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New n-substituted itaconimide polymers: synthesis, characterization and biological activity

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In this study, new Itaconimide derivatives were synthesized. A new heteropolymers (PD6, PD7 and PD8) were synthesized under nitrogen from three Itaconimide derivatives [D3-D5] in presence of Benzoyl peroxide initiator. Three drugs (Paracetamol, Carbamazepine, and Trimethoprim) carried on Itaconimide with 3-aminobenzoic acid and triethylamine. FT-IR, ^{1}H-NMR, ^{13}C-NMR, and C.H.N.S were used to analyze all of these monomers and polymers. For heteropolymers (PD6, PD7, and PD8), controlled drug release and swelling percent were investigated at pH=2 and pH=8.0. For these polymers, intrinsic viscosities were determined and used to determine solubility. For the synthesized polymers, the *in vitro* thiazolyl blue tetrazolium bromide (MTT) reduction test was examined in breast cancer cell line (MCF7), in addition to the physical characteristics and anti-bacterial activity. The results displayed increases the activity towards bacteria for synthesized compounds compared to the Carbamazepine, Paracetamol, and trimethoprine drugs. The controlled drug release and swelling % was studied in different pH values at 37 ºC. Ostwald viscometers were used to quantify intrinsic viscosities at 25 ºC and apply the solubility characteristic to these polymers.

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Introduction

Itaconamic anhydride's multifunctionality makes it a prime candidate as a precursor for many more complex molecules. Researchers have examined the homo- and copolymerization of itaconimides, as well as their thermal characteristics. Itaconic anhydride may be easily converted into N-substituted itaconimide monomers by first reacting with the appropriate amine to produce N-alkyl or N-aryl itaconamic acids, which is then followed by dehydration [1-5].

Itaconic anhydride undergoes radical polymerization as an unsaturated cyclic anhydride and by polycondensation with diols or diamines. The two processes can alternatively be carried out in the following order: radical polymerization first, followed by polycondensation, or the opposite.

There are many uses for Itaconamic anhydride and their derivatives. N-substituted Itaconimide have been used successfully as insecticidal and fungicidal agents. They have been used as cottonseed preserving agents and as modifying agents for the synthesis of rubber products. Itaconimide have been also studied for anticancer activity [2-7]. N-Substituted Itaconamic acids are strongly amphiphilic molecules [1].

Itaconic anhydride polymers and copolymers made by radical synthesis can be polyitaconic acid hydrolyzed in an alkaline solution under ring opening or can be polymerized into polymeric acid amides or esters. Homopolymers and copolymers obtained from such monomers may have wide applications [8-10]. Because itaconic acid may be obtained from renewable resources, its polymeric derivatives are becoming more and more intriguing for research and industry [11-17].

Here, our effort concentrated on the creation of N-substituted Itaconimide based polymeric pharmaceuticals to increase the medication bioactivity through the combination of polymer, and to analyze the likelihood of creating the best polymer based drugs using Itaconimide.

N-[5-benzoic Itaconimide] monomer synthesis and copolymerization using methyl methacrylate were both described. The spectral, thermal, and physical characteristics of the co-polymers have been investigated.

Material and experimental

Materials

The analytical grade solvents and reagents were from Fluka, Siga-Aldrich, CDH, and Riedel-de Haen. SDI-Samarra Company provided Trimethoprine, Carbamazepine, and Paracetamol. The melting points were calculated using SMP30 melting point equipment [18]. The UV absorbance was measured, using a PG CECIL-CE7200 double beam spectrophotometer.

The densities of prepared polymers at 25 °C were measured using a METTLER TOLEDO (Densito 30px Portable Density Meter). To determine these polymers' viscosity (η) in acetone at 25 °C, an Ostwald viscometer was employed. Chemical shifts are measured in units of (ppm) in the ^{1}H-NMR and ^{13}C-NMR spectra, which were acquired in dimethyl sulfoxide (DMSO-d_6) using a Varian INOVA 500 MHz NMR spectrometer. A Bruker Tensor II Fourier Trans Infrared Spector Promoter AT-FT-IR was used to recorded the IR spectra in the (400-4000 cm^{-1}) region.

Synthesis: compound [D_1]: N-(3-carboxyphenyl) itaconimide:[13,14]

Itaconic anhydride solution (8.2 g, 0. 1 mol) in acetone was continuously stirred for three hours while m-amino benzoic acid solution (10 g, 0.1 mol.) was added gradually.

After that, (6 mL, 0.06 mol) acetic anhydride and (0.7 g, 0.0085 mol) sodium acetate were added and heated at 90 °C for two hours to produce a clear brown liquid. The solvent was evaporated from the mixture to give a white powdery precipitate, ice water was added and stirred for an hour at room temperature, and then filtered and dried the product.

As a result of recrystallizing the precipitate from ethyl acetate, product D1 was created in (5 g, 90% Yield) with (m.p.: 244-246 °C) (Scheme 1). The reaction was followed regularly by TLC [19] (R_f = 0.4/1 hexane: 3 ethylacetate), Color: White. FT-IR (cm^{-1}): 3098 for =C-H of Itaconimide and aromatic, ~ 3500-2551 for –OH of carboxylic group, C=O of carboxylic acid appeared at 1709 and C=O of amide at 1693, C=C aromatic at 1607 and 1517, (C-N) at 1314, aromatic ring at 852.

Compound [D_2]: N-[3-(chlorocarbonyl) phenyl] itaconimide

[D1] (4.5 g, 0.0107 mol), thionyl chloride (8.6 mL), and (8.6 mL) chloroform were mixed, stirred, and heated at 65°C for 6 hrs., the mixture was cooled, and then (8.6 mL) hexane was added.

Pure dark brown crystals of acid chloride were produced by recrystallizing the residue product from Dichloromethane after the unreacted thionyl chloride had been evaporated out (Scheme 1) [20,21]. The product was obtained in 90% yield, m.p.: 136-138 °C).

The reaction process was followed regularly by TLC [19] (R_f = 0.75/1 hexane: 3 ethylacetate). FT-IR (cm^{-1}): 3165 and 3088 (=C-H), 1774 (COCl), 1714, (CONCO), 1601, and 1582 (Aromatic C=C), 1379 (C-N), 889, and 831 (=C-H).

Itaconimide -drug derivatives [D_3, D_4, and D_5]

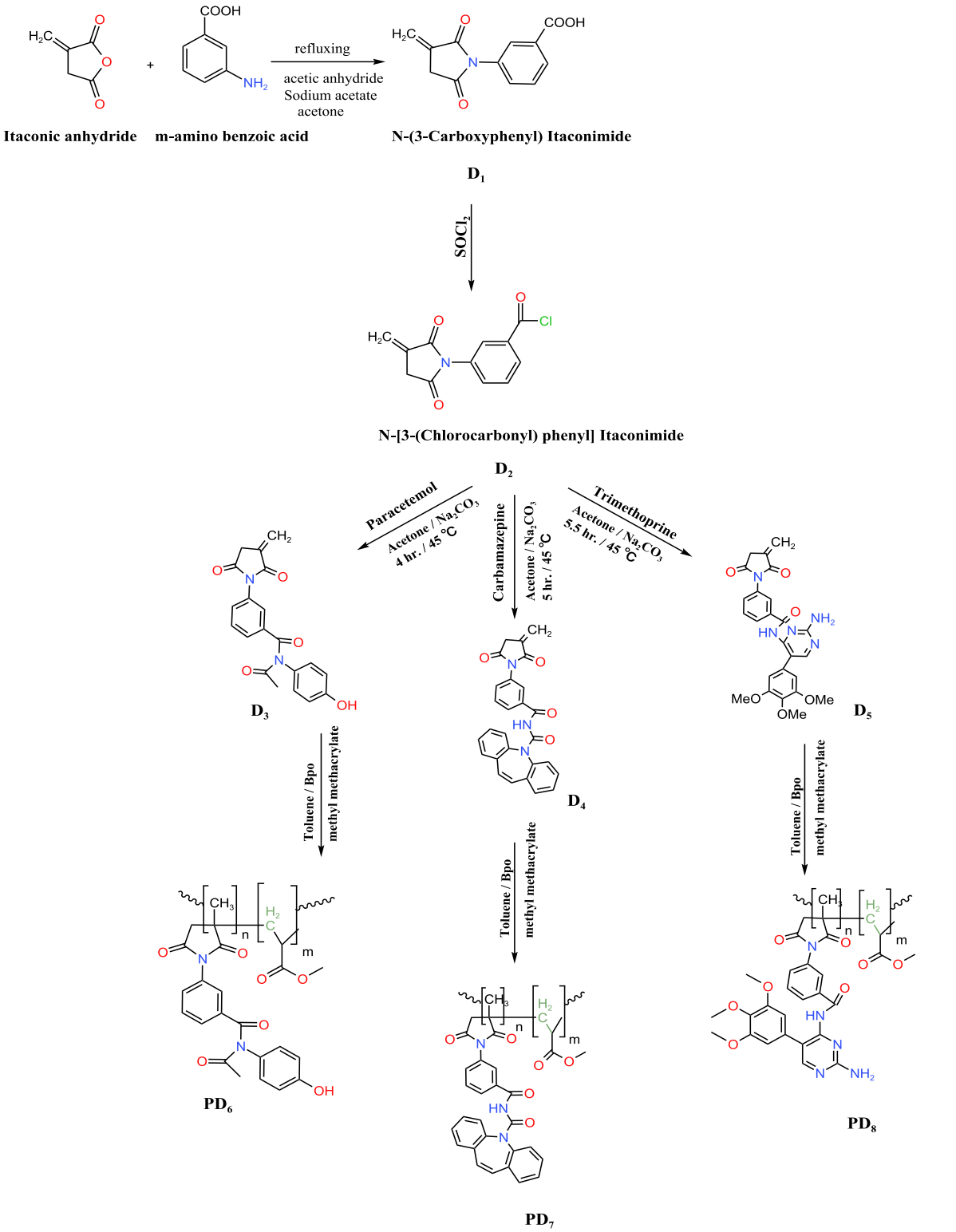
3-Itaconimide benzoyl Chloride (0.0018 mmol, 0.6 g) was added separately with stirring to (0.0018 mmol.) of drugs (Paracetamol, Carbamazepine, and trimethoprine) in 20 mL of acetone.

Thereafter, sodium carbonate (0.09 g, 0.0002 mmol) a dropwise addition was made at room temperature for (30 min) and the mixture was heated for 4-6 hrs. at 45 °C until colored precipitate had formed, dried, and recrystallized with crystallized from ethanol: water (1: 3) (Scheme 1). The reaction was followed by TLC technique [19] (D3, R_f = 0.67 / 1 hexane: 3 ethylacetate, D4, R_f = 0.64/1 hexane: 3 ethylacetate, D5, R_f = 0.89/1 hexane: 3 ethylacetate).

Hetero-polymers [PD_6, PD_7, and PD_8]

The polymerization process was achieved according to the literature [22].

In 50 mL dry two neck round bottom flask, (0.5 g, 0.0004 mmol) of monomer [D_3, D_4, and D_5] separately, Methyl methacrylate (0.0004 mmol), 3-4 drops of dry toluene and (0.01 g, 0.00023 mmol) of the initiator Benzoyl peroxide (Bpo) were mixed with stirring under nitrogen gas flow for (10 min). The flask was tightly sealed and heated on oil bath at 90 °C for 2-3 hours. After the polymerization process completed, the obtained precipitate was filtered, washed with ether, and then dried in oven at 50 °C (Scheme 1).



Scheme 1 Synthesis route of compounds (D1-D5) and (PD6-PD8)

Swelling ratio

The xerogel from heteropolymers (0.05 g) was submerged in 50 mL of three different buffer solutions (pH = 2.2 (500 mL of 0.2 M KCl and 0.86 mL of 0.2 M HCl), pH = 7.0 (H_2O), and pH = 8.0 (500 mL of 0.02 M Borax and 0.43 mL of 0.1 M HCl).

These solutions left to soak for many hours or days at a constant temperature of 37 ◦C to measure the swelling ratio.The swelling ratio of heteropolymers is listed in Tables 8,

9, and 10. Figures 1, 2, and 3 display the behavior of swelling with time (by hours and days) [23,24].

Release of drugs

UV-Visible spectrophotometer used to determine the drug release from the prepared polymers in buffer solutions (pH= 2.0, 7.0, and 8.0) at five consecutive hours and 1-3 days at 25 °C. Figures 4, 5, and 6 illustrate of the swelling behavior curve over time (in hours and days).

The polymeric solutions were prepared by dissolving (0.005 g) from each polymer in 50 mL of buffer solution at constant temperature 25 °C. After each hour (five consecutive hours) and consequently each day (three consecutive days), 5 mL from each solution were drawn and measured for the absorbance at the wave length of the free drugs dissolving in same buffer solution [25].

Measurement of swelling ratio of polymers [PD6, PD7, and PD8]

In a beaker (50 mL), (0.005 g) of prepared polymers (PD6, PD7, and PD8) were utilized at pH values of 2.0, 7.0, and 8.0, respectively. The swelling was measured for 5 consecutive hours and measured for 1-3 days and applied the following equation [26].

Swelling (%) = $(M_t - M_0)/M_0 \times 100$

M_0= Polymer weight prior to swelling

M_t= polymer weight after swelling

Measuring the viscosity and density of pharmaceutical polymers

(0.001 g) of each polymer (PD_6 , PD_7, and PD_8) dissolved in 25 mL of DMSO and then measured the density by Denstio 30px, and then measured the time of descent of distilled water in the viscometer of viscosity and was calculated for polymers.

Calculating the time of descent and intensity polymer and compare it with water alone [poise=g.s-1.cm-1] according to the equation [27]:

$$\eta_{unk} / \eta_{D.W} = d_{unk} * t_{unk} / d_{D.W} * t_{D.W}$$

Molecular weights of prepared polymers

The Ostwald technique is a straightforward method for determining viscosity, which involves comparing the flow times of two liquids of similar volume using the same viscometer. The molecular weight of the polymer is determined using a viscometer, and the molecular weight acquired using this method is referred to as viscosity average molecular weight. Because the polymer solution has a relatively high molecular weight, it has a very high viscosity when compared to pure solvent [28].

Preparation of the polymers stock solution

The concentration (10 mg/ mL) of polymers was prepared by taken of 1.25 g of each polymer and dissolved in 125 mL of Dimethylformamide. From these solutions, prepared the concentrations 8, 6, 4, and 2 mg/mL by transferring 40, 30, 20, or 10 mL, respectively, of the stock solution to a 50 mL volumetric flask and completed to the mark.

After charging with polymer solution, the viscometer was immersed vertically in the constant-temperature water bath (30 °C) for ten minutes for thermal equilibration.

The viscosity of the polymer samples might be calculated from the equation (1) by calibrating the viscometer under similar experimental circumstances with a liquid of known viscosity and density.

$$\eta / \eta_0 = \rho\, t / \rho_0 \qquad (1)$$

Where, η is the viscosity of the polymer solution, η_o is the viscosity of the solvent, and ρ and ρ_o are the densities of the polymer solution and solvent, respectively [29].

$$\eta / \eta_0 = t / t_0 \qquad (2)$$

To determine the molecular weight of the polymers conventional plots of $\eta sp/c$ vs. c,

were extrapolated to obtain the intrinsic viscosity [η] from the intercept [30].

$$\eta_{sp} = \eta / \eta_0 - 1 \quad (3)$$

The reduced viscosity ηred is given by the relation:

$$\eta_{red} = \eta / \eta_0 - 1/C \text{ or } \eta_s \quad (4)$$

The molecular weight of the polymers was obtained by substitution [η] value in the equation [31]:

$$[\eta] = K \cdot M^a \quad (5)$$

Where, [η] is intrinsic viscosity, M is molecular weight of the polymer, and K and a are constant for a particular polymer/solvent/temperature system. Values of K and a are available for poly (N-phenylmaleimide) (PNPI) give unclear linear proportionality of the intrinsic viscosity to molar mas where, K=1.34 x 10^{-4} and a=0.71 similar to the Mark-Houwink constants of methyl methacrylate/N-phenylmaleimide copolymers, K = 1.34 x 10^{-4} and a = 0.73 (115).

In the same manner, a Mark-Houwink constant was calculated for poly [N-(4-acetyl) phenyl amino] maleimideco-acrylic acid (APAMI-co-AA) using the plots of viscosities of the polymer fractions in DMF against the molecular weight (Tables 1-3)[32].

TABLE 1 Data used to determine the intrinsic viscosity and M.Wt of [PD_3]

PDA_7 polymer concentration mg/mL	**Time flow t/s**	**$\eta/\eta_o = t/t_o$**	**ηred=ηsp/c**
DMF	62	1	0
10	131	2.112903225	0.111290322
8	112	1.8064516129	0.100806451
6	101	1.629032258	0.104838709
4	89	1.4354838709	0.108870967
2	74	1.19354838	0.096774193
η=0.0955	M=8092.32D		

TABLE 2 Data used to determine the intrinsic viscosity and M.Wt of [PD_4]

PD_4 polymer concentration mg/mL	**time flow t/s**	**$\eta/\eta_o = t/t_o$**	**ηred=ηsp/c**
DMF	62	1	0
10	137	2.2096774193	0.1209677419
8	120	1.9354838709	0.1169354838
6	103	1.6612903225	0.1102150537
4	90	1.45161290322	0.1129032258
2	76	1.2258064516	0.11290322580
η=0.1101	M= 9833.49 D		

TABLE 3 Data used to determine the intrinsic viscosity and M.Wt of [PD_5]

PD_5 polymer concentration mg/mL	time flow t/s	$\eta/\eta_o = t/t_o$	$\eta red = \eta sp/c$
DMF	62	1	0
10	146	2.354838709	0.13548387
8	129	2.080645161	0.135080645
6	112	1.80645161	0.13440806
4	95	1.53225806	0.13306451
2	78	1.2580645161	0.12903225
η=0.1198	M=1039.28D		

Biological activity

Cytotoxic assay

The MTT test was carried out using 96-well plates to ascertain the cytotoxic impact of the synthesized polymers (PD6, PD7, and PD8). At a density of 1 x 10^4 cells per well, cell lines were planted. Cells were exposed to test substances at various concentrations after 24 hours or when a confluent monolayer was established. By removing the media, adding 28 µL of a 2 mg/mL solution of MTT, and incubating the cells for 2.5 h at 37 °C, cell viability was assessed after 72 hours of treatment. The residual crystals in the wells were solubilized after the MTT solution was removed by adding 130 µL of DMSO (Dimethyl Sulfoxide), which was then incubated at 37 °C for 15 min while being shaken. The experiment was carried out in triplicate, and the absorbency was measured using a micro plate reader at 492 nm [33-36].

The following equation was used to compute the percentage of cytotoxicity, or the rate at which cell growth was inhibited:

Cytotoxicity = A-B/A *100

Where, A represents the optical density of the control and B represents the optical density of the samples.

Maintenance of cell cultures

A 10% fetal bovine serum, 100 units/mL penicillin, and 100 (g/mL) streptomycin supplement was added to RPMI-1640 to sustain MCF-7 cells. Once every two weeks, cells were reseeded at 80% confluence and incubated at 37 °C after passage using trypsin-EDTA.

Results and discussion

Itaconimide compounds (D1 and D2) have been synthesized, as shown in Scheme 1. By refluxing 3-itaconimido benzoic acid in thionyl chloride at 65 °C for 6 hours, the excess $SOCl_2$ was drawn under reduced pressure and the intermediate 3-itaconimide benzoyl chloride (D2) was subsequently recrystallized from dichloromethane. The FT-IR spectra of chemical (D2) verified its structure by revealing the disappearance of carboxylic acid's wide absorption and the emergence of a prominent band at a higher frequency, 1769 cm^{-1}, which unmistakably belonged to the (COCl) group.

Characterization of Compound [D3]

Chemical Formula: $C_{19}H_{14}O_6N_2$, Color: Reddish brown, (m.p.= 111-113 °C, Yield 70%). FTIR spectrum (cm^{-1}) of (D3), as depicted in (Figure 1-1): Specific phenolic stretching vibration as broad band centered 3251 cm^{-1} and another band at 1778 cm^{-1} can be attributed to C=O Stretching vibration of the Itaconimide groups.

The band at 2985 cm^{-1} are assigned to aliphatic C-H stretching vibration of methyl groups. The aromatic C-H stretching vibration is assigned to a band around 3080 cm^{-1}. The

bands at 1712 and 1658 cm^{-1} belong to the C=O groups stretching of amide and 1510-1444 cm^{-1} are assigned to an aromatic ring. The absorption around 1373 and 1197 cm^{-1} may be traced to the bending vibrations of (C-N) and (C-O), respectively.

^{1}H-NMR (500 MHz, DMSO, δ ppm) spectrum of (D3) showed the following chemical shifts. Singlet signal at 2.51 belong to methyl group, the proton of (=CH_2) of Itaconimide appeared as singlet signal at 3.88, the proton of (CH_2) Itaconimide ring appeared at 2.23. The proton of phenol ring appeared as multiplet signals at the range of 6.65-7.0, the meta-substituted benzene protons appeared at 7.53-7.97 while the phenolic hydroxyl proton appeared 10.06.

^{13}C-NMR (125 MHz, δ, ppm) spectrum of compound (D3) showed the flowing signals

The (CH_2) in Itaconimide cyclic showing at about 40.43. The peaks with the chemical shift of 129.89 belong to the two (C=CH_2) groups of the Itaconimide ring carbon signal (O=C-CH_3) at 11.27 the two ortho- carbons of phenol ring at 108.80 and aromatic carbons at the range of 134.08-136.47. The carbon attached to phenolic -OH appeared at 142.27(1 C, Ar-C- OH), the N-acetyl carbonyl signal at 152.27, the signal at 156.09 ppm attributed to Itaconimide carbonyl, and the signal at 164.12 assigned to the amide carbonyl carbon.

Characterization of Compound [D4]

Chemical Formula: $C_{27}H_{19}O_4N_3$, Color: Brown, (m.p.= 183-185 °C, Yield 88%): shows FT-IR (cm^{-1}) spectrum of (D4). The absorption peaks at 1716 and 1685 cm^{-1} indicate the stretching vibrations of carbonyl groups (C=O) of imide ring and stretching vibration of amide, respectively. Furthermore, the absorption peak at 3147-3049 cm^{-1} indicates stretching vibration of C–H bonding of olefinic groups and aromatic rings and 1591-1489 cm^{-1} assigned to (C=C, aromatic), 1386 (C-N) stretching. Moreover, a single peak at 3483 cm^{-1} is observed, which is assigned to the stretching vibration of N-H bond of the acyclic imide. This proves the successful of imidization reaction at the amino group of carbamazepine drug by acyl of Itaconimide benzoyl chloride (Scheme 1).

^{1}H-NMR (500 MHz, DMSO, δ ppm) spectrum of (D4) showed signals at different chemical shifts as follow. The aliphatic proton of (=CH_2) of Itaconimide appeared as singlet signal at 3.59 and the proton of (CH_2) Itaconimide cyclic ring appeared at 1.92. It can be seen that the peak are observed at the chemical shifts of 7.34 and 7.40 belong to the two (-HC=CH-) groups of the seven membered ring. The aromatic protons appeared at the range of 7.54-8.14 and the acyclic imide proton (NH-C=O) appeared at 9.84.

The ^{13}C-NMR spectrum of compound (D4) shows the following chemical shifts. The (CH_2) in Itaconimide cyclic showing a range of chemical shifts at about 39.39. Furthermore, the peaks with the chemical shift of 124.25 and 135.92 belong to the two (C=CH_2) groups of the Itaconimide ring and the seven-membered ring, respectively.

The absorption peak at 160.07 assigned to two carbonyl carbons of Itaconimide ring, while the two peaks at 164.36 and 165.39 belong to the two-carbonyl carbons of acyclic imide groups. This confirms the formation of Itaconimide -drug monomer [D4], as the reaction depicted in Scheme 1.

Characterization of Compound [D5]

Chemical Formula: $C_{25}H_{23}O_6N_5$, Color: Brown, (m.p.= 228-230 °C, Yield 89%) The FT-IR spectrum (cm^{-1}) of compound [D5] shows different absorption bands as follow. Primary aromatic amine was appeared at 3404, the stretching vibration of (-NH, amide) groups appeared at 3321, the Itaconimide and aromatic (=C-H) appeared at 3172 and 2837,

the carbonyl group of Itaconimide appeared at 1708.

^{1}H-NMR (500 MHz, DMSO, δ ppm) spectrum of (D5) showed different signals. The aliphatic proton of (=CH_2) of Itaconimide appeared as singlet signal at 3.87, the aliphatic proton of (CH_2) Itaconimide ring appeared at 1.19 singlet signal at 3.55 traced to the methoxy group (s, 3H, -OCH_3). In Aniline protons: the primary amino group appeared as somewhat broad singlet signal at 5.49 ppm.

Pyrimidine protons: singlet signal at 6.25, beside to the four aromatic protons of benzene ring at about 7.0-7.6, while the two protons of aromatic ring of the drug appear at 8.01 and 8.14 and the aryl amide protons (Ar-CO-NH-, amide) occurred at 10.92.

^{13}C-NMR (125 MHz, δ, ppm) of compound (D5) showed different chemical shifts, the (CH_2) in Itaconimide cyclic indicating at about 34.41. Moreover, the three position carbons a range of chemical shifts of at 52.81, 53.41, and 65.39 belong to the (O-CH_3) substituted in the aromatic ring of drug and the peaks with the chemical shift of 113.58 and 120.98 belong to the two (C=CH_2) groups of the Itaconimide ring and the six membered ring of Itaconimide, respectively. The absorption peak at 166.01assigned to two carbonyl carbons of Itaconimide ring, while the peak at 170.48 belong to the carbonyl carbons of acyclic imide groups. The two emine and the site of substitution carbons of pyrimidine ring appeared at 143.28 and 153.94, and also the other aromatic carbons appeared in the range of 124.68-138.14. Table 4 shows the physical properties of prepared monomers [D1, D2, D3, D4, and D5).

TABLE 4 Physical properties of prepared monomers [D1, D2, D3, D4, and D5)

Compound No.	Time (h)	yield (%)	M.P. (°C)	R_f value	Color
D1	2	90%	244-246	0.4 1hexane: 3ethyl acetate	White
D2	6	90%	136-138	0.75 1hexane: 3ethyl acetate	Dark brown
D3	4.5	66%	111-113	0. 67 1Hexane: 3acetone	Reddish Brown
D4	5	88%	183-185	0.64 1Hexane: 3acetone	Brown
D5	5.5	89%	228-230	0.89 1Hexane : 3acetone	Brown

In addition, the chemical structures of all prepared compound were characterized by CHNS spectroscopies. Beside to all the results of the diagnosis other, good results are presented in Table 5.

TABLE 5 (C.H.N.S) Elementary analysis of the monomer

Compound No.	Calculated %				Found %			
	C	H	N	S	C	H	N	S
D3	62.3	3.85	7.65	-	62.10	3.71	6.50	-
D4	72.15	4.26	9.35	-	71.95	4.20	9.15	-
D5	61.34	4.74	14.31	-	61.03	4.71	13.83	-

To produce the heteropolymers polymers [PD6, PD7, and PD8], the obtained monomers were polymerized in toluene at 90 °C for 10 hours under nitrogen flow using methyl methacrylate and benzoylperoxid as an initiator. The physical properties of the prepared polymers are listed in Table 6.

Characterization of Copolymers [PD$_6$]

Color: Red. FT-IR spectra cm^{-1} of heteropolymer [PD6] showed varied peak values with the presence of different functional groups. The broad band between 3400-2700 belong to carboxylic acid hydroxyl group of drug, the phenolic –OH occurred at 3243, the =C–H of aromatic rings showed its 2682, while the aliphatic C-H bonds occurred at 2492. The wave number 1716 was assigned as characteristic absorption of imide and carboxylic acid carbonyls, the C=C of aromatic ring appeared at the range of 1655-1441, the C-N bond stretching occurred at 1377.

^{1}H-NMR spectrum of the Heteropolymer [PD6] shows varied peak values with the presence of different protons the signals at 1.1, 1.2, 2.5, and 3.5 ppm indicate the presence of different aliphatic protons for poly succinimide-Co- methyl methacrylate backbone. The aromatic protons accured at the range of 6.6-7.6 ppm. The phenolic -OH proton occurred at 8.1 ppm, the carboxylic acid protons appeared as to signals broad signal at 9.67 ppm.

Characterization of copolymers [PD$_7$]

Color: Light Yellow: The FT-IR spectrum cm^{-1} of heteropolymer [PD7], showed different bands, the amide N–H occurred at 2992, the =C–H stretching vibration band corresponding to aromatic rings and olefin showed its occurrence at 2947, while the aliphatic C-H bonds occurred at 2500. The 1722 and 1549 were assigned as characteristic absorption of imide and amid carbonyl vibration, the C=C of aromatic ring appeared at the range of 1434, the C-N bond stretching occurred at 1385.

^{1}H-NMR chemical shifts of Heteropolymer [PD7] were viewed in the multiple signals at 0.9, 1.2, and 2.5 ppm revealed the methyl, methylene, and methane protons of polysuccinimide-Co- methyl methacrylate backbone. The olefinic protons appeared at 6.01 ppm while aromatic protons accruing at the range of 7.1-7.9 ppm protons in addition to the signal at 8.2 ppm referred to the amide N-H proton in the pyridine ring.

Characterization of copolymers [PD8]

Color: Yellow: The FT-IR spectrum cm^{-1} analysis result of Heteropolymer [PD8] was demonstrated in the band at 3616 due to the aromatic amino group in the drug structure. The absorption bands 2993 assigned to -NH stretching of amide groups. The aromatic C-H absorption bands occurred at 2947 and the aliphatic C-H vibration of the polymer backbone appeared at 2900-2500. The carbonyl amide groups absorption band at 1720. The aromatic rings and the medium absorption at 1381 due to C-N stretching.

^{1}H-NMR chemical shifts of Heteropolymer [PD8] were viewed in the signals at 1.9, 2.32, 2.5, and 2.52 ppm indicated the presence of different aliphatic protons for poly succinimide-Co-methyl methacrylate backbone. The signal centered 3.34 ppm attributed to the amino group protons. The methoxy group protons occurred at 3.5, 3.7, and 3.8 ppm. The aromatic protons accruing at the range of 7.13-7.65 ppm in addition to the signal at 8.0 ppm referred to the amide N-H proton in the aromatic ring in addition to the signal at 12.9 ppm referred to the imine like protons in the pyrimidine ring.

TABLE 6 Physical properties of prepared copolymers [PD_6, PD_7, and PD_8] polymers

Polymers	Color	Time poly.(S)	Density of poly.	$\eta_{poly.}$ poise
PD_6	Red	53	0.931	0.736
PD_7	Light Yellow	54	0.934	0.755
PD_8	Yellow	56	0.935	0.783

The property of viscosity and the density of drug polymers at 25 °C, time $_{D.W}$ =61S, Density of D.W = 0.99, and $\eta_{D.w}$ poise = 0.904, where, d: density, η: viscosity, t: time for polymer and distil water.

Solubility: The solubility characteristics of produced monomers and polymers in various solvents (H_2O, ethanol, $CHCl_3$, toluene, ether, petroleum ether, DMSO, acetone, and hexane) were investigated. The solubility findings are listed in Table 7.

TABLE 7 The solubility prepared of monomers and heteropolymer in some solvents

Co.	H_2O	DMF	$CHCl_3$	Ether	Ethanol	DMSO	Hexane	THF	Acetone
D3	partial	+	-	+	partial	+	-	+	+
D4	partial	+	-	-	partial	+	-	+	+
D5	partial	+	-	-	partial	+	-	+	+
PD_6	-	+	-	-	-	+	-	Partial	Partial
PD_7	-	+	-	-	-	+	-	Partial	Partial
PD_8	-	+	-	-	-	+	-	Partial	Partial

Swelling ratio

The swelling ratio was calculated by soaking 0.05 g of polymers in 50 mL of buffer solutions (pH 2.2, 7.0, and 8.0) for times at 37 °C. The swelling ratio of hetero polymers is shown in Figures 1, 2, and 3.

Drug release

Drug release from the created polymeric hetero was assessed using a UV-Visible spectrophotometer at 37 °C in three different buffer solutions (pH 2.2, 7.0, and 8.0). Figures 4, 5, and 6 demonstrate the drug release from the synthesized polymer.

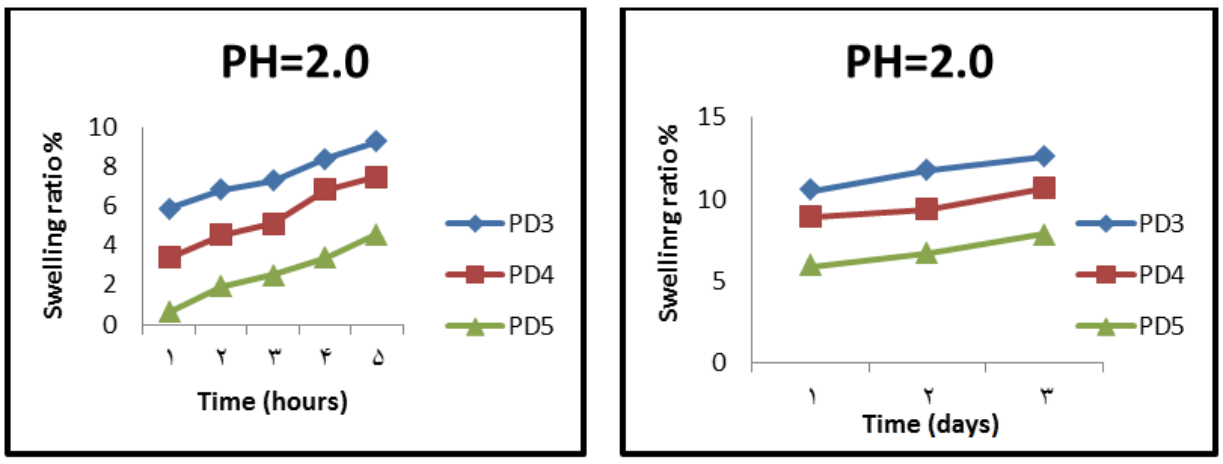


FIGURE 1 The swelling ratio of hetero polymer (PD_6, PD_7, and PD_8) in different hours and days in pH=2.0 at 37 °C

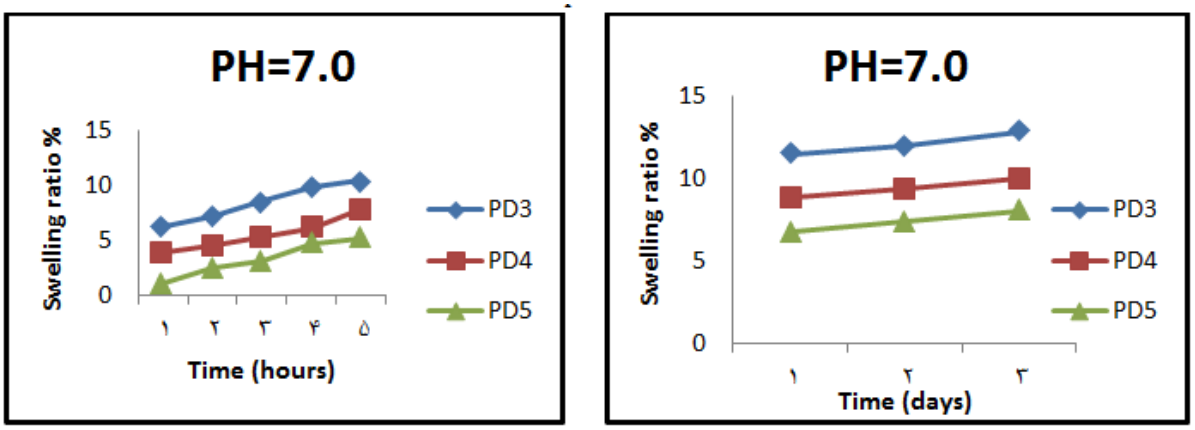


FIGURE 2 The swelling ratio of hetero polymer (PD6, PD7, and PD8) in different hours and days in pH=7.0 at 37 °C

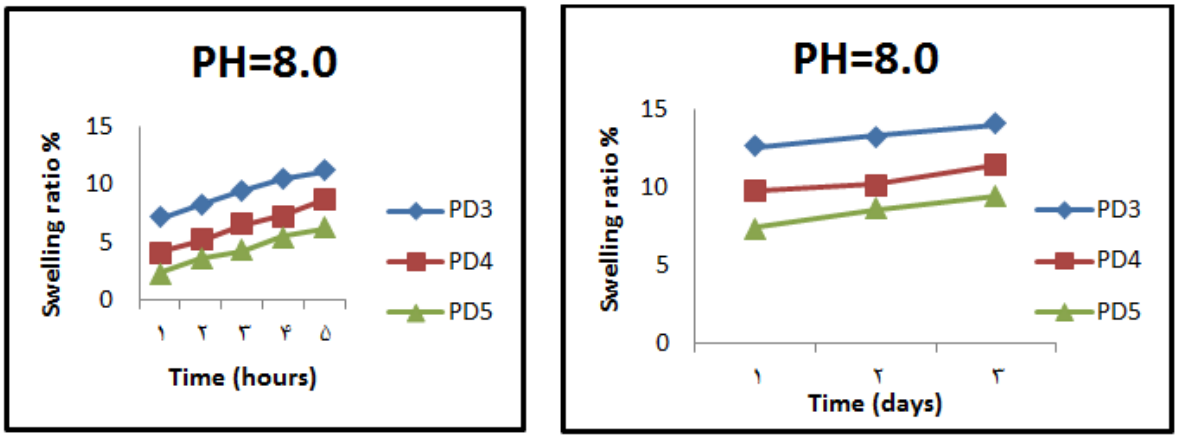


FIGURE 3 The swelling ratio of hetero polymer (PD_6, PD_7, and PD_8) in different hours and days in pH=8.0 at 37 °C

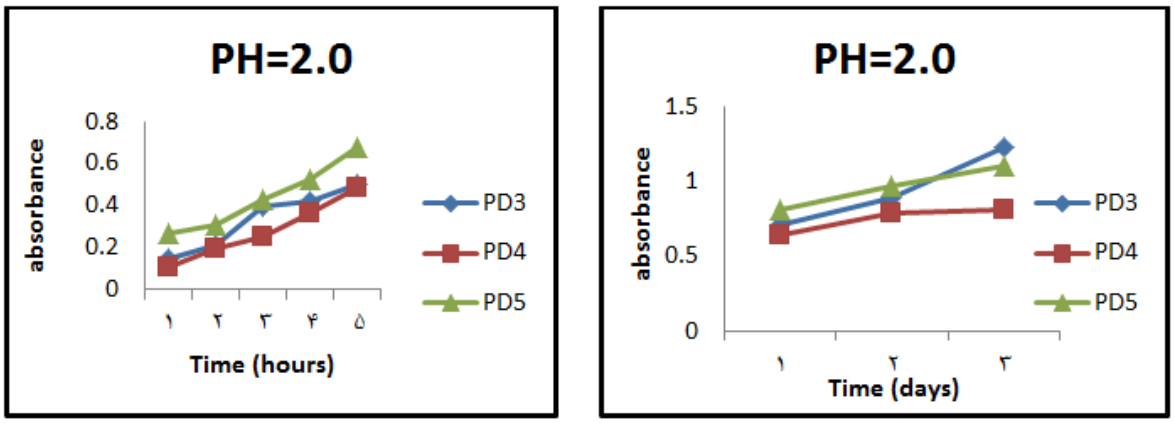


FIGURE 4 The drug release of hetero-polymer (PD6, PD7, and PD8) in different hours and days in pH=2.2 at 37 °C

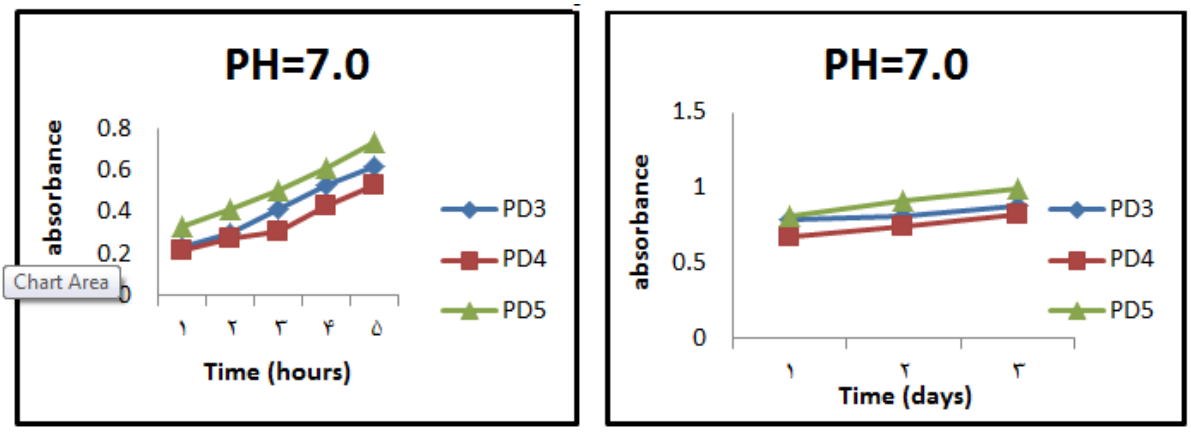


FIGURE 5 The drug release of hetero-polymer (PD6, PD7, and PD8) in different hours and days in pH=7.0 at 37 °C

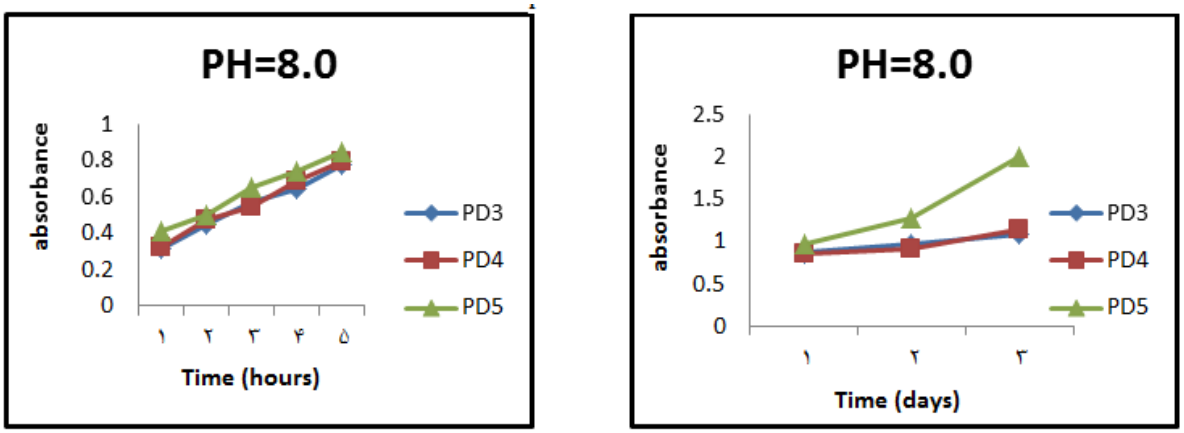


FIGURE 6 The drug release of hetero-polymer (PD6, PD7, and PD8) in different hours and days in pH=8.0 at 37 °C

The Antibacterial activity of the heteropolymers [PD6 , PD7, and PD8]

The anti-bacterial activity for the synthesized monomers and their loaded drugs were listed in Table 8.

Synergistic effects: An effect whereby two chemical substances together have more of an impact than anticipated.

Antagonist effect: is a phenomenon wherein two or more agents in combination have an overall effect that is less than their individual effects.

TABLE 8 antibacterial activities of compounds (PD6, PD7, and PD8) at 0.5 mg/mL concentration

Inhibition Zone(mm) for Staph. Coccus of Itaconimide		Inhibition Zone (mm) for Staph. Coccus of Drug		Inhibition Zone (mm)For E.Coliof Itaconimide	Inhibition Zone (mm) for E. Coli of Drug
PD6	20	Paracetemol	9	10	4
PD7	14	Carbamazepine	0	15	4
PD8	50	Trimethoprine	26	38	28
DMSO	0	DMSO	0	0	0

The disk-diffusion technique was used to test the antibacterial activity against pathogenic strains of (Escherichia coli) and (Staphylococcus aurous) using a solution of (0.5 mg) from each derivative and each loaded drug for comparison in 1 mL of DMSO. As a further negative control, the rule of DMSO was examined, but did not demonstrate any reduction of bacterial growth.

The results of antibacterial activity against Gram-positive (Staphylococcus aurous) shows synergistic effect for (PD6, PD7, and PD8), compounds were the anti-bacterial activity (inhibition zone) of these compounds are raised comparison to the corresponding loaded drugs. On the other hand most of derivatives show higher activity against Gram-negative (E. coli) than the loaded drugs do, was show synergistic effect (PD6, PD7, and PD8).

Cytotoxic Activity of Some Polymers

On the human breast cancer cell line (MCF7), the cytotoxic effects of polymers were assessed using the MTT test, and the outcomes were compared to the untreated control.

TABLE 9 Cytotoxic effect of [PD6] in MCF-7 cells

PD6	Concentration (µg/ mL)	OD	OD	OD	Avg.	Cell viability (%)
	1000	0.083	0.085	0.072	0.08	14.43174985
	500	0.165	0.172	0.174	0.170333	30.72760072
	250	0.286	0.273	0.203	0.254	45.82080577
	125	0.35	0.358	0.308	0.338667	61.0944077
	62.5	0.425	0.435	0.452	0.437333	78.89356584
	0	0.519	0.552	0.592	0.554333	100
IC_{50}	**271.125**					

TABLE 10 Cytotoxic effect of [PD7] in MCF-7 cells

PD7	Concentration (µg/mL)	OD	OD	OD	Avg.	Cell viability (%)
	1000	0.05	0.05	0.06	0.053333	9.621166566
	500	0.12	0.11	0.109	0.113	20.38484666
	250	0.198	0.201	0.225	0.208	37.52254961
	125	0.235	0.254	0.245	0.244667	44.13710162
	62.5	0.325	0.335	0.353	0.337667	60.91401082
	0	0.519	0.552	0.592	0.554333	100
IC_{50}	116.14					

TABLE 11 Cytotoxic effect of [PD8] in MCF-7 cells

PD8	Concentration (µg/mL)	OD	OD	OD	Avg.	Cell viability (%)
	1000	0.076	0.074	0.07	0.073333	13.22910403
	500	0.11	0.12	0.14	0.123333	22.24894768
	250	0.219	0.214	0.225	0.219333	39.5670475
	125	0.312	0.325	0.335	0.324	58.44858689
	62.5	0.425	0.412	0.395	0.410667	74.08298256
	0	0.519	0.552	0.592	0.554333	100
IC_{50}	**222.68**					

The cytotoxicity of synthetic polymers (PD6, PD7, and PD8) against MCF-7 cancer cells was assessed using the MTT colorimetric test (Tables 9, 10, and 11) and (Figures 7, 8, and 9). The outcomes demonstrate that, in comparison to the control, all polymers suppress cell proliferation in a concentration-dependent way.

At a concentration of 1000 (g/ mL), hetero-polymer PD6 exhibited the maximum level of inhibition, resulting in the death of 90% of the treated cells. In contrast, at a dosage of 62 (g/ mL), 40% of the cells died, and the Inhibitory Concentration value (IC_{50}) was 116.14 (g/ mL) (Figure 7).

The degree of cell inhibition at concentration 1000 (µg/mL) for PD7 (83%) while at a concentration of 62 (µg/ mL) 25 % and the inhibitory concentration value (IC_{50}), it was 222.27.

The degree of cell inhibition at concentration 1000 (µg/mL)) for PD8 (85%) while at a concentration of 62 (µg/ mL) 20% and the inhibitory concentration value (IC_{50}) was 271.125. The IC_{50} values are depicted in Figures 8 and 9.

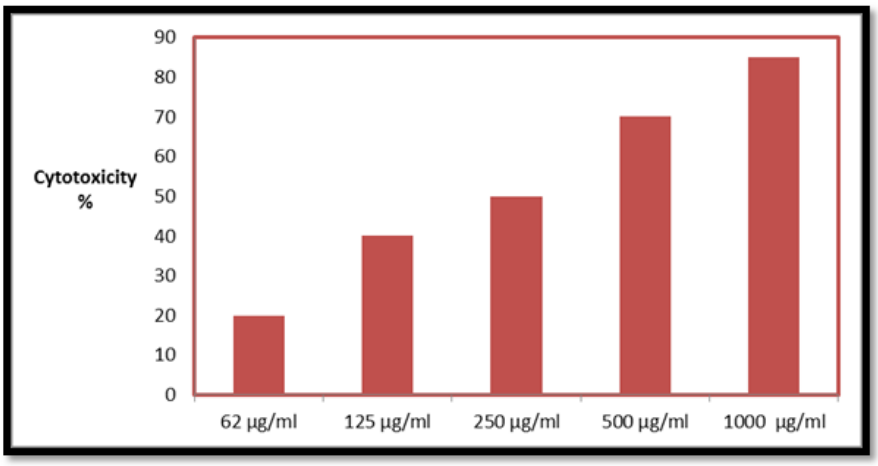


FIGURE 7 Cytotoxic effect of PD6 in MCF-7 cells, IC_{50}= 271.125 (µg/ mL)

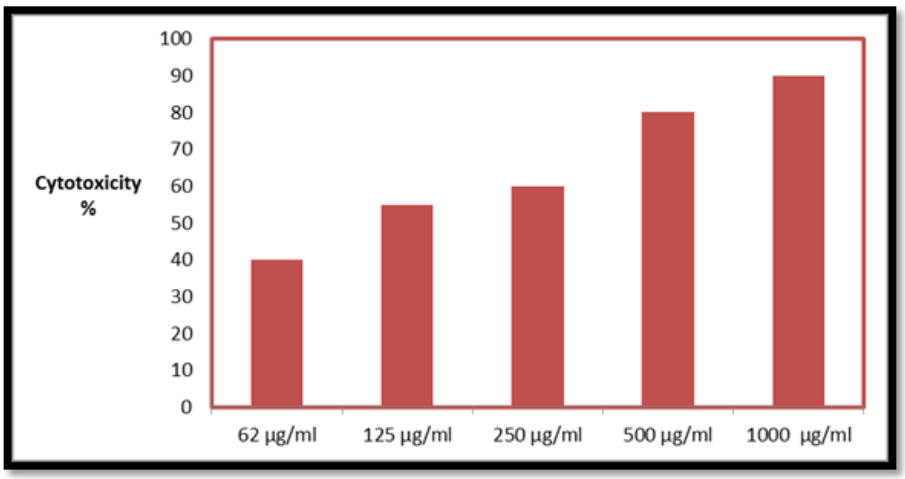


FIGURE 8 Cytotoxic effect of PD7 in MCF-7 cells, IC_{50}= 116.14 (µg/mL)

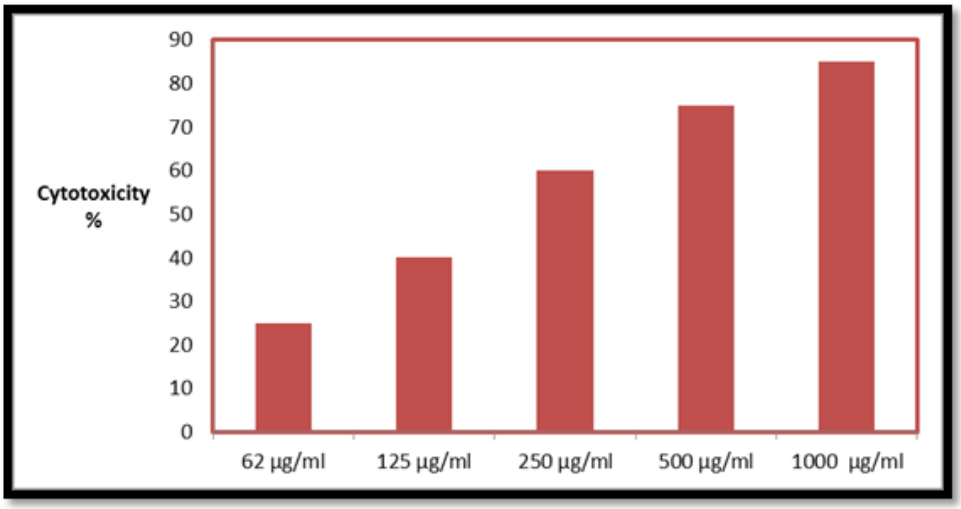


FIGURE9 Cytotoxic effect of PD8 in MCF-7 cells, IC_{50}= 222.68 (µg/mL)

Conclusion

A successful free radical polymerization procedure was used to successfully create three novel prodrug polymers based on Itaconimide-drug monomers. All the prepared compounds were diagnosed using FT-IR,^{1}H-NMR, ^{13}C-NMR, and C.H.N.S. The physical properties of the new synthesized compounds were determined and their solubility in different solvents was studied.

Antibacterial activity was screened against (*E.coli)* and (*Staph. Aurous*) bacteria. All the new compounds (PD6, PD7, and PD8) showed antibacterial activity towards *Staph.aurous* and *E. Coli* more than drugs by itself. The anticancer activity of the new compounds (PD6, PD7, and PD8) was investigated. The results revealed a highly considerable cytotoxic activity against human cell cancer ((breast cell cancer (MCF-7 cell line)). The findings reveal that (PD6, PD7, and PD8) have the potential to inhibit the diffusion of cancer cell lines.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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