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Injectable Glucose-Responsive Hydrogels as Insulin Delivery Systems for Diabetes Treatment Based on Boronic Acid−Glucose Complexation

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Abstract :

Diabetes is one of the most common chronic diseases in the world and its incidence is on the rise. Maintenance of continuous normoglycaemic conditions is the key goal for the management of both type 1 and type 2 diabetes in patients. In this study, different types of glucose-responsive polymers have been synthesized by polymerization of different monomers(Methyl acrylamide, Methyl acrylic acid, and1-vinyl2-pyrolidone) with 4-vinyl phenyl boronic acid (4-VPBA) by free radical polymerization in the presence of ethylene glycol dimethacrylate (EGDMA) and 1,6-Hexanediol diacrylate (HDODA) as a crosslinking agents and Potassium Peroxodisulfate, Sodium metabisulfite as an initiators. which has been used for glucose sensing due to its ability to form complexes with diols at different pH values and at different sugar concentrations. PBA-moiety has the ability to reverse crosslinks with glucose-diols and form the most stable boronate ester complexes. The prepared polymers were confirmed by using the FTIR and 1HNMR techniques. The Thermal stability of all polymers were also studied using the TGA and DSC techniques. In addition to the use of polymers in glucose sensing has been exploited to control the level of sugar and reduce the concentrations of high through the loading of insulin on these polymers as the large area of polymers improve the possibility of loading insulin. The release of insulin in vitro at physiological pH , different concentrations of glucose and different times were also studied. The results show the gradual release of insulin, preceded by the sudden release of insulin due to the presence of large amounts of the diols of sugar particles, as well as high release rate of high concentrations of glucose which decreases when glucose concentration decreases .The effectiveness of these polymers has also been studied on the laboratory rats , the results showed that the polymers have an evident effect in reducing the blood glucose levels in rats , as well as to avoid the problem of higher loading glucose caused by the treatment process insulin which is usually what happens as a result of the overdose in addition to reducing the number of periodic tests to monitor the level of sugar in the blood.

Keywords: 4-vinyl phenyl boronic acid, copolymerization, glucose sensitivity, physiological pH, load, control, drug release, Vitro, Vivo.

INTRODUCTION

The development of glucose-responsive controlled insulin delivery systems has attracted much attention due to its potential application in maintaining normal blood glucose levels, one of the key goals of the treatment of Type 1 diabetes [1]. Blood glucose levels in diabetics exhibit large swings throughout the day. However, current widely used devices, such as test strips and glucose meters, give only discrete time information about blood glucose level, possibly missing fluctuations involving sudden increase or decrease in glucose level [2,3]. Recently commercialized enzyme based sensors such as Medtronic CGMS® GoldTM and Dexcom® G4 Platinum can offer continuous information, but they can be problematic due to instability of the enzyme, fouling under physiological conditions, and inflammation and infection that result from breaching the skin with a needle electrode [4]. While improved enzyme based sensors that minimize some of these issues are under development [5], parallel investigation of nonenzymatic sensing modalities is of continued interest. Hydrogels have been broadly used for biomedical applications, including drug delivery and tissue engineering[6-8]. Injectable hydrogels that flow under modest pressure and exhibit self-healing recovery following cessation of pressure offer many advantages for medical applications. Specifically, injectable implantation can be self-administered and is minimally invasive, leading to improved patient compliance[9] . In addition, a number of injectable hydrogels have been evaluated preclinical or in early stage clinical trials, including hydrogels for cancer therapy and bone repair[10]. In order to prepare injectable hydrogels, a variety of cross-linking mechanisms have been leveraged, including in situ covalent cross-linking as well as physical cross-linking that include salt bridges, peptide interactions, molecular recognition motifs,and/or van der Waals forces [11-13]. Preparing hydrogels with crosslinks that can respond to a specific biologic stimulus, such as elevation of blood glucose levels in diabetes, could further expand the utility of this class of material in preparing new therapies. Early efforts to prepare glucose-responsive materials for insulin delivery evaluated the complexation of a glycosylated insulin derivative with the lectin concanavalin A (Con A), a natural carbohydrate binding protein [14-16]. The competitive binding to Con A of glucose and glycosylated insulin regulates the breakdown of the complex, leading to glucose-responsive insulin release [17-19]. However, possible immunogenicity of Con A, as well as a requirement for a special modified insulin derivative, would prove limiting to the translation of this approach[20]. Another method to prepare glucose-responsive materials utilizes the enzymatic actuation, leveraging the catalytic conversion of glucose into gluconic acid by glucose oxidase. The drop in pH that arises through this conversion can be used to trigger hydrogel swelling, leading to the release of encapsulated insulin [21-22]. This method has been widely used in a number of insulin delivery systems, including injectable networks[23-25]. However, this strategy also has risks associated with enzyme immunogenicity along with toxicity of the hydrogen peroxide by product produced in the conversion. Phenylboronic acids (PBAs) are Lewis acids that can bind reversibly to cis-1,2 or cis-1,3 diols, including glucose, to form a stable five-membered ring complex [26]. In 1959, Lorand and Edwards reported the first quantitative study describing the complexation of boronic acids and polyols[27]. Extensive studies since this time have investigated the binding affinity of boronic acids with different diols including fructose, glucose, and other sugars[26,28,29] . On the basis of previous work in the preparation of glucose responsive polymeric hydrogels from PBA−diol complexation[30] .We endeavored to design a hydrogel leveraging crosslinking between PBA and glucose-like diols that could be injectable (i.e., self-healing) . In this system, polymers containing multiple PBA groups would cross-link through interaction with multiple glucose units installed within the same polymer to form a stable hydrogel network. This PBA−glucose complexation is reversible, enabling the preparation of an injectable self-healing hydrogel as verified through rheological measurements. Herein, we report a hydrogel cross-

linked via the complexation of PBA and glucose that exhibits injectable self-healing properties, and this is accomplished through a single component polymeric material. To incorporate boronic acid and glucose functional groups within the same polymer, we designed a synthetic route that used radical polymerization . In this work, four polymers (PB1-PB4) have been synthesized by free radical polymerization of different monomers(MAA, MAAm, EC and NVP) with 4-vinyl phenyl boronic acid 4-VPBA as a function of pH and concentration of glucose. These polymers are able to respond to a stimulus that can be provided by the human body (i.e., an increase in glucose concentration). Therefore, the concept we present has promising applications in the biomedical field for the controlled delivery of insulin. Qualitative structure analysis of all prepared polymers have been carried out by the using of(FT-IR) , and (^{1}HNMR) spectroscopy, and the thermal stability were systematically investigated.

EXPERIMENTAL

Materials

4-vinyl phenyl boronic acid from (MACKLIN) ; Methyl acrylamide (MAAm), 1,6- hexandiol diacrylate from (ALDRICH); Methyl acrylic acid (MAA), Ethylene glycol dimethacrylate(EGDMA),Glutaraldehyde(GA),1-vinyl2-pyrolidone(NVP),Potassium Peroxodisulfate KPS,Sodium metabisulfite(SMBS) ,all from (MERCK); Acetone, Alloxan,all from (BDH); N,N dimethyl acetamide, N,N,N,NTetra methyl ethylene diamine (TEMED),all from(HIMEDIA) ; insulin from (Jusline) .

Instruments

FTIR 8400S, Fourier Transform infrared spectrophotometer, SHIMADZU, Japan), (Oven, Trivp International Crop. Italy),(Hot plate stir, BibbyStrlintd.UK) (Measurement of 1 HNMR Spectra : recorded NMR spectra using a type of Bruker, Ultra shield 300 Mhz, Switzerl and using (DMSO-d6) as a solvent at the university's Educational teacher-Tehran Iran),(Thermogravimetry analysis (TGA) , using a heating rate of 10ºC/min in Argon atmosphere within the temperature range of 25-600ºC).Differential thermal analysis (DSC) measurement by using apparatus (DSC) in the college of Education for pure sciences Ibn al-Haitham/ University of Baghdad). Shaking Incubator (Heidolph unimax 1010\ Germany), pH-meter(Hanna\ USA).

Preparation methods of polymers

Preparation of Copolymers (4-VPBA-CO-MAAm) (PB1)

Dissolved (3 g) of the methyl acrylamide (MAAm) in 30 mL deionized water and add to (0.2 g) 4-VPBA dissolved in 10 mL N,N- dimethyl acetamide with the addition of (1 ml) of TEMED, add 5 ml of sodium hydroxide, and The mixture was refluxing under stirring and nitrogen gas at (60) C° for 20 min , then added (0.3 g) of the cross linker of 1,6-hexan diol diacrylate and after 5 minutes was added (0.1 g) of KPS dissolved in 10 ml of deionized water and after 24 hours the polymerization process was completed. then the acetone precipitation was done to remove the solvents used and obtain the polymer.

Preparation of Copolymers (4-VPBA-CO-MAA) (PB2)

Dissolved (4 g) of MAA in 40 ml distilled water and add to 0.2 g of 4-VPBA dissolved in 10 ml N-dimethylacetamide and add 5 ml of sodium hydroxide. , The reaction was refluxing and continued under stirring ,and atmosphere of nitrogen and water-bath to raise the temperature of the reaction to 70c ° and after 20 minutes was added (0.5 g) of the crosslinker ethylene glycol dimethacrylate and after five minutes were added the initiators prepared by dissolving (0.45 g) of KPS and (0.32 g) Of sodium meta persulfate in (30, 20 ml) deionized water respectively. and after 24 hours the polymerization process was completed. then the acetone precipitation was done to remove the solvents used and obtain the polymer.

Preparation of Terpolymers (4-VPBA-CO-MAA-CO-EC) (PB3)

Dissolved (4 g) of MAA in 40 ml distilled water and add to 0.2 g of 4-VPBA dissolved in 10 ml N-dimethylacetamide , also added 0.2g from ethyl cellulose dissolved in 10ml acetic acid , and add 5 ml of sodium hydroxide. , The mixture was refluxing , then the reaction was continued under stirring ,with atmosphere of nitrogen and water-bath to raise the temperature of the reaction to 70c ° and after 20 minutes was added (0.5 g) of the crosslinker ethylene glycol dimethacrylate and after five minutes were added the initiators prepared by dissolving (0.45 g) of KPS and (0.32 g) Of sodium meta persulfate in (30, 20 ml) deionized water respectively. and after 24 hours the polymerization process was completed. then the acetone precipitation was done to remove the solvents used and obtain the polymer.

Preparation of Terpolymers (4-VPBA-CO-MAAm-CO-NVP) (PB4)

Dissolved (4 g) of the methyl acrylamide (MAAm) in 30 mL deionized water , with the adding 0.2g NVP , and add to (0.2 g) 4-VPBA dissolved in 10 mL N,N- dimethyl acetamide with the addition of (1 ml) of TEMED, add 5 ml of sodium hydroxide, The reaction was refluxing and continued under stirring and the presence of nitrogen at degree (60) C° for 20 min , then added (0.3 g) of the crossliker of 1.6 -hexan diol diacrylate and after 5 minutes was added (0.1 g) of KPS dissolved in 10 ml of deionized water and after 24 hours the polymerization process was completed. then the acetone precipitation was done to remove the solvents used and obtain the polymer. That drying in the oven at 37C°.

Determination of Maximum Wavelength of complexes

In order to determine the wavelength at which the highest absorption (λmax) was obtained for the solution of the prepared complex with glucose, it was dissolved (0.1g / ml) of each polymer in 10 ml of water. The solution was stirred until homogenization, was added to 0.2g /ml of glucose and mix well, then record the absorption spectrum of each solution using a device (UV / Vis-spectrophotometer) of the Department of Chemistry, college of Education / University of Qadisiyah, within the range (200-900 nm) and using a quartz cell thickness (1cm) where wavelengths appeared (210, 205, 214, 248) for polymers (PB1, PB2, PB3, PB4,) respectively .

Effect of pH on glucose sensitivity

Were prepared several solutions with different functions acidic (PH = 2, 4, 6, 7.4, 8, 9, 10, 12) which has been tuned by using a measuring device function pH (PH-meter), and add g \ ml 0.14 of polymers prepared (PB1- PB4) in each prepared PH solution, and added to the 0.2 g \ ml concentration of glucose, the solution was well mixed. The absorbance values were then captured by the UV/Vis-spectrophotometer of the college of Engineering / University of al- Qadisiyah for each solution at the specified maximum wavelength, graphically drawn against the acidic function to determine the effect of the change in PH values on the polymer sensitivity of the glucose.

Determination of Maximum Wavelength of insulin

In order to determine the wavelength at which the highest absorption of insulin (λmax) is obtained, the UV spectrum of the insulin solution was determined at a concentration of 25 × 10-3mg / ml using a UV- Visible Spectrophotometer double Beam of the Department of Chemistry - college of Education / University of al- Qadisiyah, within the range (200-900 nm) and using a quartz

cell thickness (1cm) where it was found that the amount (λmax) of insulin used is (246nm).

Determination of Calibration Curve for insulin

The calibration curve of insulin was determined by the preparation of a series of standard concentrations (2×10^{-3}_8×10^{-2}) mg/ml. Using the UV/Vis-spectrophotometer , and measured the absorbance of these solutions at the lambda max of insulin (246) nm, and by drawing absorbance values versus concentration was obtained calibration curve as in Figure (1):

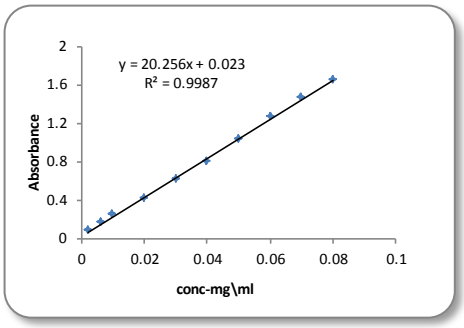


Figure (1) calibration Curve of insulin

Loading the insulin on polymers

For the purpose of loading the insulin on the prepared polymers (PB1-PB4), 140mg of each polymer was dissolved in 10 ml of deionized water. With continuous stirring, 10 mg/ml of insulin was gradually added and mixed well with shaker for approximately four hours The solutions were kept in dark glass containers special for the keeping of medicines .

In Vitro Drug Release

The insulin-loaded polymer solutions were used to study the ratio of insulin-free insulin in response to glucose. 10 mL of insulin-loading polymer solution was added to 5 mL of glucose solution at different concentrations (0.3, 0.2, 0.1 mg / ml) PH (physiological pH simulation) at 37 ° C and 39 ° C using water bath regulator of temperature . The release of insulin was followed by withdrawing 4 mL of solution and measuring absorption at different basins for 39 hours using the UV-Visible Spectrophotometer Use a quartz cell with 1cm thickness.

To calculate the concentration of liberated insulin, the following calibration curve equation was applied:

$$Concentration\ of\ drug\ (mg/ml) = (slope \times absorbance) \pm intercept \cdots \cdot (1)$$

The percentage of drug release was calculated by the following mathematical relationship:

$$percentage\ drug\ release\ (\%) = (Co - Ce)/Co \text{ x}100 \cdots\cdots\cdots\cdots\cdots\cdots (2)$$

whereas :

Ce: Concentration of the release drug (mg / mL)

V: The size of the solution (mL)

m: The weight of the polymer (mg)

Co: Primary focus of the drug (mg / mL)

The cumulative ratio was calculated by applying the following relationship:

$$Cumulative\ precentage\ release\ (\%) = volume\ of\ sample\ withdrawn\ ml/\ bath\ volume(v) \times P(t-1) + Pt \ \ldots\ldots (3)$$

Pt: percentage released at time t

P (t-1): percentage release at previous to 't

In vivo drug release

Preparation of polymers loaded with insulin

The polymers prepared (PB1, PB2, PB3, PB4) were milled into small soft granules using the mortar. After that, 0.14 g of each polymer was loaded into 20 mL of deionized water in a dark glass container With the addition of insulin (50IU) gradually and continuously stirring for about 4 hours using the electric vibrator, and were stored at a temperature of 20C ° in a glass container opaque for the preservation of medicines to be used in subsequent steps.

Experiment animals

In this study, 40 white male rats (150 ± 20 g) were used in a special room in the animal house of the college of Veterinary Medicine / Al-Qadisiyah University and the air conditioner (refrigeration equipment, air extractor) to preserve the rats under standard laboratory temperature of Living situation (23 ± 2 Celsius), with a follow-up to feed them (her diet) control. The rats were kept under these conditions for a period of five days before they started the experiment. The clinical examination of the rats was performed at the beginning of the experiment by the doctor Veterinarian in order to ensure that The good health status of all the rats was also checked for her blood glucose level by the tail vein .

Preparation of Alloxan solution

The Alloxan solution (936 mg) of Alloxan was present in 52 mL of cold normal saline solution. The dose of Alloxan was determined according to the body weight of each rat. On the other hand, each dose of Alloxan was estimated at no more than (120 mg / kg) from body weight of the rat, where the Alloxan solution was prepared to be used simultaneously and quickly within a few minutes, taking into consideration that the solution is not exposed to direct sunlight to prevent the degradation of Alloxan to its toxic secondary metabolite because Alloxan is photosensitive.

Induction of diabetes

Diabetes was induced In rats after a full night's fasting by raising food for 24 hours. Blood sugar was examined for all rats to be stimulated before injection to confirm the level of diabetes. The rats were then injected into the peritoneal membrane using an insulin syringe with one dose of Alloxan (120 mg/Kg body weight) and which prepared immediately before injection, as well as replace the drinking water of the rats with 5% glucose solution for 24 hours to reduce the shock treatment with Alloxan to prevent a sharp drop in blood sugar concentration due to pancreatic B-cell breakdown and release large amounts of insulin, the Blood sugar levels increased in rats during a period of approximately 72 hours, by conducting a blood glucose test using the on-call plus device through the tail vein . and then make sure injury male rats higher sugar levels in the blood on the third day of the induction, where considered Rats with a glucose concentration exceeding 250 mg / dL have diabetes.

Experimental testing

Before 10-day from starting the experimental, tow rats from each group were used to induce diabetes and to know the duration of the single-dose effect. In all the selected rats in this experimental study, diabetes was induced by injecting one dose of Alloxan (120 mg / kg) into the peritoneal membrane For each rat[31], after four days of injection, the blood glucose level was measured by using blood glucose test strips used on call plus [32] , and the animals that exceeded the concentration of blood glucose (250 mg / dL) were diagnosed with diabetes, after The measurement of diabetes level for all rats was given one dose of the drug(insulin-loading polymer), which was given ,the proportion of the body weight of each rat 3ml/1 kg body weight, The glucose level was measured after approximately 6 hours of administration and was (132±10 mg/dl) for all rats, followed by health status and level Diabetes for every 24 hours. After approximately 48 hours, a gradual increase in the level of diabetic rats was observed. This was repeated two times to ensure low glucose levels in the early hours of the dose and then gradually regaining after approximately 48 hours.

Experience Design

The rats were divided into six groups as follows:

1. The first group C (Control-): the control group (natural or healthy), contains six healthy rats, which were given water and natural food only throughout the experiment.
2. The second group T1 (Control +): control group (infected), and containing ten mice infected with diabetes-induced solution Alloxan by injection into the peritoneal membrane using a syringe of insulin were given water and food for the duration of the experiment.
3. The groups T2, T3, T4, T5 (treatment groups): Each of these six groups consisted of six diabetic rats induced by the alloxan solution and treated with insulin-loading polymers (PB1, PB2, PB3, PB4), where after confirmation of the injury of rats with diabetes after four days of stimulation was measured by diabetes for all rats and give them a dose of treatment solution 3 ml / 1 kg of body weight, was then measured blood sugar level every two days and were given the rest of the doses every two days for 30 days From the initiation of the experiment, which was calculated according to the body weight of each rat and the blood sugar level, which was measured using the blood glucose test strips device on call plus where blood samples were taken through a tail vein which sterilizes alcohol after each screening to prevent infection or injury, and the incidence of complications.

Statistical analysis

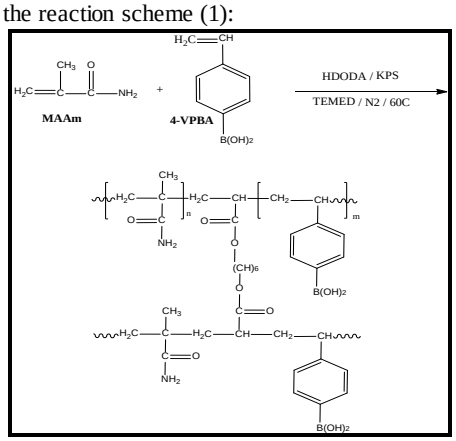
The Results were analyzed statistically for all groups by computer program SPSS. Version 17 software (2011). The methods of testing include one way ANOVA (one way analysis of variance) for group comparisons, and Probability (P <0.05) is used to record statistical significance. All data were expressed as mean ± standard error[33].

RESULTS AND DISCUSSIONS

Synthesis and Characterization of Prepared Polymers

Synthesis and Characterization of (PB1)

The polymer (PB1) was prepared through the copolymerization of MAAm with 4-VPBA , with the presence of HDODA as a crosslinker KPS as initiator , using TEMED as an accelerator for reaction and continued the reaction by refluxing for 24 hours and at 60C° under the N_2 gas to remove any dissolved oxygen, as shown in the reaction scheme (1):



Scheme 1: Synthesis of polymer (PB1)

Characterization of (PB1)

FT-IR Spectrum

The infrared spectrum showed several bands, most notably a wide range within the range (3200-3400) cm-1, which indicates the overlap between the current absorption (OH) and the current absorption (NH). The appearance of beams in the range (2890-2980 cm- 1) to the vibratory vibrations of the CH aliphatic bonds in the polymer structure. The characteristic beam at 1670 cm-1 refers to the (C = o) of the amide group, and the aromatic c = c) is shown at 1548 cm- 1). The characteristic bands within the range (1375-1450 cm-1) are due to the C-H bond for (CH_3) groups of the polymer, while the absorption bands at the frequencies (1100-1120cm-1) to the C-B, and the B-O is shown to be within the range (1220-1240 cm-1)[34,35,36] As shown in Figure 2 :

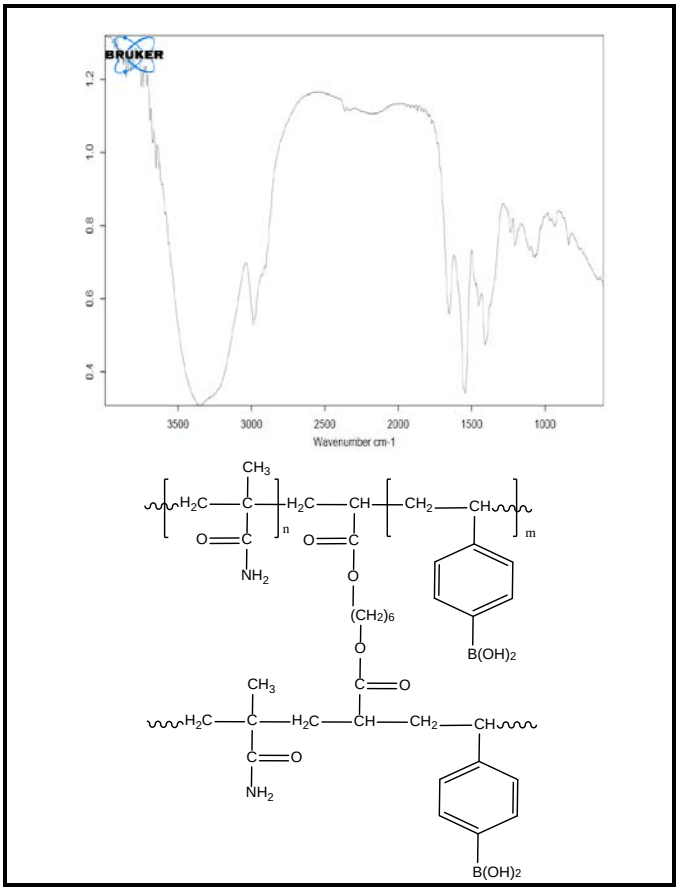


Figure 2: FTIR Spectra of (PB1)

The ^{1}H-NMR spectrum of the polymer (PB1) showed signals at (0.983-1.261 □=) returning to the CH_3 protons, as well as several signals (□= 1.81-2.25 ppm) for(CH_2), (□=2.49-2.60 ppm) for the OCH_2 protons. □=2.77 ppm" (Ppm 3.25 -3.62 □=) for (OH)protons, (□= 44.532-5.561ppm) for the protons of the(COOCH2)group , (□=7.432-7.701 ppm) for protons of aromatic ring , (□=8.623-8.308 ppm) for the (NH_2) group [35,37].

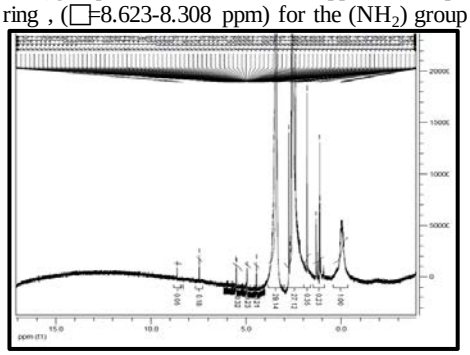
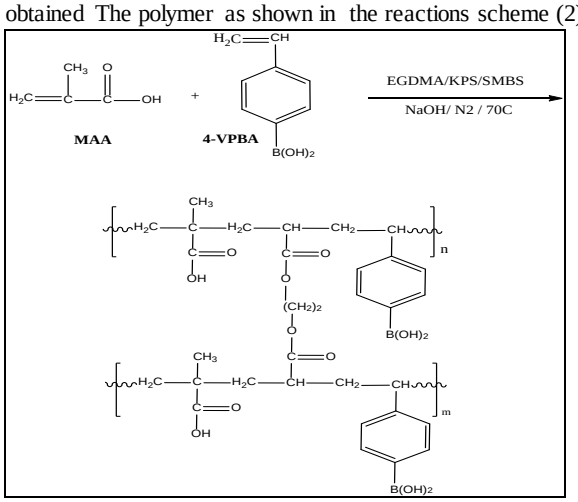


Figure 3: 1HNMR Spectra of (PB1)

Synthesis and Characterization of (PB2)

The polymer (PB2) was prepared through the copolymerization of MAA and 4-VPBA . Using the EGDMA as crosslinker, sodium meta per-sulfate and KPS solutions as initiators, the reaction was refluxed for 24 hours at a temperature of 70 ° C under an inert atmosphere of N_2 to "expel oxidizing oxygen". After

polymerization was completed, precipitations the result was done and obtained The polymer as shown in the reactions scheme (2):



Scheme 2: Synthesis of polymer (PB2)

Characterization of (PB2)

FT-IR Spectrum

The infrared spectrum showed several bands, most notably a wide range within the range (3300-3450)cm-1 which refers to the OH absorptions band . The appearance of beams in the range (2850-2990 cm-1) to the vibratory vibrations of the CH aliphatic bonds in the polymer structure. The characteristic beam at 1670 cm-1 refers to the (C = o) of the amide group, while the aromatic (c = c) is shown within the range (1475-1600 cm-1). The characteristic bands within The range (1390-1400 cm-1) are due to the C-H bond for (CH_3) groups of the polymer, while the absorption bands at the frequencies (1040-1065cm-1)to the C-B, and the B-O is shown to be within the range (1238-1245 cm-1)[34,35,36]As shown in Figure 4 :

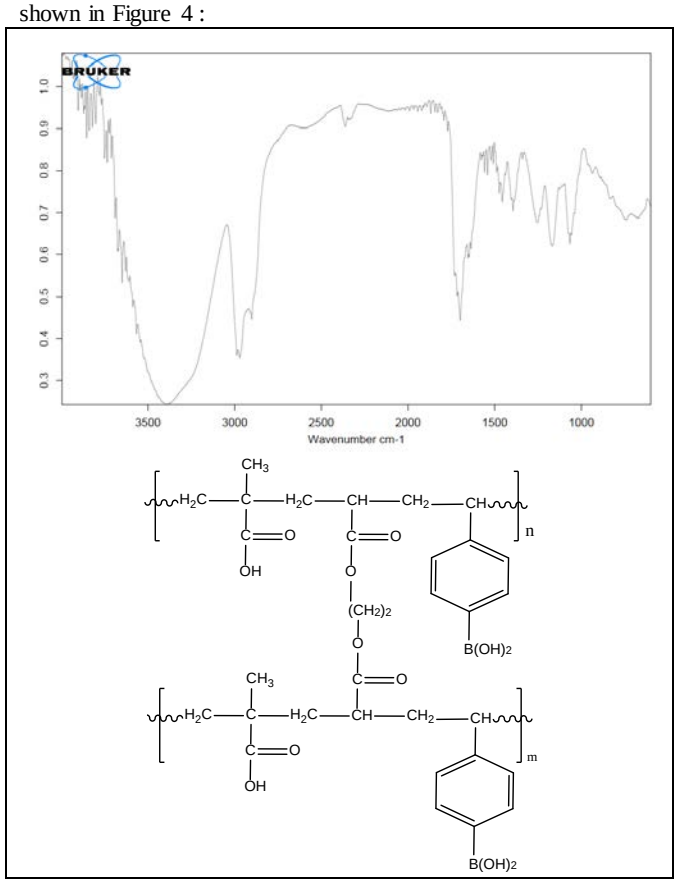


Figure 4: FTIR Spectra of (PB2)

The ^{1}H-NMR spectrum of the polymer (PB2) showed signs at (□=0.676-1.515) returning to the CH_3 protons, as well as several signals (□=1.532-2.286 ppm) for(CH**2**), (□=3.243 - 3.255 ppm) for the -OCH_2 protons . (□=3.230- 3.156)for (OH)protons, , (□=7,524-7.609ppm) for protons of aromatic ring , (□=11.470ppm) for the (COOH) group[35,37] .

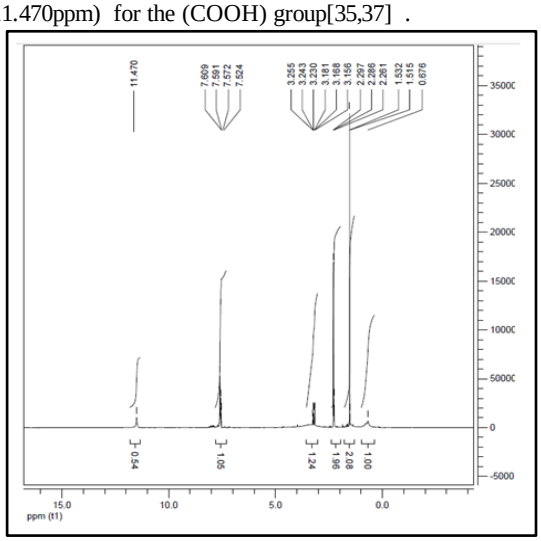
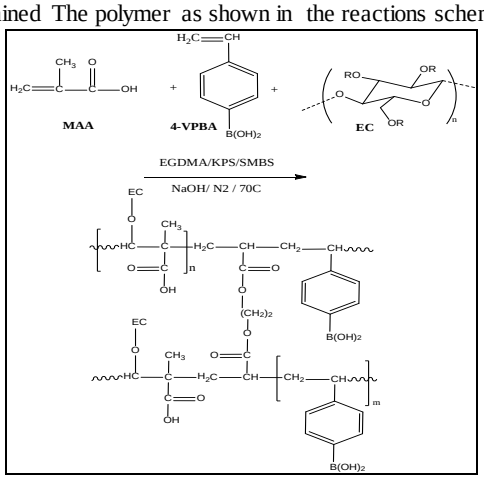


Figure 5: 1HNMR Spectra of (PB2)

Synthesis and Characterization of (PB3)

The polymer (PB3) was prepared through the copolymerization of MAA , 4-VPBA and EC monomers . Using the EGDMA as crosslinker, SMBS and KPS solutions as initiators, the reaction was refluxed for 24 hours at a temperature of 70 ° C under an inert atmosphere of N_2 to "expel oxidizing oxygen". After polymerization was completed, precipitations the result was done and obtained The polymer as shown in the reactions scheme (3):



Scheme 3: Synthesis of polymer (PB3)

Characterization of (PB3)

FT-IR Spectrum

The infrared spectrum showed several bands, most notably a wide range within the range (3220-3450) cm-1which refers to the OH absorptions band . The appearance of beams in the range (2898-2997 cm-1) to the vibratory vibrations of the CH aliphatic bonds in the polymer structure. The characteristic beam at 1700 cm-1 refers to the (C = o) of the ester group, and also The characteristic beam at 1605 cm-1 refers to the (C = o) of the carboxyl group . The characteristic bands within The range (1400-1508 cm-1) are due to the C-H bond for (CH_3) groups of the polymer, while the absorption bands at the frequencies (1063 cm-1)to the C-B, and the B-O is shown to be at the frequencies (1276 cm-1) [34,35,36]As shown in Figure 6 :

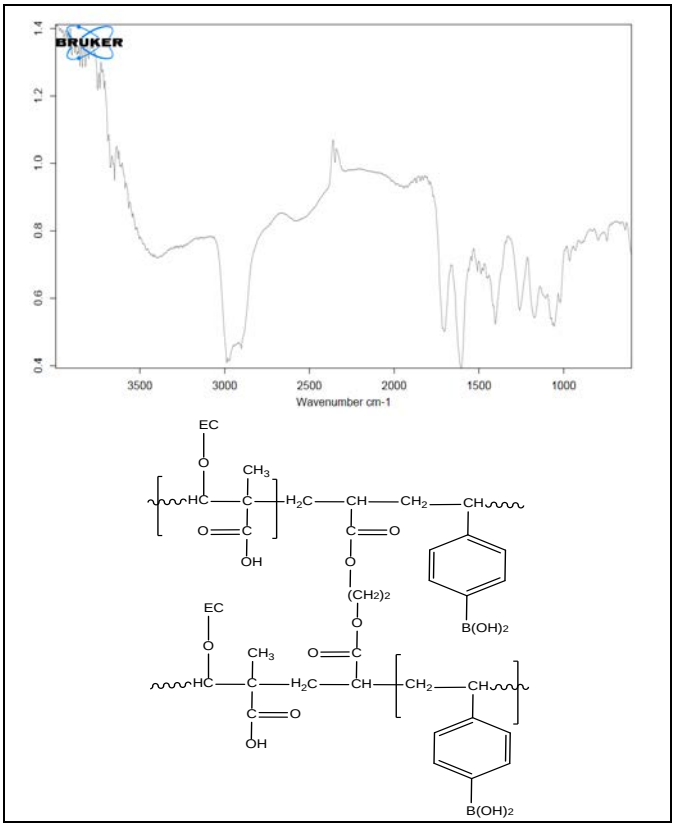


Figure 6: FTIR Spectra of (PB3)

The ^{1}H-NMR spectrum of the polymer (PB3) showed signs at (□=0.926-1.780) returning to the CH_3 protons, as well as several signals (□=1.837- 2.245ppm) for(CH_2) , (□=2.509 ppm) is due to protons of (DMSO) , (□=2.949-2.788ppm) for the -OCH_2 protons . (□=3.504–3.407ppm)for (OH)protons, , (□=7.627-7.642 ppm) for protons of aromatic ring , (□=11.431-11.445ppm) for the (COOH) group[35,37] .

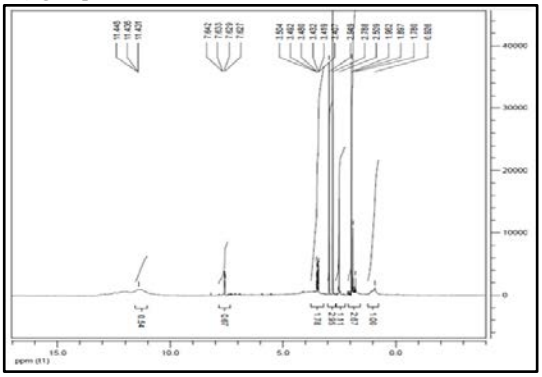


Figure 7: 1HNMR Spectra of (PB3)

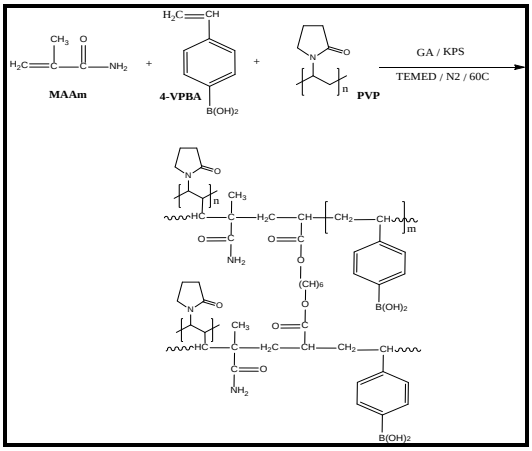
Synthesis and Characterization of (PB4)

The polymer (PB4) was prepared through the copolymerization of MAAm , 4-VPBA and PVP , with the presence of HDODA as a crosslinker and KPS as initiator , using TEMED as an accelerator for reaction and continued the reaction by refluxing for 24 hours and at 60C° under the N_2 gas to remove any dissolved oxygen, as shown in the reaction scheme (4) :

Characterization of (PB4)

FT-IR Spectrum

The infrared spectrum showed several bands, most notably The appearance of two bands at frequencies (3388 and 3297) cm-1 is due to OH and NH, respectively. The appearance of beams in the range (2898-2995 cm-1)to the vibratory vibrations of the CH aliphatic bonds in the polymer structure. The characteristic beam at1673 cm-1 refers to the (C = o) of the ester group, The characteristic beam at 1600 cm-1 refers to the (N-C=O) of the ester group , and the aromatic (c = c) is shown at (1550 cm-1). The characteristic bands within the range (1375-1450 cm-1) are due to the C-H bond for (CH_3) groups of the polymer, while the absorption bands at the frequencies (1100-1120cm-1) to the C-B, and the B-O is shown to be within the range (1220-1240 cm-1) , and The characteristic beam at 1305 cm-1 to the (C-N-C) ,[34,35,36] As shown in Figure 2 :



Scheme 4: Synthesis of polymer (PB4)

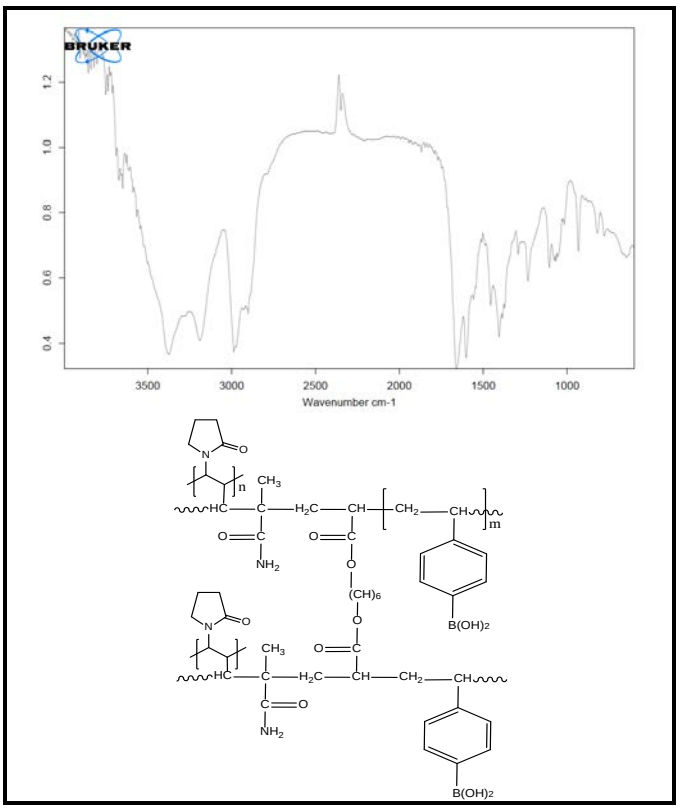


Figure 8: FTIR Spectra of (PB4)

The ^{1}H-NMR spectrum of the polymer (PB4) showed signs at (□=1.886- 2.022) returning to the CH_3 protons, as well as several signs (□=2.564-2.169 ppm) for(CH_2), (□=2.573ppm) for the –CH-N protons of pyrrolidone. (□=3.435 ppm) for (OH)protons of boron , (□=5.399–5.932 ppm) for the protons of the($COOCH_2$)group , (□=7.487 ppm) for protons of aromatic ring , (□=7.066 ppm) for the (NH_2) group[35,37] .

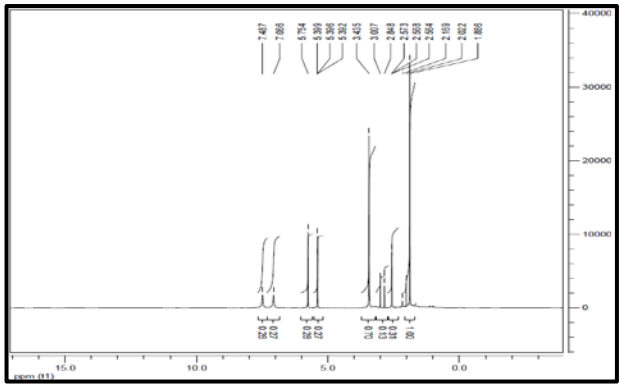


Figure 9: 1HNMR Spectra of (PB4)

Thermal Properties

Thermo gravimetric analysis (TGA) study

Thermo gravimetric Analysis (TGA) involves determining changes in mass as a function of temperature. It is commonly used to specification the degradation temperatures, absorbed content of materials, levels of organic and inorganic parts contained in a material and analysis solvent residues. It employs a sensitive electronic balance from which the sample is suspended in a furnace controlled by a temperature programmer. The thermal properties of four samples of these polymers were investigated by thermo gravimetric analysis (TGA) in Argon atmosphere at heating rate of 10 ºC/min[38,39,40,41]. in this test , several values were identified such as T*i*, T*op*, T*f*, T*50%*, % Residue at 600 ºC, and char yields at 400 ºC as shown in (Table 1).

The temperatures of 50% weight loss of (PB1- PB4) of polymers were between (458-480) ºC, The char yields of (PB1)are 48% ,(PB2)are 76%,(PB3)are 84% and,(PB4)are 59% at 500 ºC in Argon atmosphere, which indicate they could meet temperature resistant requirements, which can be used in different application . Weight residue of (PB1) are 49% , (PB2) are 77% and (PB3) are 86% , (PB4) are 61% at 600 ºC.

Table 1: Some Thermal Stability Characteristics Curves Thermal Gravimetric Analysis (TGA) of polymers

Polymers	DT/°C				$T_{50\%}$	Residue at °C600	Char % *At 500°C*
	T_i	T_{op1}	T_{op2}	T_f			
PB_1	201	180	400	599	480	49	48
PB_2	110	235	425	599	460	77	76
PB_3	110	248	425	599	465	86	84
PB_4	110	248	440	599	458	61	59

DT: Decomposition temperature.
Ti: Initial decomposition temperature.
Top: Optimum decomposition temperature.
Tf: Final decomposition temperature. The final degree of dissociation temperature
T50%: Temperature of 50% weight loss, obtained from TGA.
Char% at 400 ° C: Residual weight percentage at 500 ° C in Argon by TGA

Differential Scanning Calorimeter Analysis (DSC) Study

This type of analysis refers to the amount of energy absorbed from the sample during heating and cooling or at a constant temperature. This is done by placing the sample inside the measuring device under constant temperature and constant time, then determining the Tg, melting point (Tm) And the degree of crystallization (Tc)[42]. As shown in curves 1, 2 , 3 and 4 of the TGA and DSC curves :

Table 2: Shows the Degree of Glass Transition, Melting Point and the Degree of Crystallization in the Differential Thermal Analysis

Samples	Tg (c°)	Tm (c°)	Tc (c°)
PB_1	115	436	140
PB_2	107	438	200
PB_3	100	500	138
PB_4	110	440	141

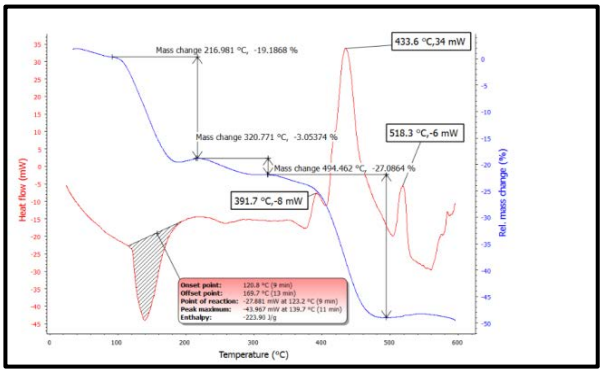


Figure 10: curves (TGA) and (DSC) for polymer (PB1)

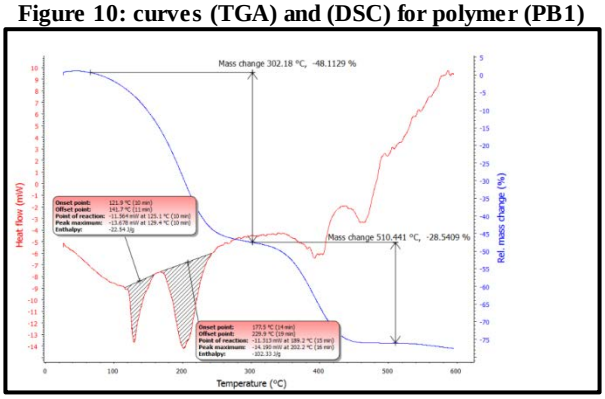


Figure 11: curves (TGA) and (DSC) for polymer (PB2)

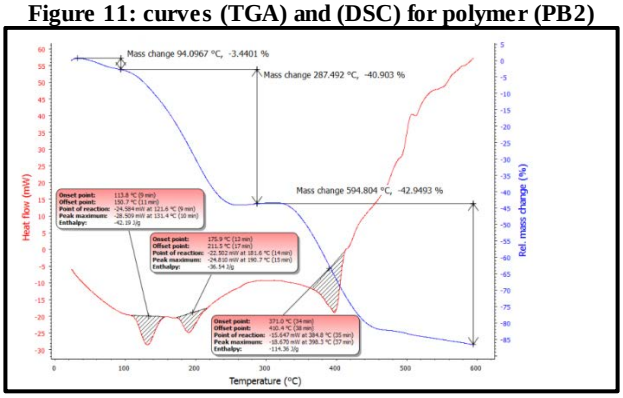


Figure 12: curves (TGA) and (DSC) for polymer (PB3)

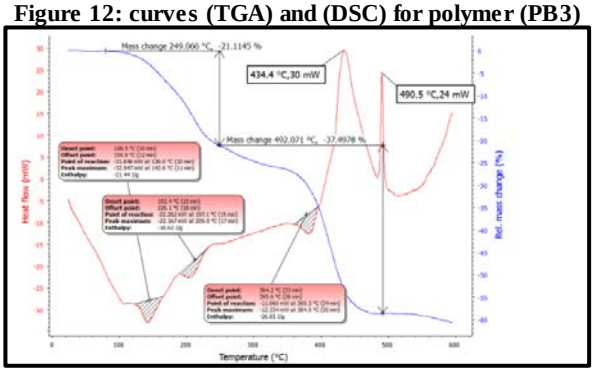


Figure 13: curves (TGA) and (DSC) for polymer (PB4)

Effect of pH on glucose sensitivity

In order to develop a polymeric system for the bioresponsive drug release , these polymers must be responsive to the pH[45], temperature[44], or chemicals[43]. The polymers containing boronic acid meet all these requirements , the versatility and unique properties of polymeric materials containing boron Due to the rapid inversion of Boron from the level of hybridization sp^2 (neutral) to tetrahedral hybridization sp^3 (anionic) , These characteristics make the boronic polymers systems very promising material for biomedical applications[46], especially on the release systems that release the drug in response to the presence of certain analytical materials such as blood sugar or catechols[47] , Phenylboronic acid and its derivatives were used for glucose sensing due to its ability to form complexes with polyols in the water solution[48,49,50] , It is also known that phenylboronic acid has a high pka value and therefore PBA alone lacks the specificity of glucose in physiological pH. In order to overcome this, two strategies have been applied which aim to reduce the pka values of phenyl boronic acid (PBA). one of which is the introduction of the electron withdrawing groups in the polymer series, and the other the insertion of the amine groups (Which act as Louis's internal bases), where the only electron pair on the nitrogen in these adjacent amine groups behaves similarly to ion hydroxide in the acid / base reaction of Lewis and filled with vacant orbitals on the boron and thus reducing the pka to about To pH7 , Which was first demonstrated by Walff [51], As the electron-withdrawing groups reduce the electrophoresis of the boron center, thus increasing the electrophilicity and Lewis acidity [52,53,54]. By following these strategies in the preparation of polymer chains with the PBA less than the value pka of the PBA, so that it is already charged too at pH 7.4, this would be the selective status of glucose to be exploited at physiological pH, where the study of the effect of acid function on Sense polymers Of glucose within a wide range of PH values, by measuring the absorption of each solution by placing the polymer in a different glucose and acid solution (PH = 2, 4, 6, 7.4, 8, 9, 10, 12), at the maximum wavelength specified for each complex, as the sensitivity was found to increase by increasing the PH , and to enable selective glucose selection (sensitivity to glucose) at the physiological pH level as shown in Fig14:

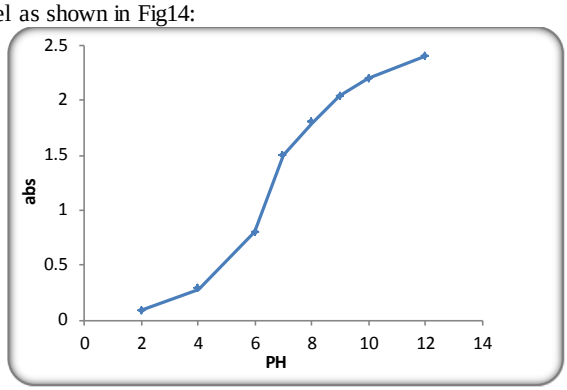
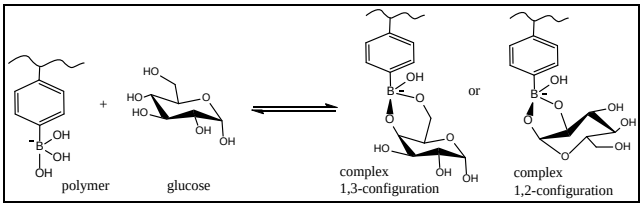


Figure 12: Effect of PH on Glucose Sensitivity

Diol complexation (Glucose-PBA)

The most important characteristic of Boronic acid is its interaction with the resulting reactions in the formation of Boronic acid complexes. This feature is most suitable for the formation of sugary complexes. [53] Boronic acids form complexes with hydroxyl groups, preferably with two hydroxyl groups in 1,2-configuration or 1,3-configuration and thus the formation of penta or hexa-ester rings [49] as in the scheme (5) [55], and many of the sugars contain the charges, but PBA has a high selectivity of the molecules of glucose, which increase by increasing its concentration, so as to increase the density of glucose bindings because it contains two sets of cis-diol and thus can bind two of the PBA simultaneously and form reversible crosslinks, and this is lacking in other sugars. Therefore, PBA shows high affinity for glucose molecules due to the presence of more than one site for the formation of diol complexes.



Scheme (5): shows how glucose is associated with PBA moiety

The association of insulin with polymer

Insulin is a key element in the treatment of both type I and type II diabetes. For insulin-dependent patients, the best therapeutic outcomes were observed when the regimented insulin administration [56]. Some patients respond well to insulin and therapy . However, most patients who rely on insulin suffer from complications that result from weak commitment in treatment or insufficient control of blood sugar [57]. Acute hypoglycemia can lead to coma or death [58]. Moreover, chronic instability in glucose levels can lead to cardiovascular disease and non-wound healing which is an independent indicator of total cardiovascular and cancer mortality [59]. Extensive efforts have been made to develop insulin release systems and to control blood sugar more accurately and longer [60,61], by developing new polymers containing glucose-sensing compounds in their structure. PBA are also known to have the possibility of reverse bonding with Cis-1,2 or cis-1,3 diols, as in glucose, which cause the stability of negative charge on boronic acid. [62] PBA groups were included within the polymer structure used to detect glucose and release insulin [63] Here, we demonstrate that Insulin with PBA-polymers provides long - term activity and response to the Long-lasting and glucose-responsive activity and the possibility of improving diabetes management strategy, self-administered insulin is one of the most important treatments to provide control of blood glucose levels in patients with type 1 diabetes. However, insulin therapy enters a number of complications . And subsequent events with control of blood glucose levels. Therefore, these bio-polymers were prepared with insulin loading as in Figure (13) to provide a self-regulating therapy system with improved control of blood sugar level .

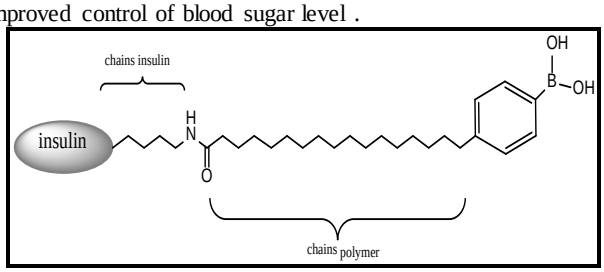


Figure 13: Insulin binding with polymer

In Vitro Drug Release

The ratio of insulin release of polymers (PB1-PB4) in response to glucose at pH 7.4 that approach to physiological pH and the degree 37c° temperature , approach to the degree of proper body , was studied . Different concentrations of glucose were taken to compare the drug release rate at the normal concentration of glucose (0.1mg / ml) with the release ratio at high concentrations of glucose (0.2mg / ml) and (0.3 mg / ml). The results shown in Fig. (14,15, 16 and17) showed that the insulin release rate at 37C° and PH = 7.4 and different concentrations of glucose (0.3 , 0.2 , 0.1) mg/ml (simulating the concentration of glucose in plasma) increases with glucose concentration where there is In the first phase of release, there is a sudden release of the drug in the first

phase of the liberalization as a result of a large increase of the diols in the solution and then continue to release the medication gradually . as These polymers show a sudden release in the first two hours followed by the slow release of insulin. When the 3mg / ml high glucose concentration is higher than the release at 2mg / ml concentration, which is higher than the concentration of 1mg / ml, it is clear that the release of insulin depends on the concentration of glucose. The results indicate that the polymers were responsive to glucose and the possibility of insulin release control Insulin is affected by two key factors, one of which is the sensitivity of glucose to the PBA moiety and the other is the molecular weight of the copolymer. When this molecular weight is low, This facilitates glucose free access to polymer and interaction with the PBA and thus the release of insulin. The results indicate that insulin is released from the interpolymeric network were not only a process of insulin diffusion but also because of relaxation in polymer chains. The results are reasonable given the overall effect of several factors. For the current system, polymer membranes are amphiphilic polymers with a relatively high sensitivity to glucose. As a result, glucose is able to propagate through the polymer's complex to form a glucose boronate complex. This eventually converts the polymer from gel to sol, which facilitates the diffusion of molecular compounds, including insulin, through the polymer complex, to achieve the release of insulin dependent on glucose. Is currently being developed a new system to provide drugs insulin based on the above result. . The cumulative percentage of release was determined at different times. And The results shown in Figs. (18 ,19,20and21) showed that the cumulative release rate was high in the high concentrations of glucose compared with the weak percentages at the lower concentrations.

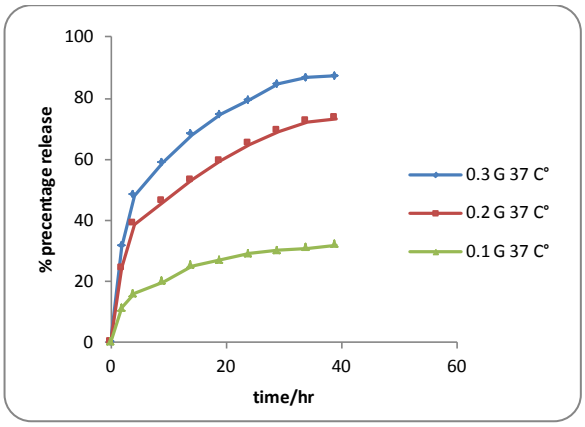


Figure 14: percentage drug release from PB1 at 37C°

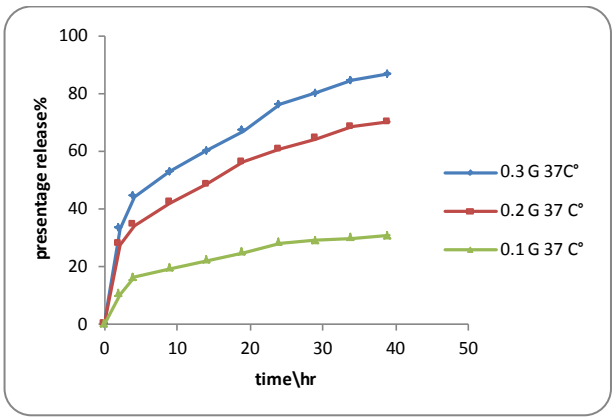


Figure 15: percentage drug release from PB2at 37C°

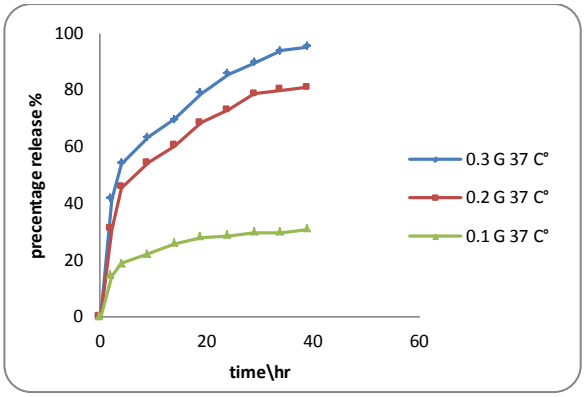


Figure 16: percentage drug release from PB3at 37C°

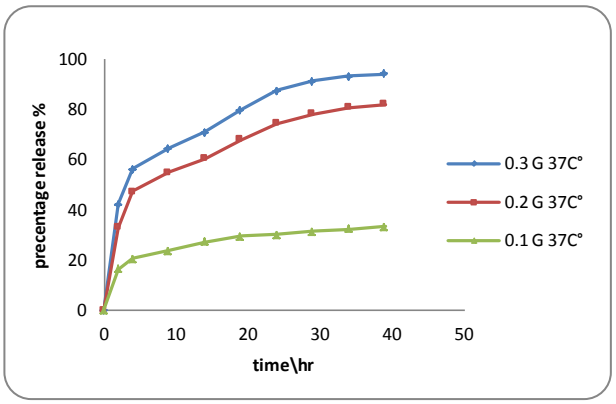


Figure 17: percentage drug release from PB4at 37C°

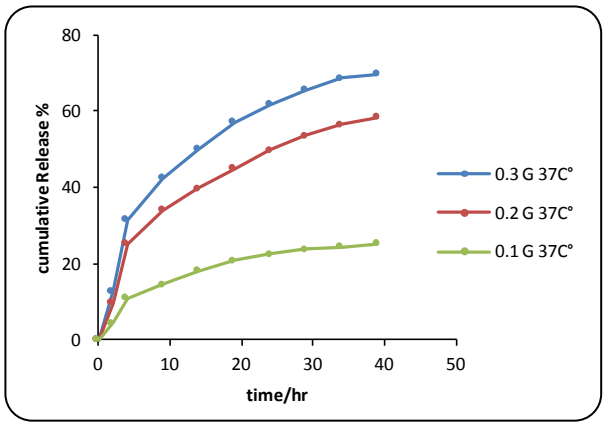


Figure18: cumulative percentage release of PB1 at 37C°

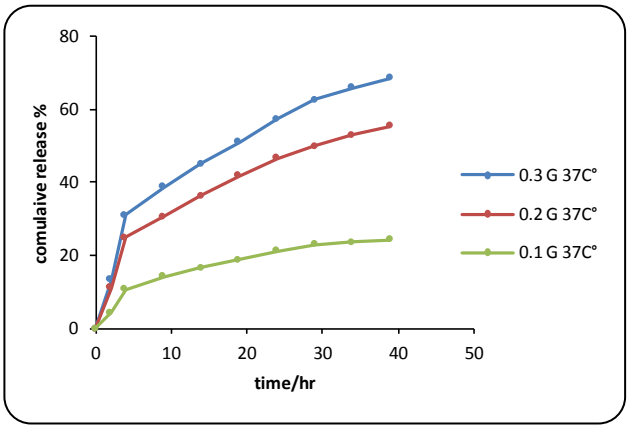


Figure19: cumulative percentage release of PB2 at 37C°

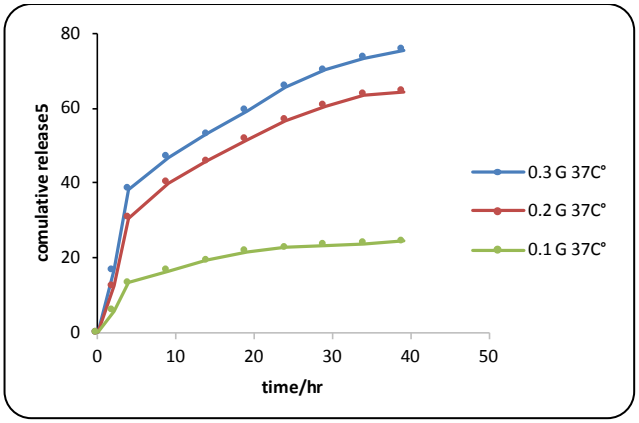


Figure20: cumulative percentage release of PB3 at 37C°

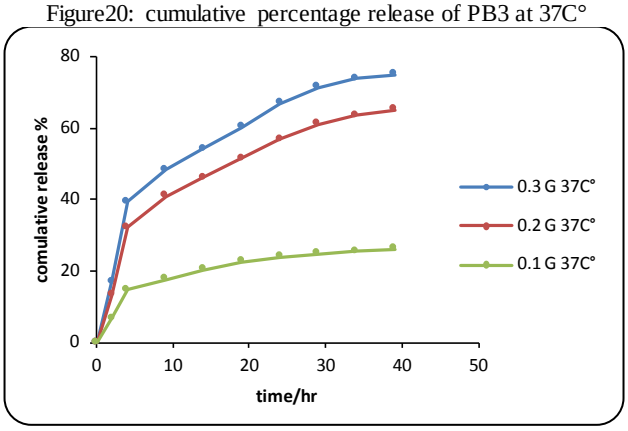


Figure21: cumulative percentage release of PB4 at 37C°

Effect of temperature on the insulin release in vitro

The effect of high temperature was studied on the release of the drug from the polymers (PB1-PB8). The release was tested at 39 ° C using the regulated water bath and (PH ~ 7.4) (simulation of physiological pH) . with The results indicated in the figures (22,23,24 and 25) that The increase in temperature increases the rate of release of the drug. This can be attributed to the increase in the rate of breakage of bonds within the polymer chains at high temperature and thus the rapid release of the insulin . Also, the high temperature causes the instability of The bond physical forces between the polymer and the drug substance that loaded and thus increases release rate of insulin within the solution [64], as well as the study of the cumulative percentage Release%, with results shown in the figures (26,27,28and29) a marked increase in the percentages at 39C ° C than at physiological temperature (37 ° C) Which increases with increasing glucose concentration.

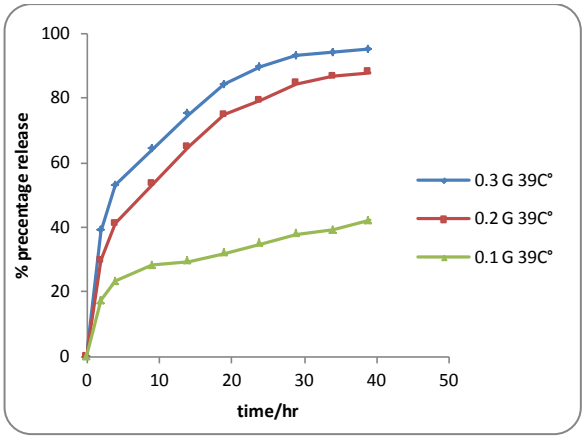


Figure 22: percentage drug release from PB1at 39C°

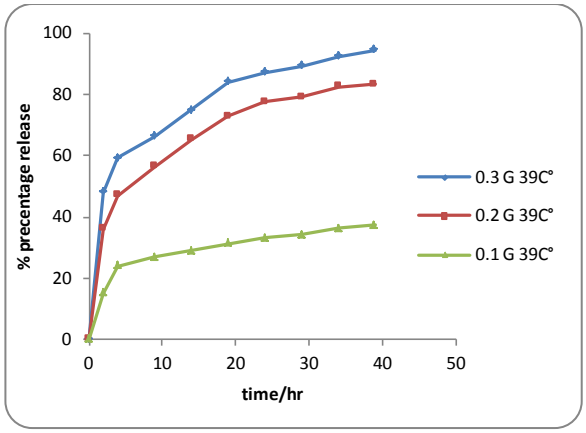


Figure 23: percentage drug release from PB2at 39C°

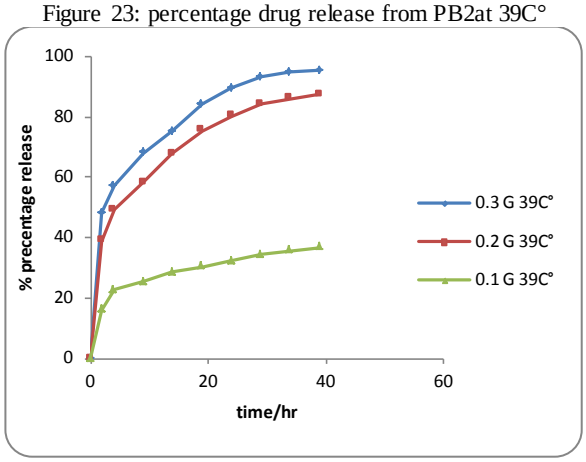


Figure 24: percentage drug release from PB3at 39C°

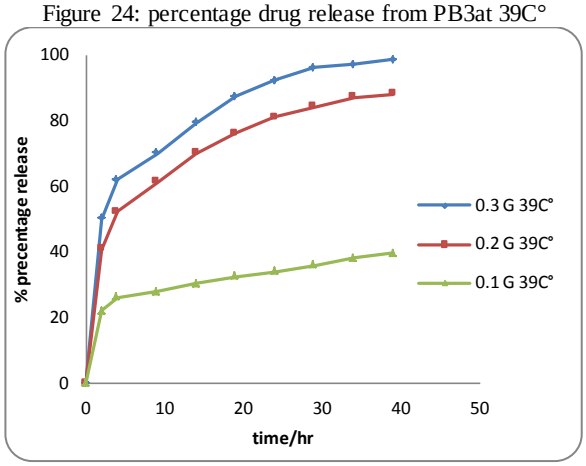


Figure 25: percentage drug release from PB4at 39C°

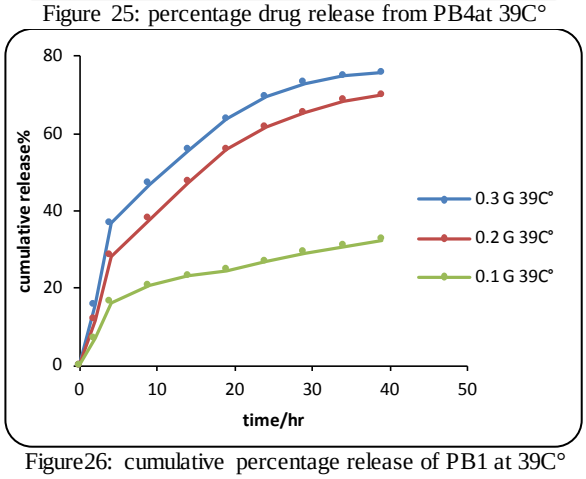


Figure26: cumulative percentage release of PB1 at 39C°

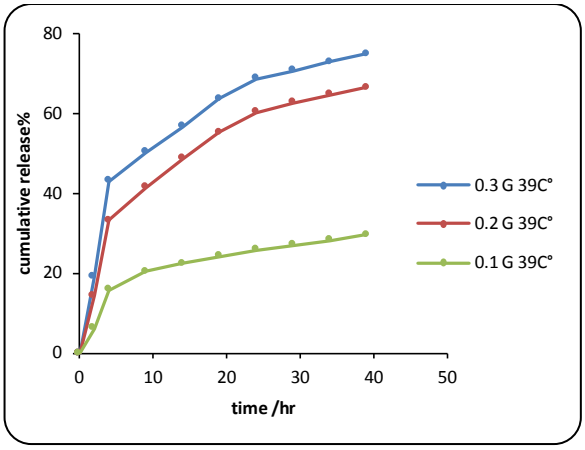


Figure27: cumulative percentage release of PB2 at 39C°

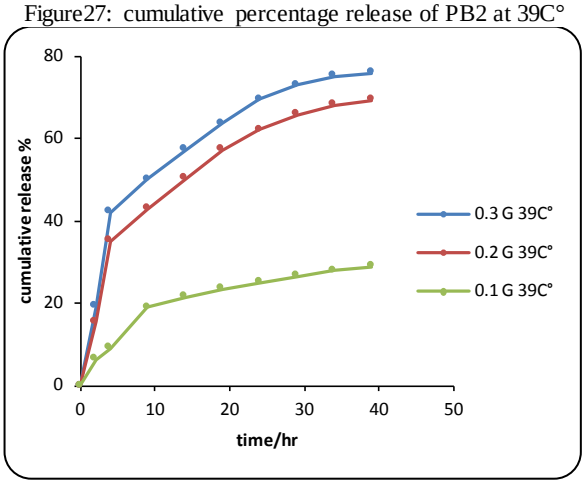


Figure28:cumulative percentage release of PB3 at 39C°

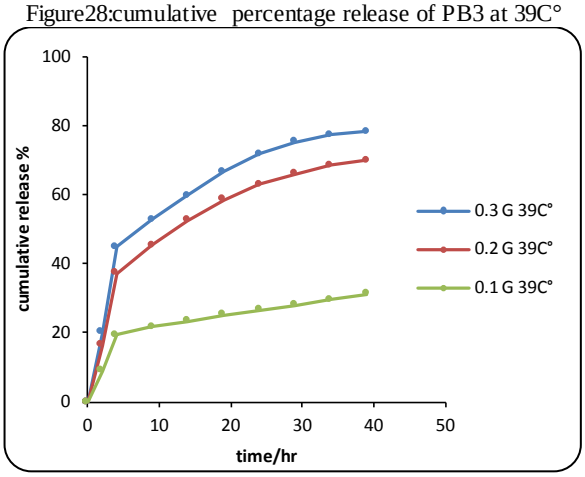


Figure29:cumulative percentage release of PB4 at 39C°

In Vivo Drug Release

The results shown in Table (3) showed significant increase below p <0.05 in the glucose concentration of male albino rats (120 mg / kg body weight by peritoneal injection) compared with healthy control rats (The results are similar to those of previous studies [67,68,69,31]) . And the reason for this is that the oxidative stress generated by the presence of Alloxan causes the generation of free radicals, which in turn attack the beta cells of the pancreatic and crashing and thus inhibit the formation of insulin first inhibitor that insulin secretion, and then the decomposition of glucose and stimulate the composition process and decomposition of glycogen stops causing high concentration of blood sugar . While led the treatment of rats exposed to alloxan by polymers loaded with insulin to a positive result of a clear reduction in the negative impact of alloxan through for a significant decrease significantly below the level of p <0.05 in the concentration of sugar glucose, which was much more than it is in the case Alloxan group alone . Where the treatment of diabetic rats (induced by Alloxan) with the prepared solutions of polymers loaded with insulin through intra-peritoneal injection with a dose of 3 ml / kg body weight, which was studied in the experimental test to determine the approximate duration of one-dose effect in infected rats with diabetes by alloxan , Where was given a single dose of rats from each group (T2-T5), after 3 days of induction of diabetes, where the amount of blood glucose level (320±6.2) mg/dl after as the (92.1±0.15)mg/dl , and then follow-up the glucose level in rats for every 24 hours, where it reached the level of glucose mg / dl (3.15 ± 128.6) after 24 hours of treatment , as noted the resumption of the rise to the level of glucose extent mg / dl (2.1 ± 268) after 48 hours of treatment, suggesting a decline in the influence of dosage and adopted as a dose used to evaluate the effect of liberation of insulin loaded on polymers in reducing the level of glucose in the rat experiment [66] treatment by polymers loaded with insulin (T2-T5) for 30 days . The results indicated in Table (3) showed a significant decrease in the level of glucose in the treatment rats (T2-T5) on the seventh day compared with the alloxan-stimulating group (T1) while still significantly higher than the healthy control group (C), which reduced in the tenth day of treatment , and consistently treatment there is a significant decrease compared with the animals stimulating alloxan- group (T1) and a healthy control group (C) , where was noted in the day 30 of treatment the presence of a large significant decrease in the level of sugar in the rats treated (T2-T5) compared to With the alloxan-stimulating group (T1) , while there is no significant difference compared to healthy control groups (C) , which indicates improved control of the state of hyperglycemia in rats, and the effectiveness of treatment in reducing the level of diabetes significantly for all treatment groups (T2-T5) compared with Alloxan group (T1) as shown in the Table (4), these results are consistent with previous studies [65] and [48] which confirmed the effective effect of PBA- polymers loaded with insulin in the gradual release of insulin and reduced blood sugar level in treated rats.

Table (3) glucose Levels of healthy and Diabetic Male Rats

Time / groups	1day	4day	7day	10day	21day	30day
C	90.66± 0.24 a	90.66± 0.24 a	90.66 ±0.24 a	90.66± 0.24 a	90.66± 0.24 a	90.66± 0.24 a
T_1	90.97± 0.23 a	345.8±2 0 b	350.8±2 0 b	363.00± 20 b	396.5±1 b	418.5± 1.36 b
T_2	91.00± 0.23 a	344.3±9. 7 b	276.66± 2.2 c	262.3± 3.37 c	158.8±2. 7 c	93.0± 1.0 a
T_3	90.47± 0.15 a	346.6±4. 1 b	277.1±4. 4 c	265.5± 6.4 c	159.1±2. 9 c	94.1± 1.35 a
T_4	90.8 ±0.28 a	348± 3.6 b	275± 2.8 c	263.1± 3.9 c	156± 2.7 c	92.8± 1.2 a
T_5	91.1± 0.43 a	349.5±1 1 b	279±1.8 5 c	261.8±3. 1 c	158.6±2. 3 c	91.3± 0.88 a

Numbers represent the mean ± standard error.
The different letters indicate a significant difference, while the similarity indicates that there is no significant difference.
C: represent the healthy control group.
T1: represents the group of rats in which diabetes was introduced by Alloxan.
T2: represents rats in which diabetes was introduced by Alloxan and treatment with the insulin-loaded polymer PB1 group.
T3: represents rats in which diabetes was introduced by Alloxan and treatment with the insulin-loaded polymer PB2 group
T4: represents rats in which diabetes was introduced by Alloxan and treatment with the insulin-loaded polymer PB3 group.
T5: represents rats in which diabetes was introduced by Alloxan and treatment with the insulin-loaded polymer PB4 group.

Table (4): The significant differences in treatment groups compared with the alloxan group during the days

Group / time	T_1	T_2	T_3	T_4	T_5
1day	90.97± 0.23 a	91.0± 0.23 a	90.47± 0.15 a	90.8± 0.28 a	91.1± 0.43 a
4day	345.8± 20 b	344.3± 9.7 b	346.6± 4.1 b	348± 3.6 b	349.5± 11 b
7day	350.8± 20 b	276.66± 2.2 c	277.1± 4.4 c	275± 2.8 c	279± 1.85 c
10day	363.0± 20 b	262.3± 3.3 d	265.5± 6.4 d	23.1± 3.9 d	261.8± 3.1 d
21day	396.5± 1 c	158.8± 2.7 e	159.1± 2.9 e	156± 2.7 e	158.6± 2.3 e
30day	418.5± 1.36 c	93.0± 1.0 a	94.1± 1.35 a	92.8± 1.2 a	91.3± 0.88 a

CONCLUSIONS

In this study, polymers prepared showed a significant response to high concentrations of glucose at physiological pH and temperature (pH 7.4, temperature 37°C), as the increase of diols at high concentrations of glucose(0.3, 0.2mg /dl) increases reversible crosslinks between PBA-moiety and glucose molecules. The polymers sensing for glucose and insulin release gradually at different time intervals . where there was a release of large amounts of insulin in the early hours of liberation due to the presence of large amounts of diols and then slow gradual release . And there found a clear effect of polymers loaded with insulin in lowering blood sugar levels in laboratory rats with diabetes induced by alloxan .The proposed system can be applied in a broad temperature range and may have significant benefits compared to earlier reported glucose-responsive systems which comprise the use of proteins (concanavalin A) or (enzymes glucose oxidase) which may easily denature. Further research aims to design polymers which disassemble at elevated glucose concentrations at more physiological pH (i.e., 7.4) using polymerizable phenylboronic acid derivatives having a pKa situated near the physiological pH.

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