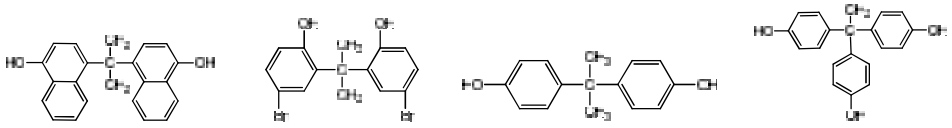
*Synthesis And characterization Of New Type OfBiodegradable Polyurethane Containing Azo Derivatives Of Benzocaine

Received :9/1/2014 Accepted :18/3/2014

Mohammed A. Mutar , Sabrean F. Jawad
Department of Chemistry , Faculty of Education , University Of Al-Qadisyia .

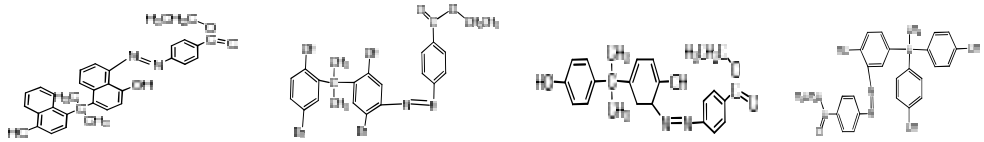
Abstract

In this work a series of new types of biodegradable and pH-sensitive polyurethane containing azo derivatives of Benzocaine were synthesized by the condensation polymerization of 1,6-hexamethylene diisocyanate at the temperature 80 °C with the azo monomers . The monomers synthesized from the reaction of (1-naphthole M1, 4-bromophenol M2, phenol M3 , and p-hydroxy acetophenone M4):



M1 M2 M3 M4

With acetone in presence of HCl as catalyst by refluxing for 6 hrs at 60°C.The azo monomers were synthesized from the reaction of monomers : (M1 ,M2 ,M3 ,M4) with Benzocaine in presence of HCl , NaNO2 and NaOH 10% by stirring for one hour in ice bath at (0-5 °C) .



AZO1 AZO2 AZO3 AZO4

polyurethane azo were synthesized from the reaction between the azo monomers with 1,6- hexamethylene diisocyanate in oil bath by refluxing for 8 hours at 80 oC in dry nitrogene. U.V-Visible , FT-IR , H1NMR techniques were used to confirm the chemical structure of the synthesized Polyurethane azo.

A calibration curve between absorbance and concentration was constructed to stock solution of pure drug. the concentration of Benzocaine release was calculated by extrapolation of the result on the calibration curve every 24 hrs. the detection limit for Benzocaine was minimum used 0.001 gm/mol wavelength at 293 nm.

Benzocaine was loaded into the polymeric matrix during in situ polymerization . the drug release from the Benzocaine loaded was studied in two medias (pH=7.8, pH=4) at body temperature(37°C). also the total amount of released Benzocaine was measured at room temperature and pH=4,pH=7.8 .

Chemical Classification QD 241-441

***The Research is apart of on MSC.Thesis in the case of the second researcher**

1- INTRODUCTION

One of the most important methods to increase the therapeutic effects and decrease the side effects of drug is by controlled drug release . Therefore, it has attracted great interest in recent years and application of polymers has been also extended in this field [1].Polymers are the most important and dominant biomaterials, because they can be designed and prepared in a wide range of compositions and properties, from hard and hydrophobic systems to highly hydrophilic and soft biomaterials when they are in contact with the physiological fluids [2]. Polymers may be used as carriers for pharmaceutical agents [3]. Therefore, the drug is released in certain parts of the body in a required dose and desirable specific rate which itself consists of various techniques [4].

Synthetic polymers used in biomedical applications are making a significant contribution to the progress currently being achieved in health care and in this regard the combination of pharmacologically active compounds with polymers via chemical reactions has been attracting an increasing degree of attention during recent years. The major objectives in such studies are aimed at prolonging the duration of drug activity by controlling the release of drug . Bioactive agents have also been chemically bonded to preformed synthetic or naturally occurring polymers by allowing them, or one of their derivatives, to react with the polymers, functional groups [5].An alternative to direct drug-polymer linkage is the incorporation of a spacer group between the drug and polymer chain. The use of suitable spacer arms can increase the mobility of drug on the polymer chain and enhance the sensitivity of conjugates to undergo chemical or enzymatic hydrolysis [6].

The facility with which the drug can be converted to polymerizable derivatives depends to a large extent on their functionality . The value of the hydrolysis rate constant depends, on the strength and chemical nature of the agent-polymer chemical bonds, the polymer structure and the surrounding condition [7].The design of the Polyurethane controlled-release forms for therapeutic drug administration is the subject of intense interest. Such systems are being used for sustained and controlled delivery of various pharmaceutical agents such as prednisolon, morphine, caffeine ,prostaglandin and theophylline

Urethane-based polymers were prepared based on the reaction of diisocyanates with azo monomers to deliver drug.[8] .

Colon is known to be a reductive medium in which azo groups are reduced to the corresponding amines[9-12]. It has been shown that polymeric azo compounds could be used for colon targeting since reduction and subsequent splitting of the azo bond occurs only in the large intestine, and therefore they are highly site-specific [13-15].

In this research, the synthesis of a pH-sensitive polymer network consisting of polyurethane azo(PU AZO) is described. Complex-forming constituents of the polymer were covalently linked to each other and to the drug-linked monomer, and drug release properties of the polyurethane azo were studied in simulated gastric fluid (SGF, pH 4) and simulated intestinal fluid (SIF, pH 7.8) are used without purification .

EXPERIMENTAL

2.1 Materials

Benzocaine (Sigma Aldrich) , 3-bromophenol (FLUKA) , Phenol (CARLO ERBA) , 1-Naphthole (GRIFFIN) , ,N-dimethyl formamide (DMF) , Para acetophenone , acetone (BDH) , Hydrochloric acid (BDH) , Sodium hydroxide (BDH) , Sodium nitrite (SIGMA-ALDRICH) , Methanol (BDH) , buffer solution with pH=4 and pH=7.8 .

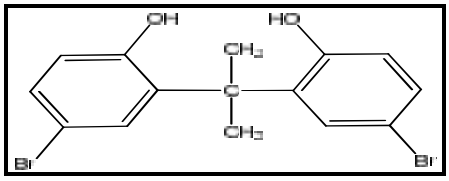
2.2 Instruments

Infra-red spectra were taken on a shimadzu Japan spectrophotometer (model) using KBr pellet. 1H NMR spectra were recorded on a Brker, Ultra, Shiel 300 MHz spectrophotometer using DMSO as solvent. Ultra-violet spectra were taken on a shimadzu 1650 PC spectrophotometer. pH meter, Hanna, Romania, Fume Hood , K & K Scientific suppler , Korea . measuring the degree of fusion (Melting Point) smp30 . Vacuum drying Oven , K-vo27 scientific suppler Korea . water still , Labtech , Korea . Oven ,Trivp International Crop . Italy . Hot plate stir, Bibby Strlintd. UK .water still , Labtech , Korea .

2.3 Monomer Synthesis

2.3.1 synthesis of 2,2'-(propane-2,2-diyl)bis(4-bromophenol) (M1):

A mixture of 4-bromo phenol (94g , 5.4mmol) , acetone (12.75g , 2.1mmol) and HCl catalyst (37.5 g , 10mmol) were placed in a three necked-round bottom flask equipped with a condenser , mechanical stirrer and thermometer and it was kept in a thermostat bath at 60oC for 6 hr. After a definite period of time , the reaction mixture was transferred to cold water to quench the reaction at the given time . then , the product was washed ,A dried and weighted [16]. to give (45%) of dark brown crystals , m.p= (98-103^{O}C).

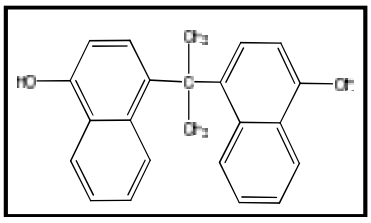


Scheme 2.1 *Structure of M1*

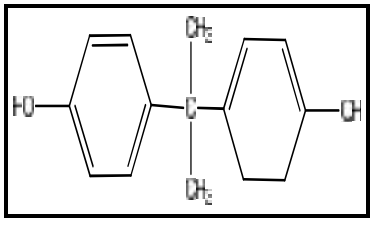
The other monomer was prepared by the same procedure as above using 1-naphthole , phenol , para- hydroxyl acetophenone

Table2.1 *represents the color, yield and melting point for preparing of monomers*

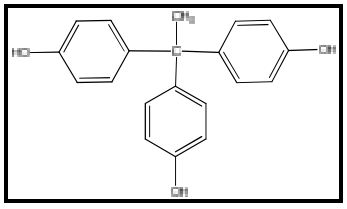
NO	Monomer	Substance		Color	Yield%	M.P/ c
1	PBN1	Acetone 12.75g (21.mmol)	1-naph. 94g (65 mmol)	Black	75	119
2	PDDP	Acetone 12.75g (21 mmol)	Ph. 94g (21 mmol)	Orange	79	112
3	ETTP	P-HAP 94g (69mmol)	Acetone 12.75g (21mmol)	Brown	65	112



Scheme 2.2 Structure of M2



Scheme 2.3 Structure of M3

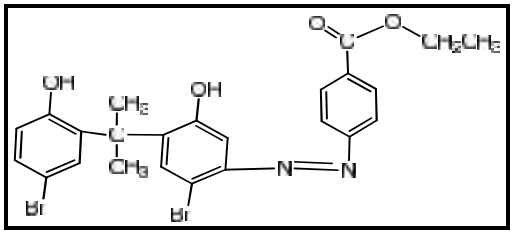


Scheme 2.4 *Structure of M4*

2.4 Azo Monomer synthesis

2.4.1 synthesis of p-[3-bromo-6-hydroxy phenyl azo]ethyl-p-amino benzoate (AZO1):

In 500 mL conical flask, Benzocaine (1.53 g, 10 mmol) was dissolved in a mixture of concentrated hydrochloric acid (10 mL), water (25 mL) and ice (25 g). The solution was cooled with stirring in an ice bath to 0ºC until it become clear, then a solution of sodium nitrite (3.45 g, 50 mmol) in water (7.5 mL) was added dropwise during 10 min, and the reaction mixture was further stirred for 20 min in an ice bath at 0–5 ºC. The solution was added dropwise to monomers that shown in table 2.1 (1.38 g, 10 mmol), in 10% sodium hydroxide solution (25 mL) with stirring in an ice bath for a further 1 h. The produced monomers was precipitated. The product was collected by filtration and washed with water and dried under *vacuum* at room temperature overnight[17] to give (91%) of red to crystals , m.p= (137 ^{O}C) .

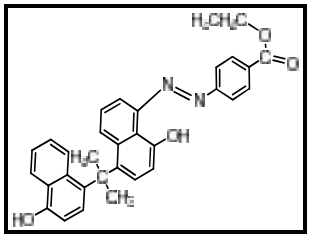


Scheme 2.5 *Structure of AZOl*

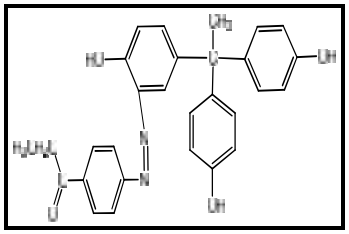
The other compounds were prepared by the same procedure as above using AZO (2-4) are shown in Table (2.2) .

Table 2.2 : *synthesis and details data of prepared azo monomers:*

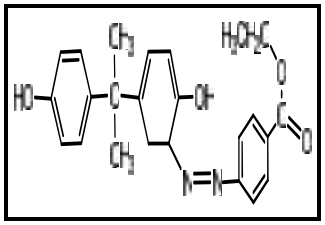
NO.	Monomer	Colore	Yield%
1	AZO 2	Black	93
2	AZO 3	High orange	41
3	AZO 4	High brown	48



Scheme 2.6 *structure of AZO2*



Scheme 2.7 *structure of AZO3*

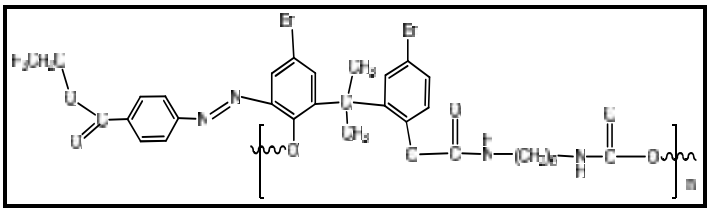


Scheme 2.8 *structure of AZO4*

2.5 Synthesis of Azo-polyurethane:

2.5.1 synthesis of azo polyurethane from (AZO1)(PU AZO1):

In a three-neck round-bottom flask, a solution of the above monomers azo (3.01 g , 10 mmol), in N ,N- dimethylformamide (DMF, 30 mL) was added dropwise to a solution of 1,6-hexamethylenediisocyanate (HDI, 1.68 g , 10 mmol), in dry DMF (20 mL), under a dry nitrogen atmosphere at room temperature. Then the reaction mixture was stirred at 80 ºC for 8 h. The solution was poured into cold methanol to precipitate the polymer. The solid product was collected by filtration, washed with methanol , and dried under *vacuum* at room temperature [17] , yielded 75% Black crystals.

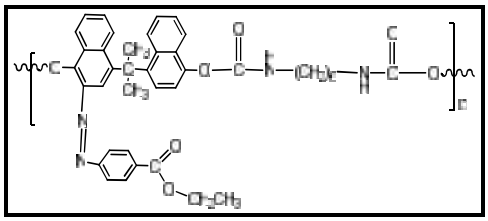


Scheme2.9 *Structure of PU AZO1*

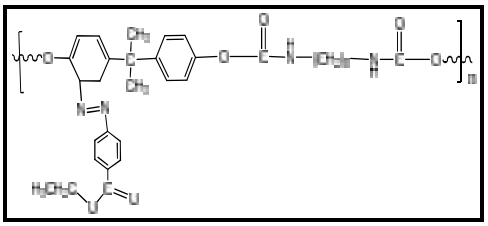
The other compounds were prepared by the same procedure as above using AZO (2-4) are shown in table (2.3) .

Table 2.3 **:** *synthesis and details data of prepared azo polyurethane:*

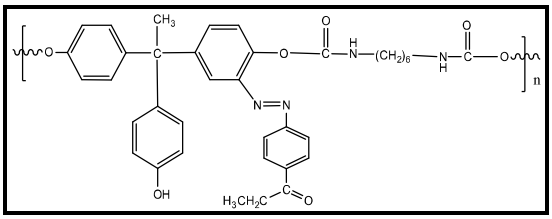
NO.	Polymer	Color	Yield%
1	PU2	Black	92
2	PU3	High brown	89
3	PU4	Black	88



Scheme2.10 *Structure of PU2*



Scheme2.11 *Structure of PU AZO3*



Scheme2.12 *Structure of PU AZO4*

2.6 UV-visible spectrophotometric analysis

The polyurethane azo solutions were prepared by dissolving in (10ml) DMF as solvent .U.V-Vis spectral absorption bands were obtained using Shimadzu, Japan UV–vis spectrometer at room temperature using DMF as solvent in quartz photochemical cell (1cm) length bath .

A known concentration of polymer was introduced in to the cell and The absorption spectra were recorded between 200-700 nm [18] .

2.7 preparation of calibration curve

A standard curve for Benzocaine was constructed In the range of (0.001 to 0.04) $g.L^{-1}$. the solution were prepared by stock solution using deionized water as a solvent. The absorbance of the resulting solution was measured at λ max 293 nm using distilled water as a blank . The standard curve was plotted in the range of (0.001-0.04)$g.L^{-1}$,and the regression analysis shows the liner relationship between the concentration of the Benzocaine and the absorbance [19].

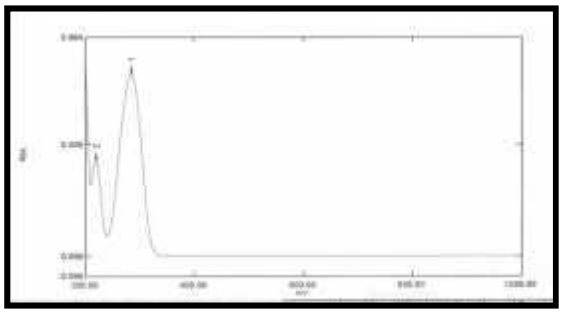


Figure 2.1 *UV spectra of Benzocaine*

2.8 Preparation of Phosphate Buffer (PB) Solution

Phosphate buffer solution (PB) was prepared as previously described as following : a pH 7.8 buffer solution was prepared by dissolving sodium phosphate dibasic (21.7 g) and

potassium phosphate monobasic (2.6 g) and the pH was adjusted to 7.8 using 0.1 N sodium hydroxide. A pH 4.0 buffer solution was prepared by dissolving sodium phosphate dibasic (21.7 g) and potassium phosphate monobasic (2.6 g) in deionized water (1 L) [17] .

2.9 Drug (Benzocaine) release

in deionized water (1 L)

The release of Benzocaine was followed as a function of time using a UV spectrophotometer at λ max=293nm . the procedure was used as follow: polymer (10mg) was placed in aqueous buffer solution of pH= 7.8 and pH=4 at 37 ^{0}C (50 mL) . At specific intervals , aliquots of the buffer (3 mL) were collected for analysis each 5 hrs. for 45hrs [17]

2.10 Determination of the Total Benzocaine Content

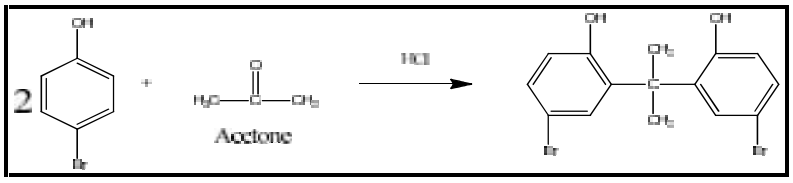
A sample of polymers (10 ppm) was suspended in PB (30 mL, pH 7.8 and pH 4). The mixture was heated at 60 ºC, and the amount of Benzocaine released was determined using UV spectrophotometry at λ max = 293 nm [17]

RESULT AND DISCUSSION :

3.1 Synthesis of Monomers :

3.1.1 Synthesis and Characterization of (M1):

The (M1) was synthesized from the reaction of 4-bromophenol with acetone in presence of HCl as catalyst by condensation for 6 hrs at 60^{O}C. this reaction was shown in Scheme (3.1). The FTIR spectra of (M1) shows the absorption band of C=C Aromatic at 1590cm-1, C-H Aromatic at 3150 cm-1, OH phenolic at 3300 cm-1, C-H alkane at 2900 cm-1, and C-Br at 660 cm-1 [20].

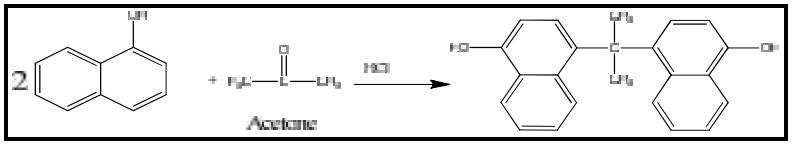


Scheme 3.1 *synthesis of M1*

3.1.2 Synthesis and Characterization of (M2)

The (M2) was synthesized from the reaction of 1-Naphthole with acetone in presence of HCl as catalyst by condensation for 6 hrs at 60^{O}C. this reaction was shown in Scheme (3.2). The FTIR spectra of (M2) shows the

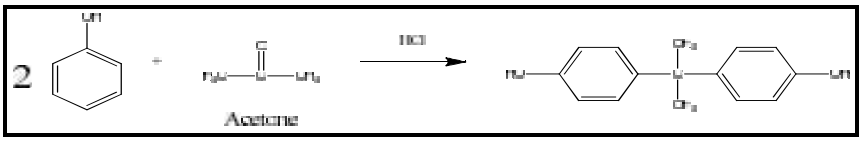
absorption band of C=C Aromatic at 1550cm-1, C-H Aromatic at 3100 cm-1 , OH phenolic at 3300 cm-1 , and C-H alkane at 2900 cm-1 [20] .



Scheme 3.2 *synthesis of M2*

3.1.3 Synthesis and Characterization of (M3):

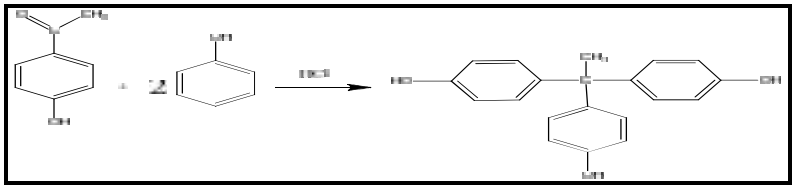
The (M3) was synthesized from the reaction of phenol with acetone in presence of HCl as catalyst by condensation for 6 hrs at 60^{O}C. this reaction was shown in Scheme (3.3). The FTIR spectra of (M3) shows the absorption band of C=C Aromatic at 1596cm^{-1}, C-H Aromatic at 3178 cm^{-1}, OH phenolic at 3255 cm^{-1} and C-H alkane at 2970 cm^{-1} [20].



Scheme 3.3 *synthesis of M3*

3.1.4 Synthesis and Characterization of (M4):

The (M4) was synthesized from reaction of p-hydroxy acetophenon with phenol in presence of HCl as catalyst by condensation for 6 hrs at 60^{o}C. the reaction was shown in Scheme (3.4) . The FTIR spectra of (M4) shows the absorption band of C=C Aromatic at 1573 cm^{-1}, OH phenolic at 3309 cm^{-1} , C=C alkene at 1666 cm^{-1}, and C=C-H alkene at 2993, 2800 cm^{-1} [20].



Scheme 3.4 *synthesis of M4*

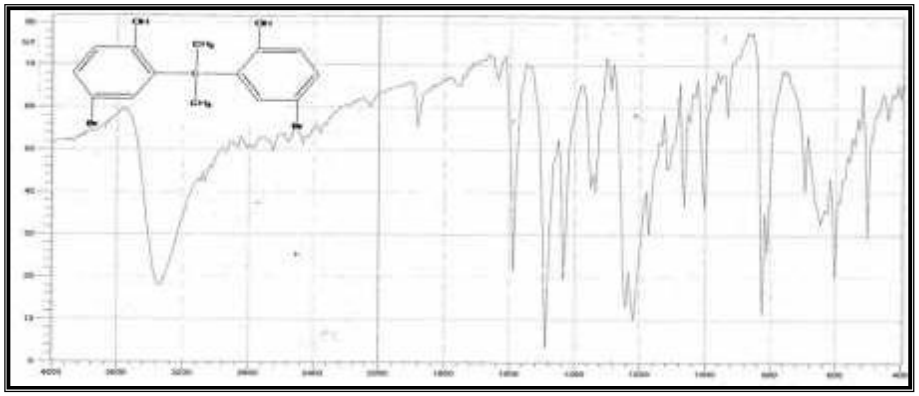


Figure 3.1 FTIR spectra of(M1)

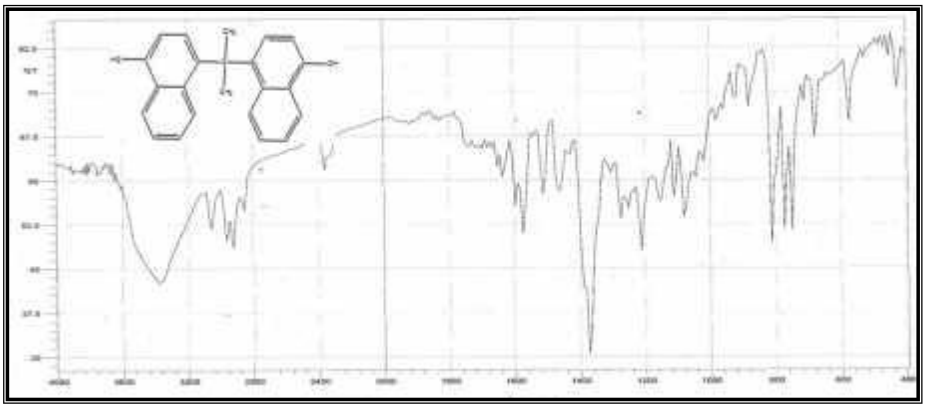


Figure 3.2 FTIR spectra of(M2)

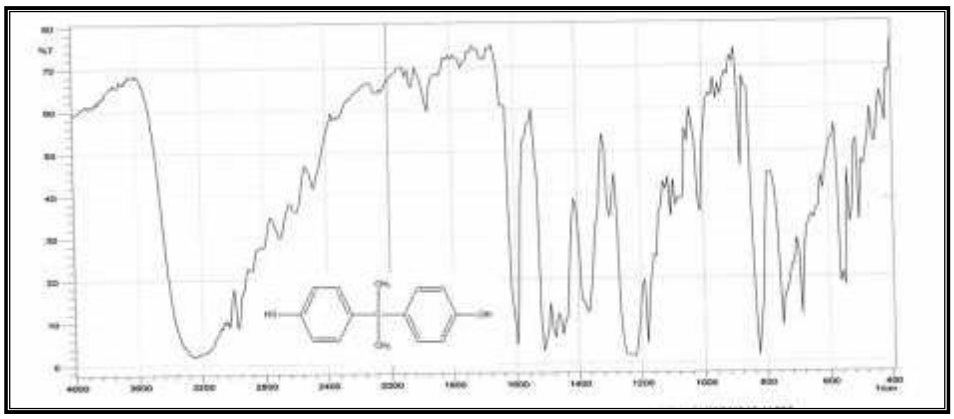


Figure 3.3 FTIR spectra of(M3)

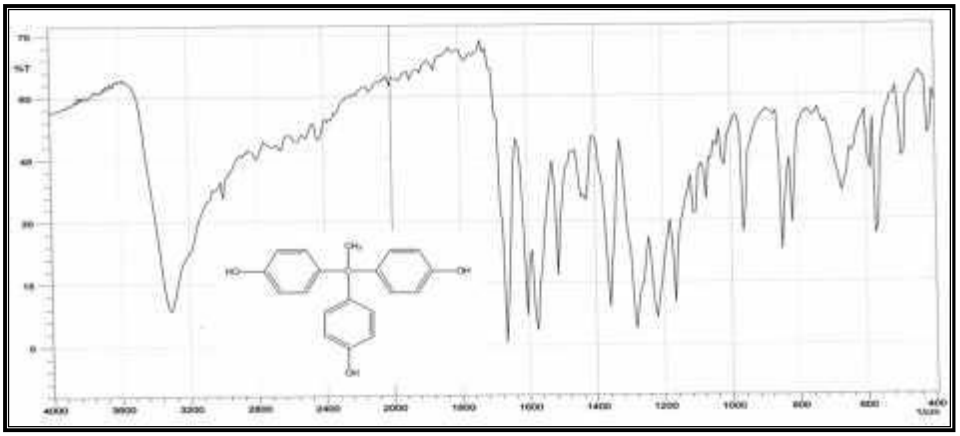


Figure 3.4 FTIR spectra of(M4)

3.2 Syntheses and Characterization of Azo monomers

3.2.1 Synthesis and Characterization of p-[3-bromo-6-hydroxy phenyl azo]ethyl-p-amino benzoate (AZO1):

The p-[3-bromo-6-hydroxy phenyl azo]ethyl-p-amino benzoat were prepared by the coupling of AZO1 with the diazonium salt of Benzocaine, for 1 hr. at (0-5^{o}C) in ice bath .This reaction was shown in scheme (3.5) . The FTIR Spectrum of AZO2 show the following peaks :

3402 cm^{-1} (-OH phenolic stretching vibration) ; str. 3070, 3155 cm^{-1} (C-H vibration) ; 1697 cm^{-1} (ester group C=O) ; 1488 , 1542 cm^{-1} (azo group N=N) ; 1365, 1388 cm^{-1} (C-O-C phenol) ; 516 cm^{-1} (C-Br) and 1589 cm^{-1} (C=C phenol) [21].

3.2.2 Synthesis and Characterization of ethyl4-((5-hydroxy-8-(2-(4-hydroxy naphthalen-1 -yl) propan-2-yl) naphthalen-4-yl)diazenyl)benzoate (AZO2):

The ethyl4-((5-hydroxy-8-(2-(4-hydroxy naphthalen-1 -yl) propan-2-yl) naphthalen-4-yl)diazenyl)benzoate were prepared by the coupling of AZO2 with the diazonium salt of Benzocaine, for 1 hr. at (0-5^{o}C) in ice bath .This reaction was shown in scheme (3.5) . The FTIR Spectrum of AZO2 show the following peaks :

3379 cm^{-1} (-OH phenolic stretching vibration) ; str. 2970, 2927 cm^{-1} (C-H vibration) ; 1712 cm^{-1} (ester group C=O) ; 1427 , 1473 cm^{-1} (azo group N=N) ;; 1373 cm^{-1} (C-N) and 1666 cm^{-1} (C=C phenol) .

3.2.3 Synthesis and Characterization of p-[2-hydroxy-5-prppanyl-4-hydroxy phenyl azo]ethyl-p-aminobenzoat (AZO3):

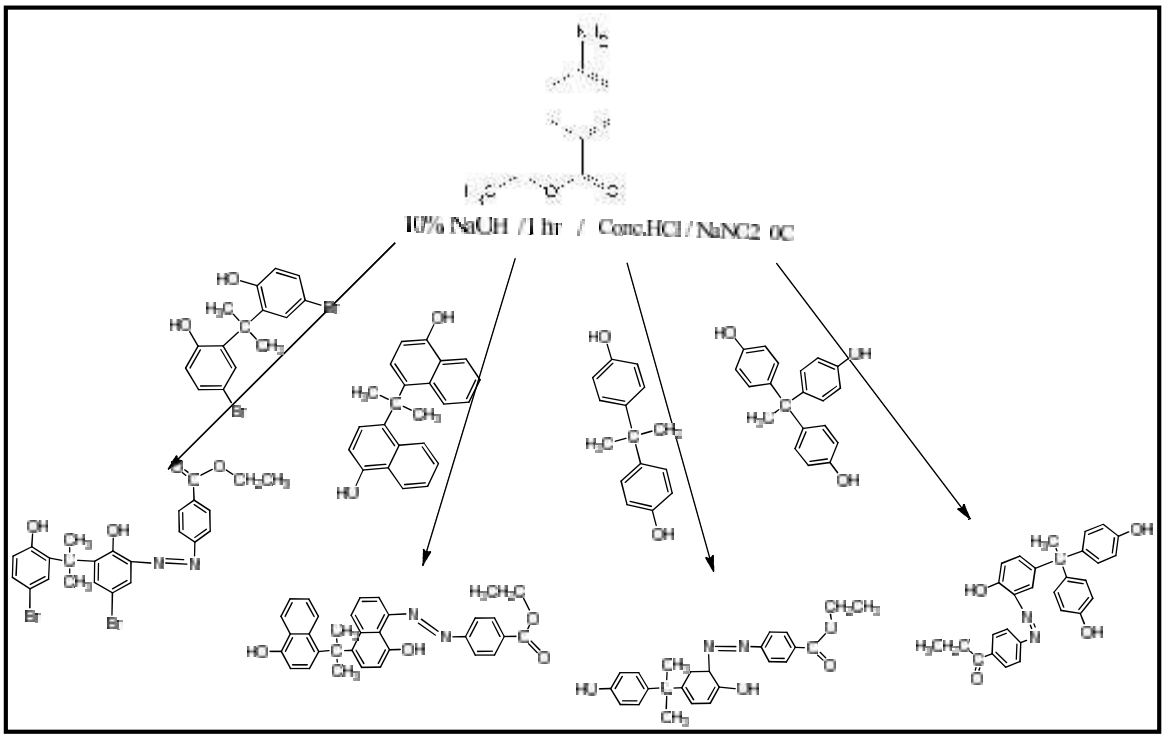
The p-[2-hydroxy-5-prppanyl-4-hydroxy phenyl azo]ethyl-p-aminobenzoat were prepared by the coupling of AZO3 with the diazonium salt of Benzocaine , for 1 hr. at (0-5^{o}C) in ice bath .This reaction was shown in scheme (3.5) . The FTIR Spectrum of AZO6 show the following peaks :

3394 cm^{-1} (-OH phenolic stretching vibration) ; str. 2970,2931, cm^{-1} (C-H vibration) ; 1743 cm^{-1} (ester group C=O) ; 1427 , 1465 cm^{-1} (azo group N=N) ; ; 1365 cm^{-1} (C-N) and 1650 cm^{-1} (C=C phenol) .

3.2.4 Synthesis and Characterization of 1-(4-((5-(1,1-bis(4-hydroxy phenyl) ethyl) -2-hydroxyphenyl)diazenyl)phenyl)propan-1-one (AZO4):

The 1-(4-((5-(1,1-bis(4-hydroxy phenyl) ethyl) -2-hydroxy phenyl) diazenyl) phenyl) propan -1-one were prepared by the coupling of AZO4 with the diazonium salt of Benzocaine , for 1 hr. at (0-5°C) in ice bath .This reaction was shown in scheme (3.5). The FTIR Spectrum of AZO4 show the following peaks :

3332 cm^{-1} (-OH phenolic stretching vibration) ; str. 2985 cm^{-1} (C-H vibration) ; 1712 cm^{-1} (ester group C=O) ; 1427 , 1488 cm^{-1} (azo group N=N) ; 1365 cm^{-1} (C-N) and 1604 cm^{-1} (C=C phenol) .



Scheme 3.5 *synthesis of AZO (1-4)*

3.3 Syntheses and Characterization of Azo polyurethane

3.3.1 Synthesis and Characterization of (PU1):

Biodegradable azo-containing polyurethanes (PU AZO1) were prepared by polycondensation of HDI with AZO1 at 80 ºC for 8 hrs. under a dry nitrogen atmosphere .This reaction was shown in scheme (3.6) . H^1NMR (400 MHz, DMSO) :δ 1.33-1.38(m,3H,CH3) ; δ 2.50 (s,1H, DMSO) ; δ 2.89 (m,3H,CH_2-NH_2) ; δ 3.31 (s,1H, H_2O) ; δ 4.33 (s,1H,OCH_2-OH) ; δ 7.31 (s ,1H, pH-O) ; δ 8.11 (s,1H, N-H) [22]

. The FTIR Spectrum of (PU AZO1) 3325 cm^{-1} (-OH phenolic stretching vibration) ;3580 (N-H stretching vibration) ; str. 2931, 2854 cm^{-1} (C-H vibration of polymer backbone) ; 1712 cm^{-1} (ester group C=O) ; 1481 ,1566 cm^{-1} (azo group N=N) ; 1334, 1380 cm^{-1} (C-N) and 1666 cm^{-1} (C=C phenol) ; 2252 cm^{-1} isocyanides and 632 (C-Br vibration).

shows the following peaks :

3.3.2 Synthesis and Characterization of (PU2):

Biodegradable azo-containing polyurethanes (PU AZO2) were prepared by polycondensation of HDI with AZO2 at 80 ºC for 8 hrs. under a dry nitrogen atmosphere .This reaction was shown in scheme (3.6) . H^1NMR (400MHz,DMSO): δ1.37(m,3H,CH_3) δ1.68(s,1H,CH_2CH_3) ; δ 2.5 (s,1H, DMSO) ; δ 2.93 (d,2H, OCH_2) ; δ 3.32 (s,1H,H_2O) ; δ 4.33 (s, H, CH_2-O) ; δ 7.74- 7.75 (m, 3H, phenyl) ; δ 8.62 (s,1H, N-H). The FTIR Spectrum of (PU AZO2) shows the following peaks : 3332 cm^{-1} (-OH phenolic stretching vibration) ;3550 (N-H stretching vibration) ; str. 2931, 2854 cm^{-1} (C-H vibration of polymer backbone) ; 1712 cm^{-1} (ester group C=O) ; 1458 , 1473 cm^{-1} (azo group N=N) ; 1373 cm^{-1} (C-N) and 1666 cm^{-1} (C=C phenol) ; 2229 cm^{-1} isocyanides

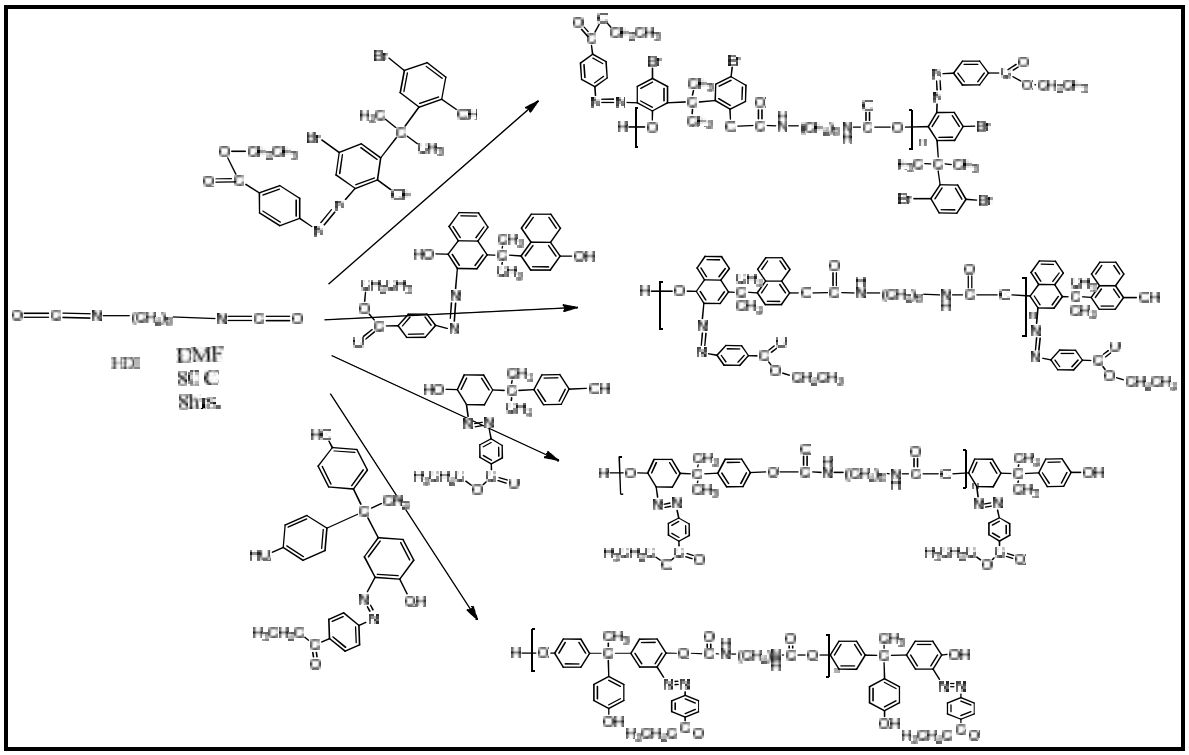
3.3.3 Synthesis and Characterization of (PU3):

Biodegradable azo-containing polyurethanes (PU AZO3); were prepared by polycondensation of HDI with AZO3, at 80 ºC for 8 hrs. under a dry nitrogen atmosphere .This reaction was shown in scheme (3.6) . H^1NMR (400 MHz, DMSO) : δ1.61 (m,4H, CH_3); δ 2.5 (s,1H, DMSO) ; δ 2.95(s,1H,OCH2-NH) ; δ 3.32(s,1H,H_2O) ; δ 4.36 (s,1H,OCH_2) ; δ 6.95-7.34 (s,2H,phO-C=O) ; δ 7.84-7.87 (m,3H, O-Ph) and δ 9.11(s,1H,NH) . The FTIR Spectrum of (PU AZO3) shows the following peaks :

3325 cm^{-1} (-OH phenolic stretching vibration) ;3533 (N-H stretching vibration) ; str. 2931, 2854 cm^{-1} (C-H vibration of polymer backbone) ; 1704 cm^{-1} (ester group C=O) ; 1458 , 1496 cm^{-1} (azo group N=N) ; 1342, 1365 cm^{-1} (C-N) and 1573 cm^{-1} (C=C phenol);2268cm^{-1}isocyanides.

3.3.4 Synthesis and Characterization of (PU4):

Biodegradable azo-containing polyurethanes (PU AZO4); were prepared by polycondensation of HDI with AZO4, at 80 ºC for 8 hrs. under a dry nitrogen atmosphere .This reaction was shown in scheme (3.6). The FTIR Spectrum of (PU AZO4) show the following peaks: 3332 cm^{-1} (-OH phenolic stretching vibration) ;3550 (N-H stretching vibration) ; str. 2931, 2854 cm^{-1} (C-H vibration of polymer backbone); 1720 cm^{-1} (ester group C=O) ; 1458, 1481 cm^{-1} (azo group N=N) ; 1404 cm^{-1} (C-N) and 1620 cm^{-1} (C=C phenol) and 2534 cm^{-1} isocyanides



Scheme 3.6 *synthesis of polyurethane AZO(1-4)*

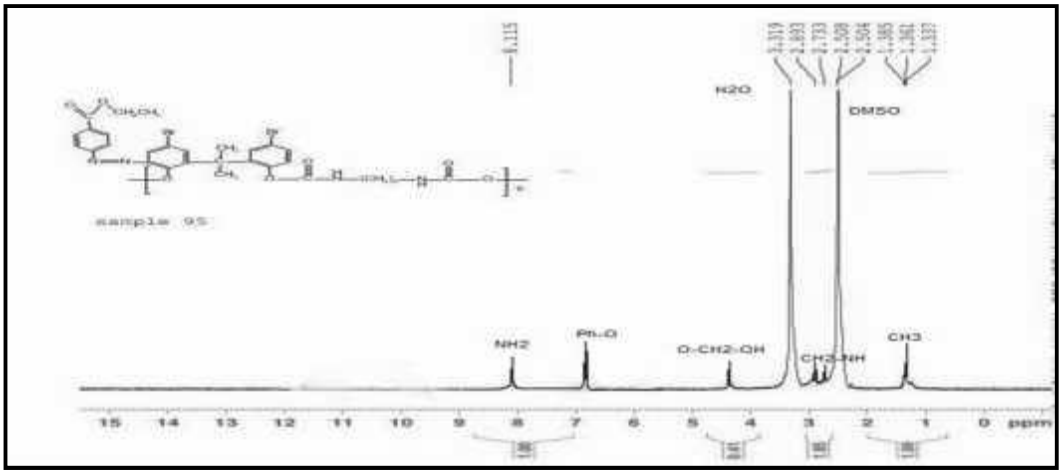


Figure 3.5 *H1NMR spectra of PU1*

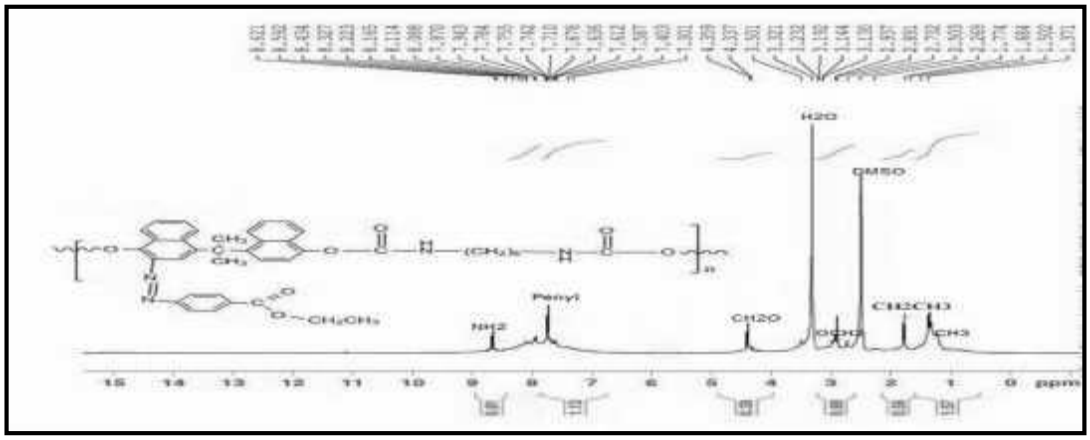


Figure 3.6 *H1NMR spectra of PU2*

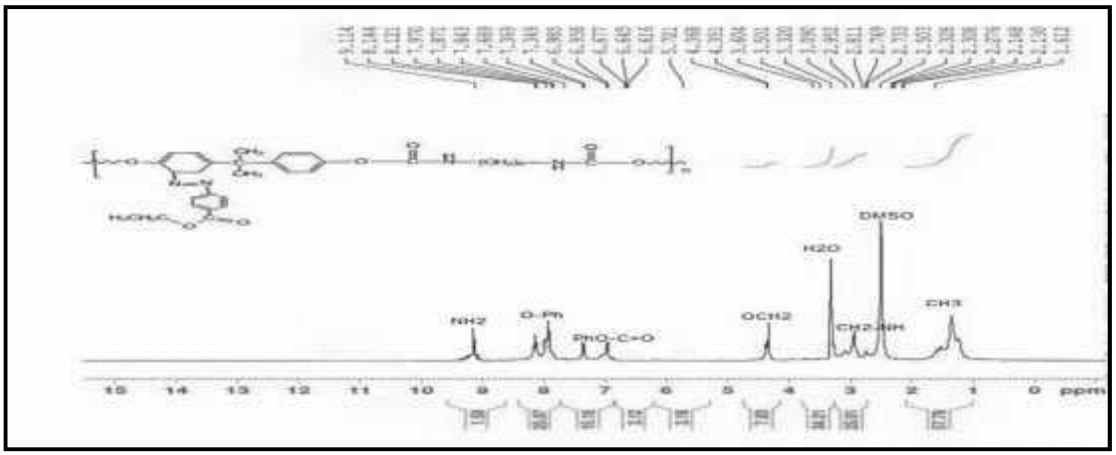


Figure 3.7 *H1NMR spectra of PU3*

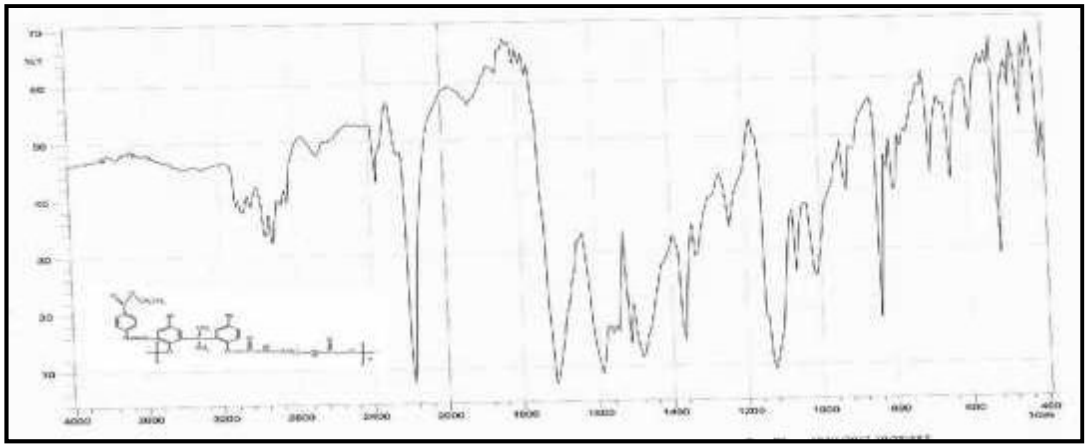


Figure 3.8 *FTIR spectra of PU1*

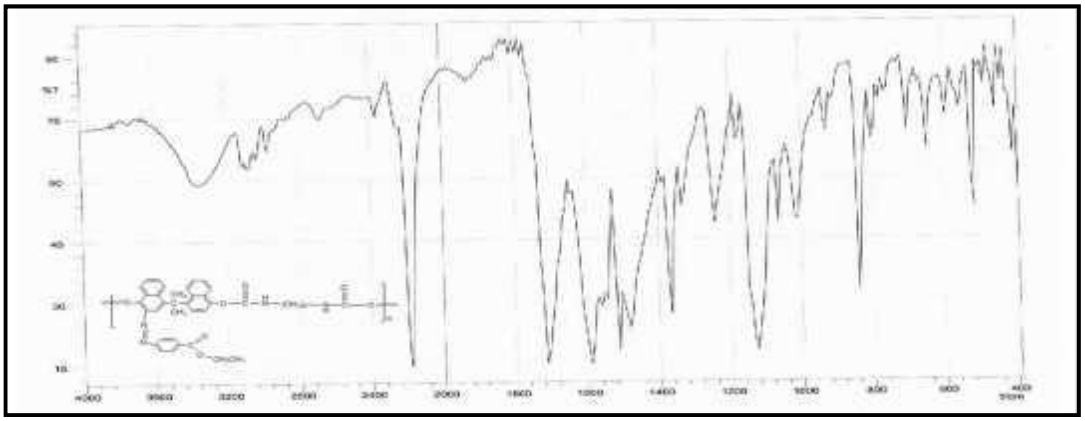


Figure 3.9 *FTIR spectra of PU2*

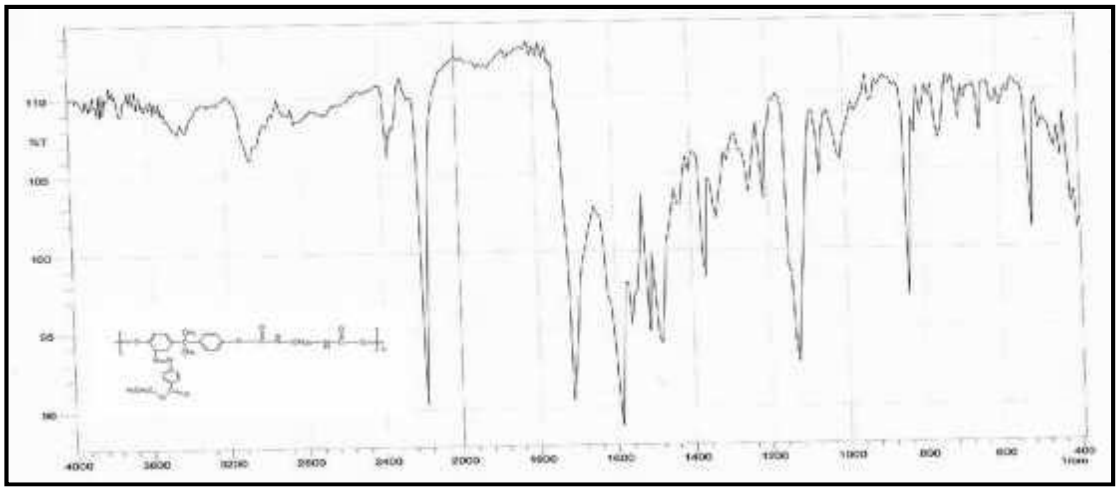


Figure 3.10 *FTIR spectra of PU3*

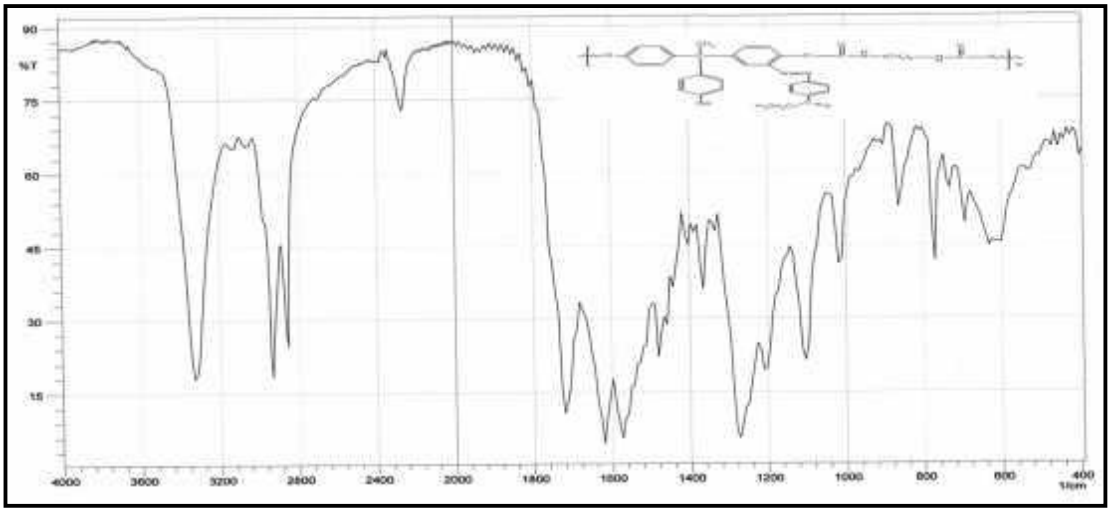


Figure 3.11 *FTIR spectra of PU4*

3.4 The spectra of synthesized Polyurethane azo:

3.4.1 The Ultraviolet spectra of (PUAZO1) as in figure (3. 4), shows the following peaks :

1) $\pi \rightarrow \pi^*$ at 499 mμ to carbonyl group , phenyl ring and amine.
2) $n \rightarrow \pi^*$ at 335 mμ to azo compound [18].

3.4.2 The Ultraviolet spectra of (PU AZO2) as in figure (3.5) , shows the following peaks :

1) $\pi \rightarrow \pi^*$ at 251 mμ to carbonyl group , phenyl ring .
2) $n \rightarrow \pi^*$ at 491 mμ to azo compound [18].

3.4.3 The Ultraviolet spectra of (PU AZO3), as in figure (3.6) , shows the following peaks :

1) $\pi \rightarrow \pi^*$ at 363 mμ to carbonyl group .
2) $n \rightarrow \pi^*$ at 421 mμ to charge transfer on aromatic ring [18] .

3.4.4 The Ultraviolet spectra of (PU AZO4), as in figure (3.7) , shows the following peaks :

1) $n \rightarrow \pi^*$ at 480 mμ to carbonyl group .
2) $\pi \rightarrow \pi^*$ at 251 mμ to azo compound [18].

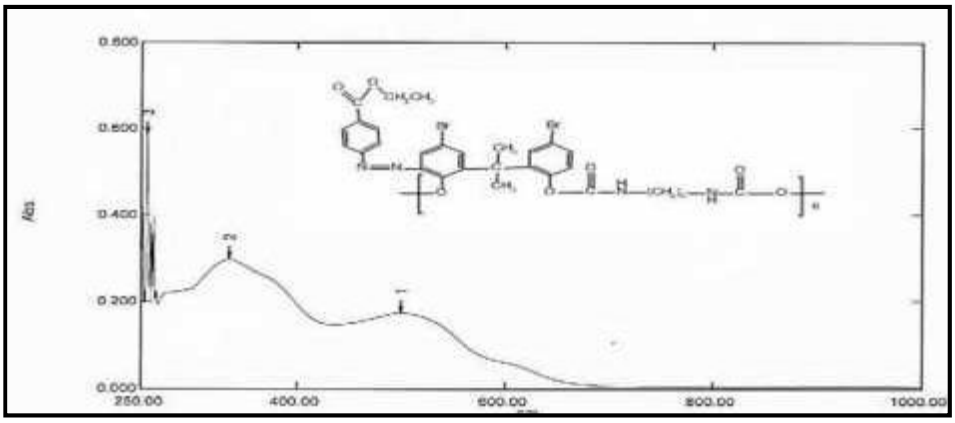


Figure 3.4 *U.V-Visible spectra of PU 1*

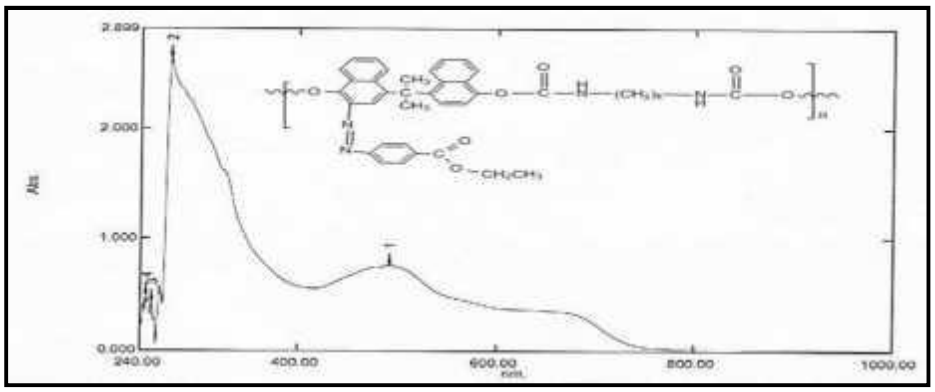


Figure 3.5 *U.V-Visible spectra of PU 2*

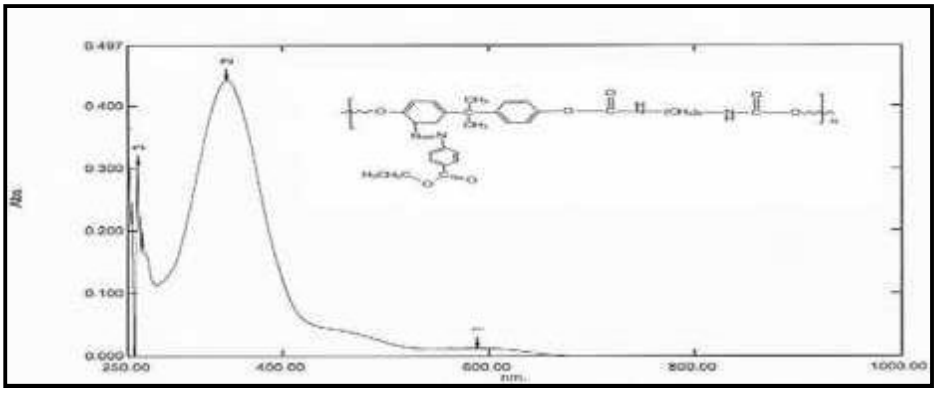


Figure 3.5 *U.V-Visible spectra of PU3*

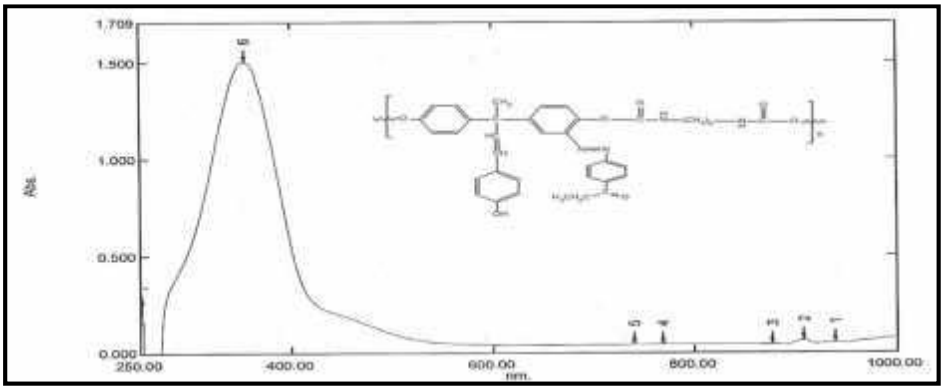


Figure 3.6 *U.V-Visible spectra of PU4*

3.5 Drug release

3.5.1 Method of analysis (Benzocaine)

Accuracy and precision for the method of analysis is very important in drug related studies. Therefore, initially UV-visible method for the analysis of (Benzocaine) was developed, to analyze these components in drug release medium.

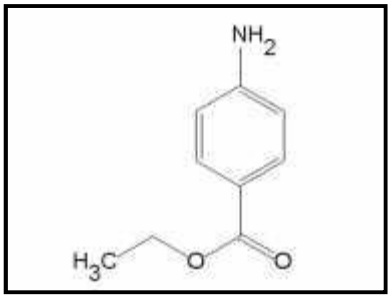
Benzocaine supplied as white to pale yellow crystalline powder. Benzocaine is sparingly soluble in water; it is more soluble in dilute acids and very soluble in ethanol, chloroformand ethyl ether. The melting point of benzocaine is 88-90 °C [23]. Since the solubility of basic compounds in water is pH dependent, this property was determined at pH 7.4. At this pH, BZC has its lowest solubility because the undissociated molecular compound is predominant [24].

Benzocaine (BZC) used as topical drug on the skin and mucous membranes. These applications are based on its physicochemical properties, especially its aqueous solubility and partition coefficient, that is, this compound has low aqueous solubility and a high octanol /water partition coefficient; that is,BZC is a lipophilic drug [25].

Most local anesthetics structures have amino-ester or an amino-amide group which are linked to hydrophilic (secondary or tertiary amine) and to hydrophobic group (aromatics) on the other sid. The ester can be hydrolysed in plasma by the enzyme pseudocholinesterase into para-aminobenzoic acid. Amide is stable for longer acting and more systemic distribution [26].

when (Benzocaine) is administered orally, a large amount of the drug is absorbed from the upper gastrointestinal tract (GIT), and causes systemic side effects. Therefore, it is preferable to deliver the drug site-specifically to the target [27].

Formal name : Ethyl p-aminobenzoate; 4-Aminobenzoic acid ethyl ester Molecular Formula : C9H11NO2 .Molecular Weight : 165.2 g/mol [28].



Scheme 3.7 *structure of Benzocaine*

3.6 UV-visible spectrophotometric analysis

Generally, molecules that absorb in the UV region at a certain wavelength will contain suitable chromophore. The spectrum consisting , of a plot absorbance , percent transmittance as a function of wavelength is automatically obtained using a scanning spectrophotometer. The absorptivity or molar absorptivity of many substance at specified wavelength is listed in various tables in literature

.

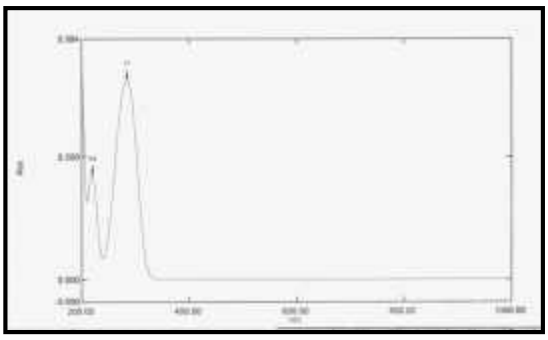


Figure 3.7 *U.V-Visible spectra of Benzocaine*

The molar absorptivity of polymers has been calculated by two methods:

1. From a single absorptive measurement of a solution of known concentration by direct substitution in Beer's law.
2. By plotting of absorbance as a function of concentration. If a linear straight line is obtained, the slope of the straight line can be used to calculate the molar absorptivity.

The first procedure is recommended. Concentration based upon single measurement are not statistically sound, because it is easy to make measurement error that can not be detected with single measurement. So the 2nd more precise route for calculation concentration is followed [29].

3.7 Calibration curve for (Benzocaine)

A standard curve was constructed by varying the amount of (Benzocaine) in the range of (0.001 to 0.04) g. L^{-1}. The solutions was prepared from stock solution using distilled water as solvent. The absorbance of the solution was measured at λmax 293.0

nm against solvent. The regression analysis shows the linear relationship between the concentration of the (Benzocaine) and the absorbance, the plot is shown in figure (3.8). The results indicate that the method is quite suitable for the analysis of the drug in this concentration range [17].

Table (3.1) *The absorbance of (Benzocaine) in various concentration :*

CONC./M	ABS.
0	*0*
$6.05*10^{-4}$	*0.076*
$1.81*10^{-5}$	*0.443*
$3.02*10^{-5}$	*0.5*
$4.23*10^{-5}$	*0.801*
$6.05*10^{-5}$	*1.034*
$7.86*10^{-5}$	*1.40*
$9.08*10^{-5}$	*1.502*
$1.02*10^{-4}$	*1.675*
$1.21*10^{-4}$	*1.866*

These data are represented in figure (3.8)

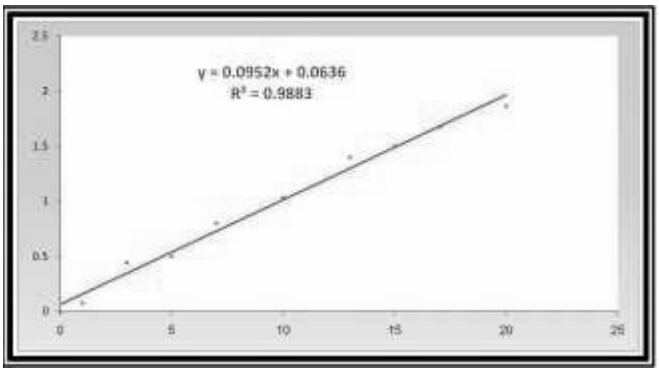


Figure (3.8)
The *working calibration curve for the data of (Benzocaine), (the absorbance in 1 cm cell) at λmax 293.0 nm*

3.8 In Vitro Drug release :

To study potential applications of these polyurethanes containing azo derivatives of Benzocaine as a pharmaceutically active compound, in vitro degradation of the polymers was studied in physiological conditions. Hydrolytic degradation products of polymers upon cleavage of urethane bonds was measured by the appearance of AZO monomer in degradation media using UV. Degradation of Poly urethane -AZO at 37°C under acidic (pH 4), neutral (pH 7.8) conditions were performed over 45 hrs [30].

It has been widely demonstrated that the side chain hydrolysis of drug pendant polymers depends on the strength and chemical nature of the polymer structure and the surrounding condition . The hydrolysis of a linkage is also dependent on its distance from the polymer backbone [31].

The length and hydrophilicity of the spacer unit between the drug and polymer chain can effect the release rate . In order to study the possible applications of this type of polymer as a pharmacologically active compound, we have studied from a chemical point of view the hydrolysis of the synthesized polymer in buffer solution with pH=4 and pH=7.8 at 37 ^{o}C [32].

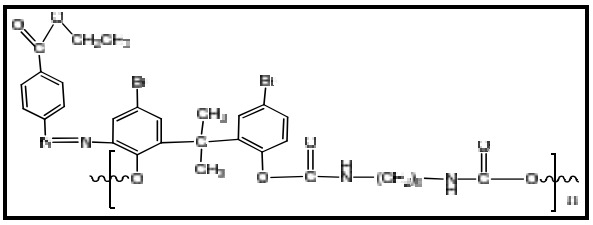
The rate of Benzocaine released from the polymers was measured at pH 7.8 and 4 at 37 ºC. The rate of release show dependence on the pH of the medium and on the polymer microstructure. Generally, it was found that the rate of release of Benzocaine increased as the pH increased in alkaline medium (i.e., within the colon pH) [17].

At pH 7.8, polymer 1 released 82% ,polymer 2 released 73% , polymer 3 released 78%, polymer 4 released 79% of Benzocaine respectively, after 45 hours at 37 ºC.

At pH 4, polymer 1 released 76% ,polymer 2 released 49%, polymer 3 released 60%, polymer 4 released 70% of Benzocaine respectively, after 45 hours at 37 ºC.

This means that more than 90% of the drug will pass to the colon without hydrolysis. Therefore, this indicated that the system will be useful for colon drug targeting. Generally, as the number of polar groups on the monomeric units along the polymer chain increased, the hydrophilicity of the polymer increased, and the rate of hydrolysis increased, and consequently, the amount of Benzocaine released increased [17]

As shown in table (3.2-3.5), the degradation of PU-AZO at 37°C under acidic (pH 4) conditions was slowest. At pH 7.8, AZO was released gradually and the polymer degradation was complete by 45 hrs. Base catalyzed hydrolysis of the urethane bonds of the polymer is much more rapid under basic conditions than acid conditions

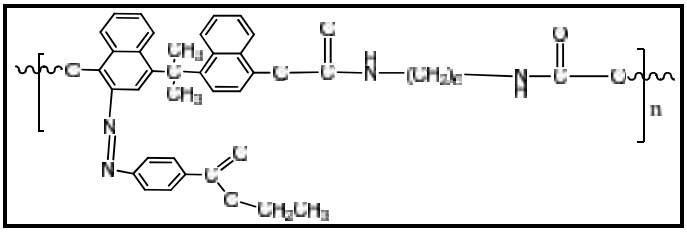


Scheme 3.8 *Polyurethane AZO1*

	pH=7.8				*pH=4*		
No.	***Abs.***	***Conc.***	***Drug release%***	***Time***	***Abs.***	***Conc.***	***Drug release%***
1	*0.066*	*0.028*	*28*	*1*	*0.066*	*0.03*	*30*
2	*0.067*	*0.036*	*36*	*5*	*0.067*	*0.037*	*37*
3	*0.067*	*0.04*	*40*	*10*	*0.067*	*0.04*	*40*
4	*0.068*	*0.047*	*47*	*15*	*0.068*	*0.048*	*48*
5	*0.069*	*0.058*	*58*	*20*	*0.068*	*0.051*	*51*
6	*0.07*	*0.062*	*62*	*25*	*0.069*	*0.055*	*55*
7	*0.071*	*0.075*	*75*	*30*	*0.069*	*0.06*	*60*

8	0.071	0.079	79	35	0.07	0.064	64
9	0.071	0.082	82	40	0.071	0.076	76
10	0.071	0.082	82	45	0.071	0.076	76

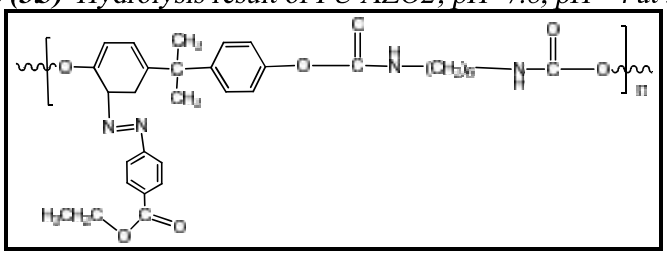
Table (3.2) Hydrolysis result of PU AZO1, pH=7.8, pH =4 at 37 ^{o}C



Scheme 3.9 Polyurethane AZO2

	pH=7.8				pH=4		
No.	**Abs.**	**Conc.**	**Drug release%**	**Time**	**Abs.**	**Conc.**	**Drug release%**
1	0.065	0.018	18	1	0.064	0.014	14
2	0.066	0.029	29	5	0.065	0.018	18
3	0.068	0.043	43	10	0.065	0.021	21
4	0.069	0.052	52	15	0.066	0.026	26
5	0.069	0.058	58	20	0.066	0.03	30
6	0.07	0.065	65	25	0.066	0.035	35
7	0.07	0.069	69	30	0.067	0.041	41
8	0.07	0.071	71	35	0.067	0.044	44
9	0.071	0.073	73	40	0.068	0.049	49
10	0.071	0.073	73	45	0.068	0.049	49

Table (3.3) Hydrolysis result of PU AZO2, pH=7.8, pH =4 at 37 ^{o}C

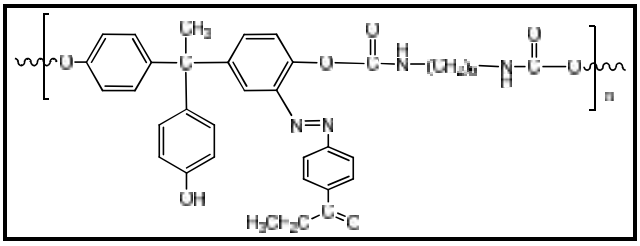


Scheme 3.10 Polyurethane AZO3

	pH=7.8				pH=4		
No.	**Abs.**	**Conc.**	**Drug release%**	**Time**	**Abs.**	**Conc.**	**Drug release%**
1	0.065	0.017	17	1	0.065	0.012	12
2	0.066	0.027	27	5	0.065	0.014	14
3	0.068	0.048	48	10	0.066	0.026	26
4	0.069	0.053	53	15	0.066	0.029	29
5	0.069	0.06	60	20	0.067	0.031	31
6	0.07	0.064	64	25	0.067	0.036	36
7	0.071	0.073	73	30	0.068	0.044	44
8	0.071	0.077	77	35	0.069	0.054	54
9	0.071	0.078	78	40	0.069	0.06	60

10	0.071	0.078	78	45	0.069	0.06	60

Table (3.4) *Hydrolysis result of PU AZO3, pH= 7.8, pH =4 at 37 oC*



Scheme 3.11 *Polyurethane AZO4*

	pH=7.8				pH=4		
No.	**Abs.**	**Conc.**	**Drug release%**	**Time**	**Abs.**	**Conc.**	**Drug release%**
1	0.067	0.036	36	1	0.066	0.029	29
2	0.068	0.041	41	5	0.067	0.036	36
3	0.068	0.048	48	10	0.067	0.038	38
4	0.069	0.059	59	15	0.068	0.047	47
5	0.07	0.066	66	20	0.069	0.056	56
6	0.07	0.069	69	25	0.07	0.063	63
7	0.07	0.071	71	30	0.07	0.067	67
8	0.071	0.076	76	35	0.07	0.069	69
9	0.071	0.079	79	40	0.07	0.07	70
10	0.071	0.079	79	45	0.07	0.07	70

Table (3.5) *Hydrolysis result of PU AZO4, pH= 7.8, pH =4 at 37 oC*

3.10 Determination of the Total Benzocaine Content

To determine the total content of the polymers derived from Benzocaine, it was necessary to carry out a fast hydrolysis of the drug from the polymers. Heating the polymeric systems at 60 ºC in phosphate buffer with pH 7.8 and pH 4 hydrolyzed the drug-polymer bond. The fast hydrolysis of Benzocaine in buffer solution led to total Benzocaine contents . At pH 7.8 at 37 ºC, the total amounts of Benzocaine released after two days were found to be 85%, 80%, 84% ,83% for polymers (PU1-PU4) respectively. At pH=4 and 37 ºC temperature, the total amounts of Benzocaine released after two days were found to be 77%, 70%, 69% , 79% for polymers (PU1-PU4) respectively

.

Table (3.6) *The total amount released of Benzocaine at pH=7.8, pH =4 at 37 °C*

	pH=7.8				pH=4		
No.	**Abs.**	**Conc.**	**Drug release%**	**polymer**	**Abs.**	**Conc.**	**Drug release%**
1	0.072	0.085	85	**PU1**	0.071	0.077	77
2	0.071	0.08	80	**PU2**	0.07	0.064	64
3	0.072	0.084	84	**PU3**	0.07	0.067	67
4	0.072	0.083	83	**PU4**	0.071	0.075	75

Conclusion

Polyurethanes containing azo-Benzocaine in the main chain were synthesized by incorporating bioactive Benzocaine molecules into the polymer backbone and characterized as polymeric biodegradable prodrug systems.

The rate of degradation was mainly determined by the number of azo bonds and not by the type of azo derivative . Hydrolytic degradation is pH-dependant and is slow in acidic conditions. pH= 7.8 > pH= 4.0

The rate of release depends on the pH of the medium and on the polymer structure. It was found that the rate of release of Benzocaine increased as the pH increased in alkaline medium (i .e. within the colon pH). the number of polar groups on monomeric units along the polymer chain increased, the hydrophilicity of the polymer increased, and the rate of hydrolysis increased and consequently, the amount of Benzocaine released increased. Due to the pH-dependent degradation of the polymers,

these polyurethane derivatives of Benzocaine could be effective in treating gastrointestinal disease where it is important to release the drug at the desired site, the colon (basic conditions), rather than the upper intestine or stomach which are neutral or acidic, respectively.

References

1. L. García, M. Rosa Aguilar, and J. San Román **(2010)** ; Biodegradable Hydrogels for Controlled Drug Release , Media , New York Dordrecht Heidelberg London , (2) : 147-148 .
2. R. A. Khalil and B. Z. Al-Khiro**(2006)**, "Surfactant Effect on Kinetic of Reaction of Some Sulphonamides With p -Dimethylaminobenzaldehyde: Surfactant-Modified Determination of Sulphonamides in Aqueous Solution", J. Chin. Chem. Soc., 53, p. 637.
3. Roman J.S . and Gallardo A., Macromol Symp **(1994)** , A Novel Synthesis and Characterization of Poly [4-imino(N--4-ethylbenzoate)benzene

p-styrenesulphonate] and the Investigation on Polymer Ability for Drug Release, 84: 145–148 .

4. Roman 1.S. and Madruga E.L. **(1989)** , Polymer, 5: 949–954 .
5. W. Mark Saltzman , Vladimir P. Torchilin **(2008)**; Drug delivery systems article.
6. Arshadi,R. **(1993)**, A Novel Synthesis and Characterization of Poly[4-imino(N--4-ethylbenzoate)benzene p-styrenesulphonate] and the Investigation on Polymer Ability for Drug Release , Adv. Polym. Sci, (1) : 111.
7. Li Yan Qiu1, and You Han Bae **(2005)** , Polymer Architecture and Drug Delivery,421 , 315 .
8. Syed K. H. Gulrez, Saphwan Al-Assaf and Glyn O Phillips **(2011)** , Hydrogels: Methods of Preparation, Characterisation and Applications , Prof. Angelo Carpi (Ed.).
9. Alireza Garjani, SoodABE H davaran, MohammadReza Rashidi and Nasrin Maliki **(2004)** , Protective Effect of some azo derivatives of 5-aminosalicylic acid and their pegylated prodrugs on acetic acid – induced rat colites , (1) :24-25
10. Yerkesh Batyrbekov and Rinat Iskakov **(2012)** , Polyurethane as Carriers of Antituberculosis Drugs , (8) : 147-148 .
11. Yasser Fakri Mustafa **(2012)** , Synthesis and in vitro kinetic study of new mutual prodrug for colon cancer associated with constipation , 8(1) :35-36 .
12. Mehrdad Mahkam,a Reihaneh Mohammadia and Seyed Omid Ranaei Siadatb **(2006)**, Synthesis and Evaluation of Biocompatible pH-sensitive Hydrogels asColon-specific Drug Delivery Systems , , 53, 727-733 .
13. Farah Maria Drumond Chequer, Daniel Junqueira Dorta and Danielle Palma de Oliveira **(2011)**. Azo Dyes andTheir Metabolites: Does the Discharge of the Azo Dye into Water Bodies Represent Human and EcologicalRisks , Peter Hauser (Ed.) .
14. Brown, J.P . **(1981)** Reduction of polymeric azo and nitro dyes by intestinal bacteria. Appl. Environ. Microbiol . 41 :1 283 -1286.
15. (a) K.Hunger, Industrial Dyes: Chemistry, Properties, Applications **(2003)** , ed. Wiley-VCH, Weinheim , Germany; (b) H. Zollinger, Color Chemistry **(1983)**: Syntheses, Properties and Applications of Organic Dyes and Pigments(1987) , VCH, NY, p. 85; (c)P.F.GordonandP. Gregory, Organic Chemistry in Colour, Springer, NY,p .95 .
16. Azam Rahimi' and Satwa Farhangzadeh **(2001)** , Kinetics Study of Bisphenol A Synthesis by ondensation Reaction , ranian PolymerJournal 10 , 1 .
17. El-Refaie Kenawy , Salem S. Al-Deyab and Mohamed H. El-Newehy **(2005)** , Controlled Release of 5-Aminosalicylic Acid (5-ASA) from New Biodegradable Polyurethanes , Molecules 15, 2257-2268 .
18. Salah A. Naman, Ayad H. Jassim, Mahasin F. Alias **(2002)** , Photodecomposition of molybdenum (II) and tungsten(II) carbonyl omplexes with triazole, benz-imidazole, and

oxadiazole cetylinic derivatives , Journal of Photochemistry and Photobiology A: Chemistry 150: –48 .

19. Vicki Barwick **(2003)** , Preparation of calibration Curves , LGC/VAM/032 .
20. P. Crews, J. Rodriguez, M. Jaspars, (Organic structure analysis),University of California, santa Cruz, 1998 .
21. John F. Casale , Minh C. Nguyen **(2010)** , N-Acetylbenzocaine: Formation via Transacetylation of Benzocaine and Acetylsalicylic Acid in a Cocaine Exhibit , Microgram Journal, 1: 7 .
22. Jun Cao , Niancao Chen , Yuanwei Chen and Xianglin Luo **(2010)** , Synthesis of a Novel Biodegradable Polyurethanewith Phosphatidylcholines , Int. J. Mol. Sci. , 11(4), 1870-1877 .
23. A. T. Florence and D. Attwood **(1998)** physicochemical Principles of Pharmacy, 3rd edn., (MacMillan, London,) .
24. Carolina M.Avila and Fleming Mart´ınez **(2002)** , Thermodynamic Study of the Solubility of Benzocaine in some Organic and Aqueous Solvents ournal of Solution Chemistry, 31, 12.
25. W. Catterall and K. Mackie, inGoodman & Gilman's **(2001)**The Pharmacological Basis of Therapeutics, 10th edn., J. G. Hardman, L. E. Limbird, and A. G. Gilman, eds. (McGraw-Hill, New York,) .
26. Gregory M. Lipkind and Harry A. Fozzard **(2005)** , Molecular Modeling of Local Anesthetic Drug Binding by Voltage-Gated Sodium hannels , 10.1124/mol.105.014803 .
27. Zou, M. J., Cheng, G., Okamoto, H., Hao, X. H., An, F., Cui, F. D. and Danjo, K. **(2005)**. Colon-specific drug delivery systems based on cyclodextrin prodrugs: In vivo evaluation of 5-amino salicylic acid from its cyclodextrin conjugates. World J. Gastroenterol, 11(47): 7457-7460
28. Sigma-Aldrich.com .
29. Range, H. P. and Dale, M. M. **(1995)**. Pharmacology, 3rd ed., Churchill Livingstone, New York. 590-592.
30. SOODABEHDAVARAN ANDMOHAMMAD R. R ASHIDI **(2006)** , Synthesis and Degradation Characteristics of Polyurethanes Containing AZO Derivatives of 5-Amino Salicylic Acid , Journal of BIOACTIVE AND COMPATIBLEPOLYMERS, Vol. 21 .
31. Roman J.S . and Gallardo A., Macromol Symp **(1994)** , A Novel Synthesis and Characterization of Poly[4-imino(N--4-ethylbenzoate)benzene p-styrenesulphonate] and the Investigation on Polymer Ability for Drug Release, 84: 145–148 .

تخليق وتشخيص وتقييم أنواع جديدة من البولي يورثين القابلة للتفكك الاحيائي والمحتوي على مشتقات الآزو لمادة البنزوكائين

تاريخ الاستلام : 2014/1/9 تاريخ القبول :2014/3/18

محمد علي مطر ، صدبرين فرحان جواد
قسم الكيمياء، كلية التربية ، جامعة القادسية

الخلاصة

تضمن هذا البحث تحضير سلسلة أنواع جديدة من البولي يوريثين المتفكك احيائيا والحساس لـلدالة الحامضية والذي يحتوي على مشتقات البنزوكائين المتضمنة مجموعة الازو بالبلمرة التكاثفية لـ 1,6-hexamethylene diisocyanate في درجة حرارة 80 م مع مونمرات الازو.

استعملت تقنيات الأشعة فوق البنفسجية ، والأشعة تحت الحمراء ،وطيف الرنين النووي المغناطيسي لتأكيد التراكيب الكيميائية المحضرة للبولي يوريثين الآزو .

تم رسم المنحني المعاير بين الامتصاص والتركيز والذي كان مبني على محلول الدواء النقي . تركيز البنزوكايين المتحرر يحسب بواسطة استقرار نتيجة منحني المعايرة لكل 24 ساعة . اقل قيمة لتركيز البنزوكائين استخدمت هو 0.001 غم/مول عند الطول الموجي 293 نانوميتر.

حُمّل البنزوكائين داخل الشبكة البوليمرية في عملية البلمرة ودُرس الدواء المحمل في وسطين عند (pH=4 , pH=7.8)عند درجة حرارة الجسم 37 م . وكذلك قيست الكمية الكلية المتحررة من البنزوكائين عند درجة حرارة الغرفة وبوسط حامضي 4 و7.8 .

- البحث مستل من رسالة ماجستير للباحث الثاني