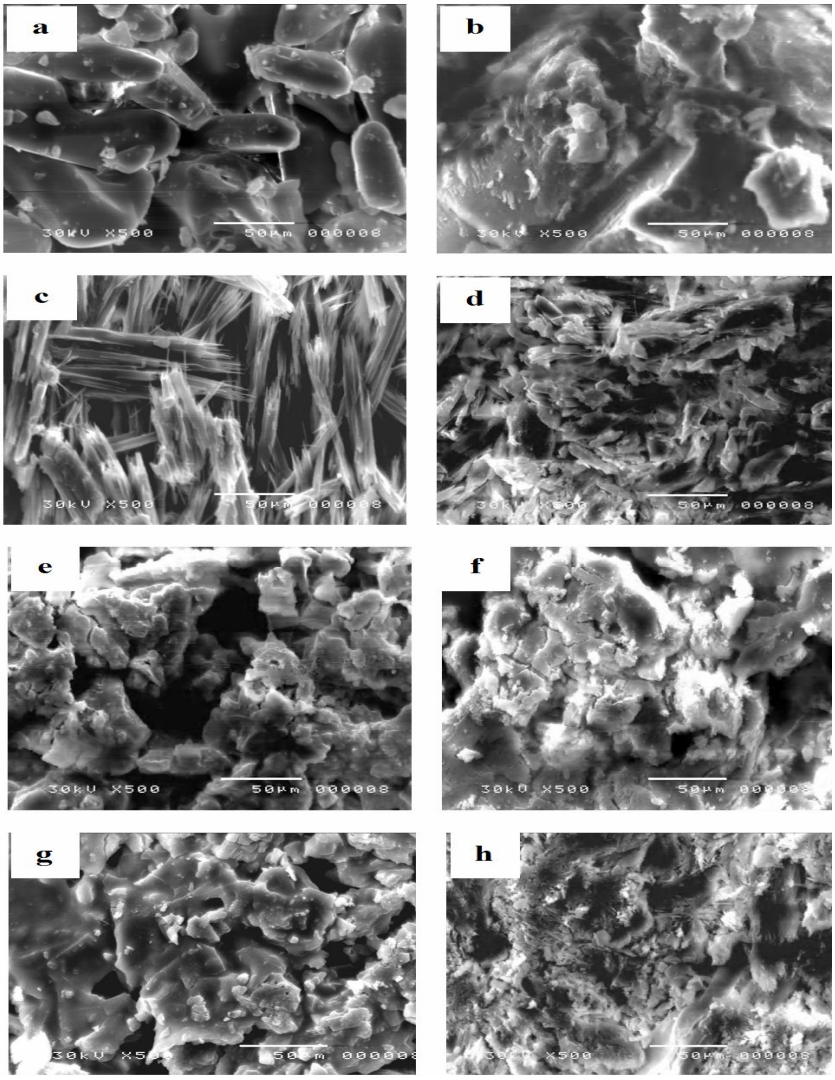


Figure 7: Scanning electron micrographs of (a) 2-(4-methoxy-benzylidene)-malononitrile (MBM), (b) P(MBM-co-NVP) and P(MBM-co-NVP) modified with (c)thiourea, (d) thiosemicarbazide, (e) 2-aminothioxazole, (f) 4-amino-N-[5-methyl-3-isoxazolyl]benzene sulfonamide, (g) N-Glycyl glycine and (h) 4-amino-N-[4-methyl-2-pyrimidinyl] benzene sulfonamide.

The results suggest that the embedding of organic compounds containing sulfur in the matrix of copolymer increase the change in surface morphology. Consequently, the crystallinity of the modified copolymer may also increase and it became more efficient towards various microorganisms.

Acknowledgement

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4. CONCLUSIONS

The copolymer was prepared by radiation polymerization of NVP with MBM monomer using γ-irradiation. From the discussion of the experimental results, the synthesized polymer revealed that the reactivity of α-methylene function of NVP towards some organic compounds containing sulfur has been investigated. The results confirmed by elemental analysis, GPC, DSC, TGA and SEM measurements. The thermal stability of modified copolymer with thiosemicarazide is higher than that modified with thiourea. Organic compounds containing sulfur show a rescannable biological activity. The biological activity increases with the amount of modified moiety increase. However, after incubation of the nutrient broth inoculated with the bacterial strains and copolymers. It was observed that the bacterial cells precipitated after 24h due to the deproteinization the bacterial cells. Also, the results suggest that embedding of organic compounds containing sulfur in the matrix of that prepared copolymer increase the change in surface morphology.

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