

Ring-opening Polymerization (ROP) of Poly Lactid by Cellulose Diacetate and Substitution with Different Amino Acids and Studying Release of Drug

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Abstract

Novel and promising macromolecular were directly synthesized by ring opening of poly lactid by hydroxyl groups in cellulose diacetate as biodegradable polymers and amino acids substitution for the development of a polymeric implantable delivery carrier The remain of carboxylic groups of amino acid substitution with different drug. The toxic of the synthesized matrices were evaluated using a bacterial *Staphylococcus aureuse* , *Micrococcus* , *Pseudomonas aeruginosa*, *klebsiella*, *Streptococcus*. A two new amid bond was formed between the carboxyl end-groups of the synthesized polymer matrices and an amine group of amino acids , The structure of the polymeric conjugates was characterized by various spectroscopy techniques. A study of hydrogen nuclear magnetic resonance ($^1\text{H-NMR}$) and differential scanning calorimetry (DSC) thermodiagrams indicated that the presence of drug pendant groups in the macromolecule structures increased the polymer's rigidity alongside increasing thermal stability. It has been found that the kinetic release of amino acids and drugs from the obtained macromolecular conjugates, tested *in vitro* under different conditions, is strongly dependent on the physico-chemical properties of polymeric matrices.

Keywords: controlled release drug, biological active, methyl acetate, trimethoprim, sayluthamole.

الخلاصة

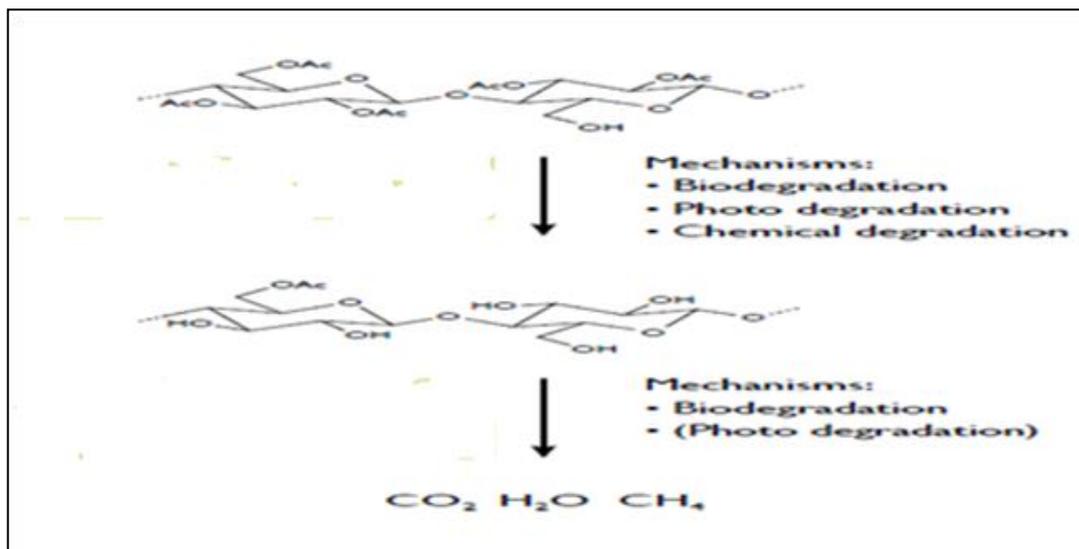
تضمن هذا البحث فتح حلقة اللاكتايد باستخدام مجموعة الهيدروكسيل في ثنائي خلات السيلولوز ثم تعويض احد مجاميع الهيدروكسيل باستخدام امينية مختلفة اما المجموعة الكاربوكسيلية المتبقية تم تعويضها بادوية مختلفة (تراي مثيريم، وساليوثامور). وقد تم دراسة الفعالية البيولوجية للمركبات المحضرة وتم تشخيصها طيفياً باستخدام طيف (H.NMR، IR) وقد تم دراسة الاستقرارية الحرارية للمركبات وكذلك التحلل الدوائي.

الكلمات المفتاحية: التحرر الدوائي، الفعالية البيولوجية ، ميثايل اسيتيت، ثلاثي مثيروبريم ساليوثامور

Introduction

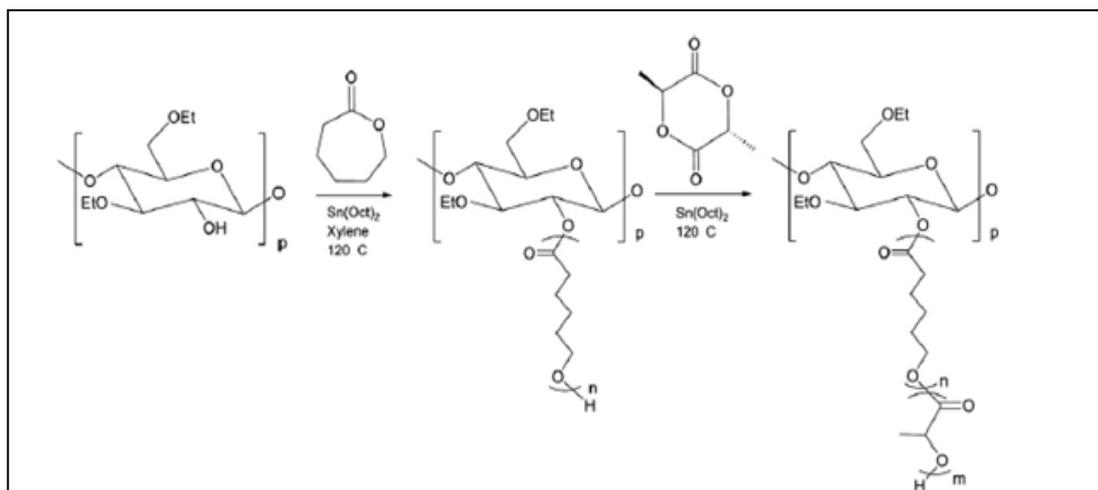
Cellulose acetate (CA) is often used in these attempts, since this polymer is a commercial product and can be handled relatively easily compared to cellulose or to most of the other natural polymers. Low cost and high productivity, the serious problems facing cellulose acetate are its poor heat stability at high temperatures as well as its hydrophilic nature [YuD, 2013]. Cellulose acetate (CA) has been used for the applications in diverse areas such as fibers, films, laminates, adhesives, coatings, and plastic. Their disposal by burning produces a considerable increase in carbon dioxide (CO_2) and, in some cases, toxic gases, which contribute to global pollution or green house effects [Kim, 2006]. As a result, there is considerable interest in biodegradable polymers, which can be used as alternatives to traditional plastics, thus reducing the

amount of wastes. Hence in order to control environmental pollution, Products. Cellulose acetate polymer is chemically degraded by a process called hydrolysis, which means breaking a part by the addition of water) [Khatri, 2012]. This is a chemical reaction in which water is added to the cellulose acetate polymer causing the release of acetic acid molecules. This process proceeds stepwise until all the acetate groups are released resulting in the reformation of cellulose.[Barud, 2008] This chemical degradation occurs naturally during composting and during other environmental processes in which mild acidity is present. The resulting acetic acid and cellulose can serve as food sources for soil microorganisms and thereby it is in synergy with biodegradation [Södergard, 2002].



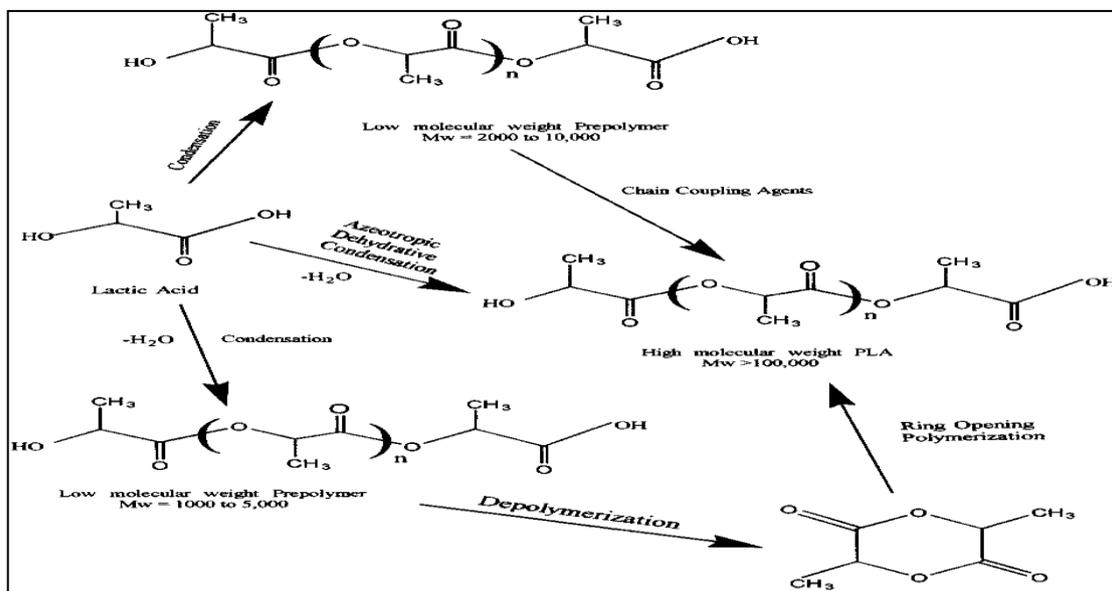
Scheme (1) Degradation of Cellulose acetate

Grafted CDA with PLA through ROP of LA, both in solution and in bulk [Vert, M., 2002]. The results showed that longer side chains are formed when the polymerisation was performed in bulk as compared to in solution. It was also found that the polymer chain length could be controlled by the amount of monomer added to the reaction mixture [Choi, 2003, Zare, 2013].



Scheme(2) Grafted of cellulose diacetate with poly lactic anhydride

Poly(lactic acid) (PLA) belongs to the family of aliphatic polyesters commonly made from acids, which include polyglycolic acid or polylactic polyPLA is a thermoplastic, high-strength, high-modulus polyPLA is a thermoplastic, high-strength, high-modulus [Reed, 1982; Branden, 2000] to yield articles for use in either the industrial packaging field or the biocompatible/bioabsorbable medical is one of the few polymers in which the stereo chemical structure can easily be modified by polymerizing a controlled mixture of the L- or D-isomers to yield high molecular weight amorphous or crystalline polymers that molecular-weight amorphous or crystalline polymers that of the ester bond and does not require the presence of enzymes to catalyze this hydrolysis Poly(lactic acid) homo polymers have a glass transition temperature of about 55°C and 175°C, respectively. They require processing temperature in excess of 185–190°C [Discher, 2002; Cardinaux, 1977].



Scheme(3) Synthesis of polylactic anhydride[13]

Materials and Methods

Cellulose diacetate (CDA) was purchased from Aldrich with a degree of polymerization and degree of substitution 160 and 2,4 respectively. Polylactic anhydride was purchased from Aldrich. Amino acids (alanin, valine) and other solvent from Aldrich.

A -Modification of cellulose diacetate:- (B1)

The modified polymers were prepared by gradually addition (0.2mol) of polylactic anhydride (PLA) to a solution of cellulose acetate (4 mol) dissolved in 50 ml of cyclohexanone. Solutions had been prepared under nitrogen atmosphere, reaction was allowed to occur for 3 h at 70 °C The reaction product (B1) was precipitated in diethyl ether, filtered, and washed with hot methanol. The recovered polymer was then dried under vacuum at 40°C.

B-Substitution of poly CDA-co-PLA with amino acid :- (B2,B3,B4,B5)

In around conical flask about (0.9gm, 3mol) of CDA-co-PLA was dissolved in 10ml of DMF, (0.3gm, 2mol) of alanin dissolved (10ml of NaOH: ethanol) the mixture was refluxed with magnetic stirring at room temperature for 2 hrs. The brown

viscous polymer was reprecipitate by diethylether and then washed with ethanol, then dried under vacuum at 40° C. 2gm of B2 was dissolved in 7ml of DMF ,1ml of thionylchloride was added and 1.33gm of methepime dissolved in 10 ml of acetone and then added to B2. The mixture was reflux about 1hr, the red precipitate was washed by diethylether dried under vacuum at 40C⁰.

Table (1)Physical properties of compounds B1, B2, B3, B4, B5

No. Com-pounds	polymer	Sifting point	Color	Yield %
B1		252	yellowish	82
B2		271	brown	73
B3		255	orange	61
B4		283	red	67
B5		Dark brown	265	66

Controlled Drug Release Studying of polymer prepared:-

A 50mg of drug polymer(B5) was kept in a cylinder containing 50 ml water in different pH values at 37 °C without stirring. A released sample periodically withdrawn and analyzed by UV. Spectroscopy at specific rang λ_{max} 236-320nm to determine the amount of the released drug unite. A calibration curve was constructed with a software built in the computerized UV spectrophotometer, the amount of the released drug was determined directly from the software for many days, using the calibration curve in different pH values.[Kurtoglu, 2010; Oledzka, 2014]

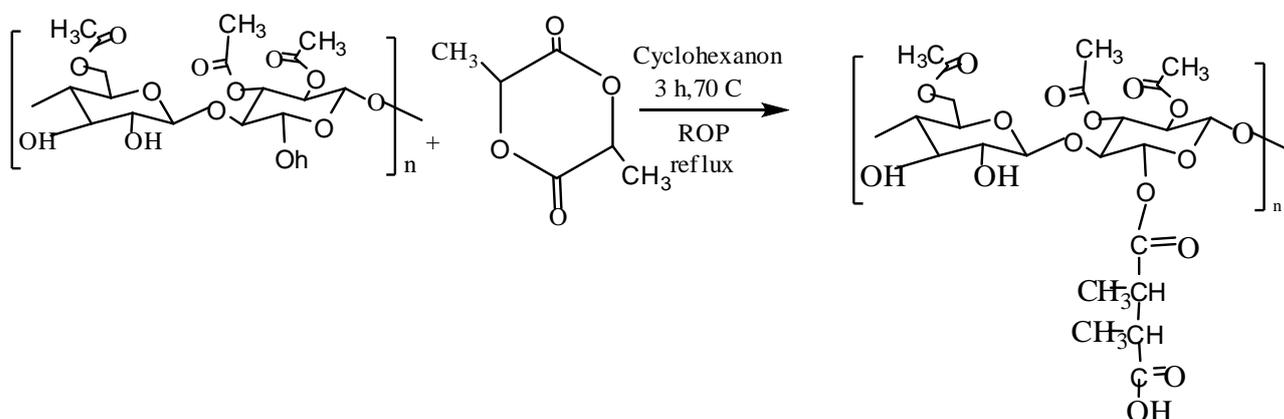
Result and Discussion

In order to investigate the specific interactions of lactic anhydride with CA, is shown in equation (1).FTIR spectral studies were carried out for pure CA and in (Figure 1). FT-IR spectrum of pure CA shows characteristic peaks at 3445 due to the OH 1737, 1369 and 1224 cm-1 due to the C=O, C-CH3 and C-O-C stretching, re-

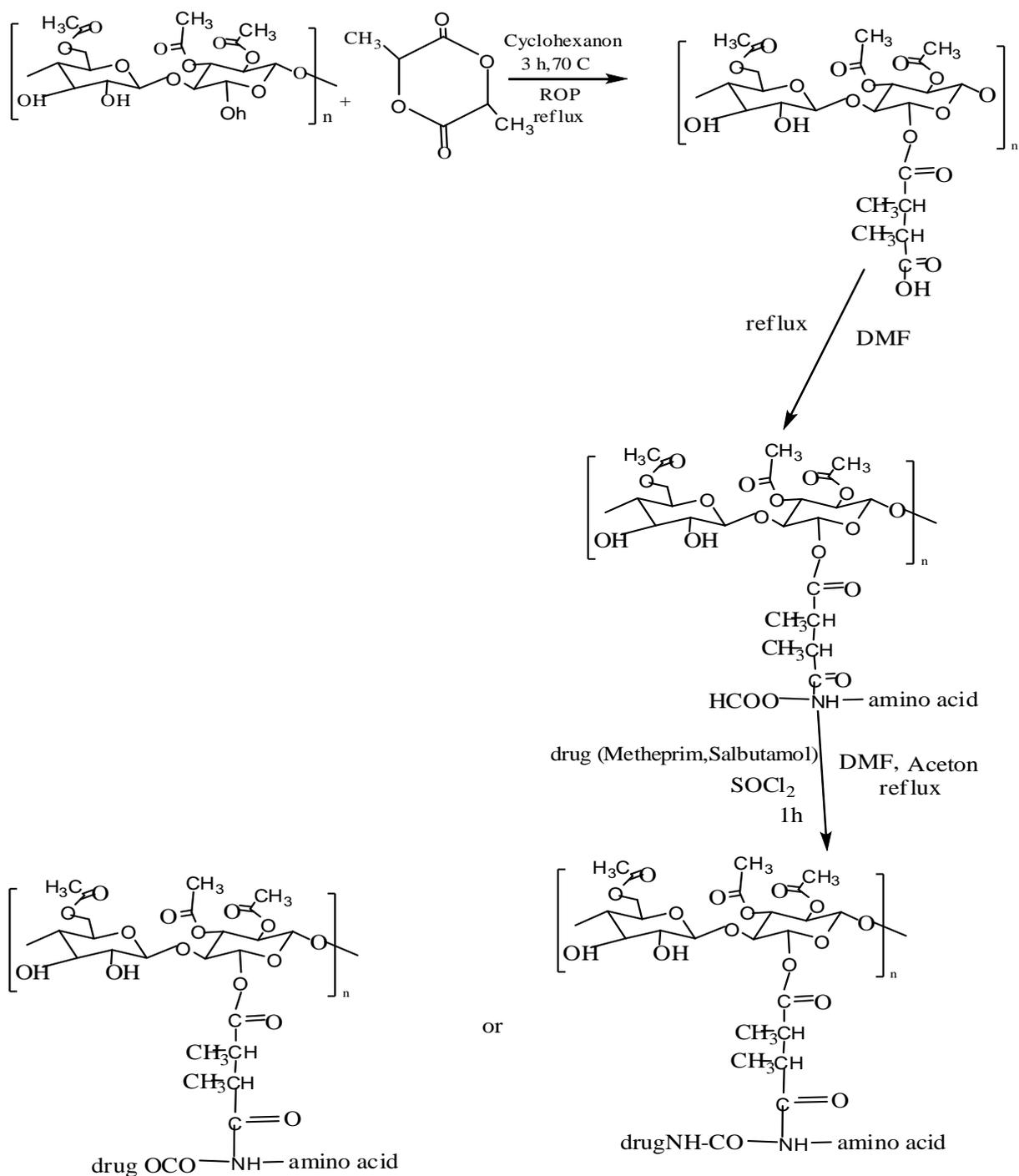
spectively. In Figure 2, FT-IR spectra of the CA-co-PLA revealed the presence of functional groups whose absorption frequencies correspond to C=O anhydride (1745cm⁻¹) and peak was slightly shifted from 1737 to 1732cm⁻¹) due to the C=O ester [Silverstien R., 1976, Silversyein R.M., 2005]. The absorption band broadening of carboxyl groups for CA-co-PLA samples was also observed, which indicates gen interactions between CA and PLA other compounds were listed in table(2)

Table(2) FT-IR spectrum of compounds

No. of com.	C=O cm-1	N-H	C-H cm-1 Aliph.	O-H cm-1	C=C cm-1	C=O amid
B1	1737	-	2931 2887	3500	1452	-
B2	1722	3263	2959- 2800	3400	1450	1656
B3	1718	3258	2933- 2877	3367	1437	1656
B4	1712	3244	2925- 2833	3366	1446	1634
B5	1732 from ester	3252	2922- 2831	3340	1466	1656



Equation (1) Ring opening polymerization (ROP) of lactide to produce (CA-co-PLA) [Oledzka, 2013]



Scheme(4) Substitution of amino acid and drug with CA-co-PLA [Field L.D.,2008, R. Abdul Kider,2001]

$^1\text{H-NMR}$ spectra of some prepared drug polymers were obtained DMSO- d_6 as solvent with TMS as internal standard. Signal at (δ 3.8) (q,2H, CH_2) and at (δ 2.844-4.018) (s,1H, C=ONH) [18-19] The $^1\text{H.NMR}$ spectrum of compounds (B1) shows characteristic signals at (δ 7.66-8.501) due to v3.644-4.018) (s,3H, C=ONH) Also signal at (δ

3.024) (s,3H,CH₃) . Also signal at (δ 3.5) (q,2H,CH₂) and at (δ 3.1(s1H,C=ONH)).
¹H.NMR spectrum of compounds (B2: 0.79 CH₃, 2.34 CH₃, 3.65CH₃, 4.9 OH, 5.8 NH, 6.4-6.8CH, 8.55COOH), ¹H.NMR spectrum of compounds (B3: 1.2-2.4CH₃, 3.43CH, 6.6OH, 7.4NH, 8.1 COOH), ¹H.NMR spectrum of compounds (B4: 1.9CH₃, 4.2CH₃, 7.1CH. 7.3-8.3 CH ar., 8.7 NH, 10.02 OH), ¹H.NMR spectrum of compounds (B5: 6.3OH, 7.2-7.8H ar., 9.14NH).

Thermal analysis DSC

The thermal stability was examined, under nitrogen, using a Perkin-Elmer (DSC) thermo gravimetric analyzer from room temperature to 500 °C at a heating rate of 10°C. The prepared drug-polymers have high thermal stability .[Ku, 2011; Lee, 2004]

Biological activity

Antibacterial activities (in vitro study) of these compound were tested in the present studies and results are presented in table (3) .the antimicrobial activity of compounds against a different isolates :(*Staphylococcus aureuse* ,*Micrococcus* ,*Pseudmonas aeruginosa*,*klebsiella*,*Streptococcus*) of bacteria ,from the zone of inhibition ,it has observed that the (B2 and B3.B4,B5)more active than the(B1). The result showed high activity towered Gram-positive *Staphylococcus aureus* and Gram –negative *pseudomonas aeruginosa* bacteria which agree with other result [Tachibna,2004].

Table (3)Biological activity of compounds (B2, B3, B4, and B5)

Bacterial isolated	DMSO	B2	B3	B4	B5
<i>Staphylococcus aureuse</i>	-	25	20	35	33
<i>Micrococcus</i>	-	18	15	20	19
<i>Pseudmonas aeruginosa</i>	-	23	20	42	40
<i>klebsiella</i>	-	15	14	19	16
<i>Streptococcus</i>	-	13	14	19	17

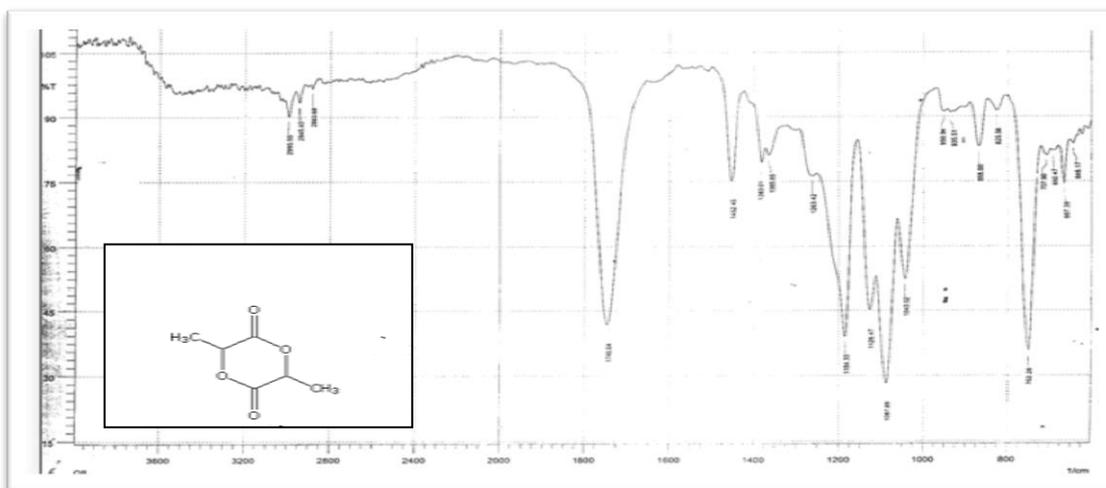


Figure (1) FT-IR spectrum of Lactied

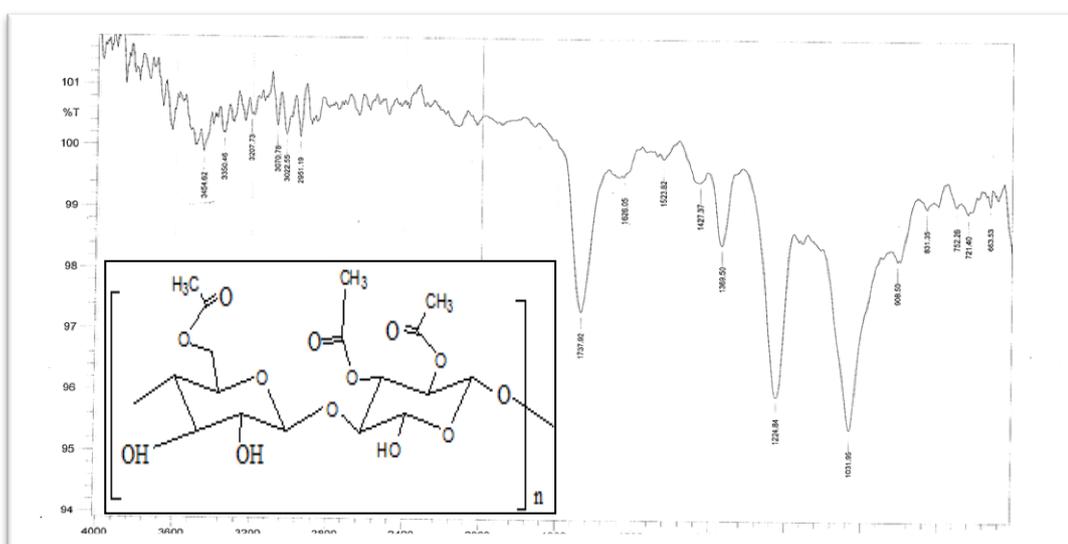


Figure (2) FT-IR spectrum of Cellulose acetate

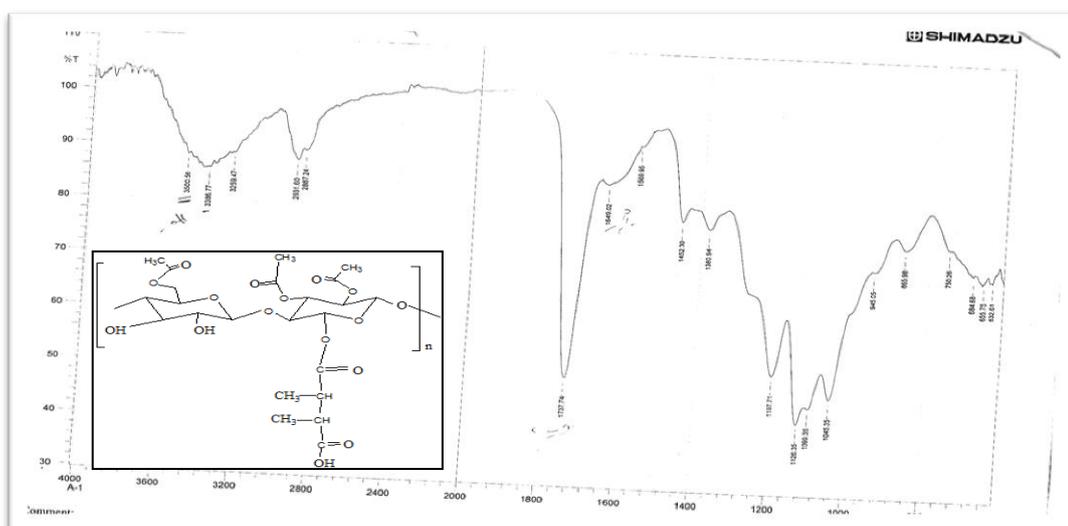


Figure (3) FT-IR spectrum of B1

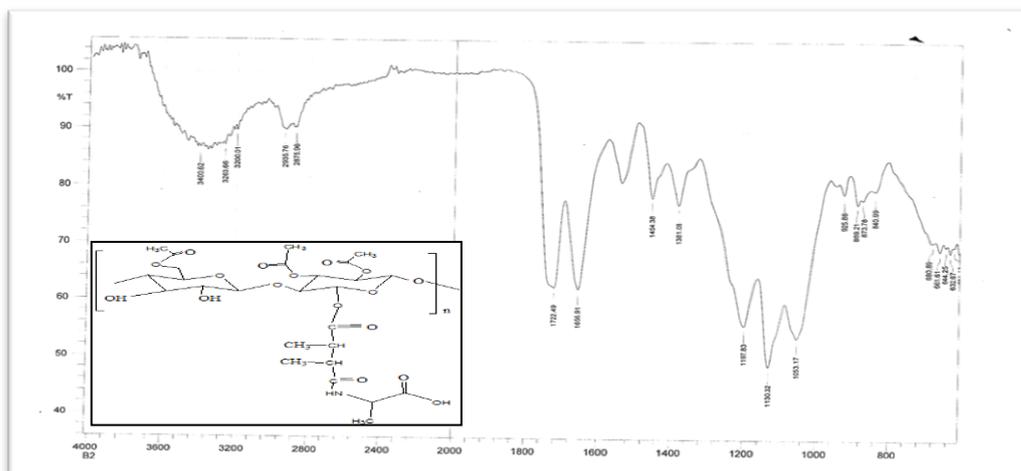


Figure (4) FT-IR spectrum of B2

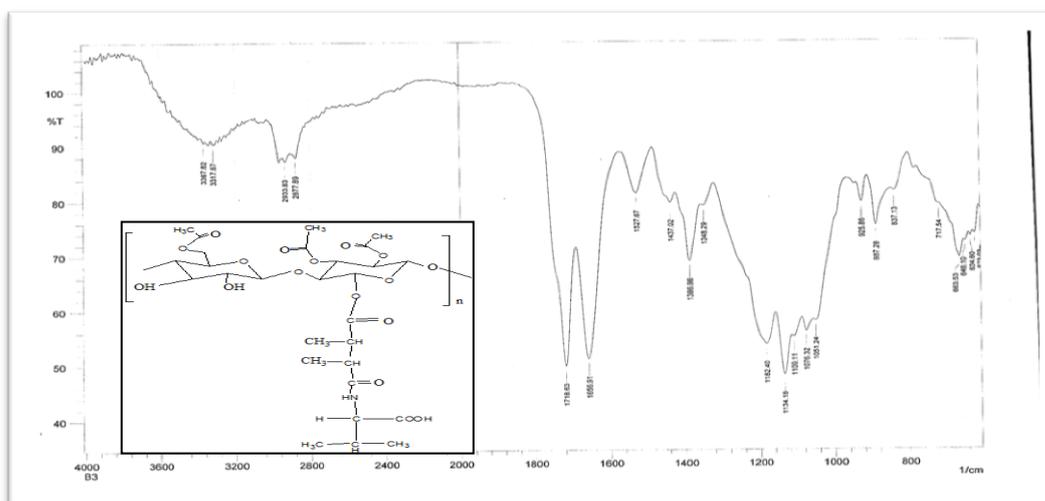


Figure (5) FT-IR spectrum of B3

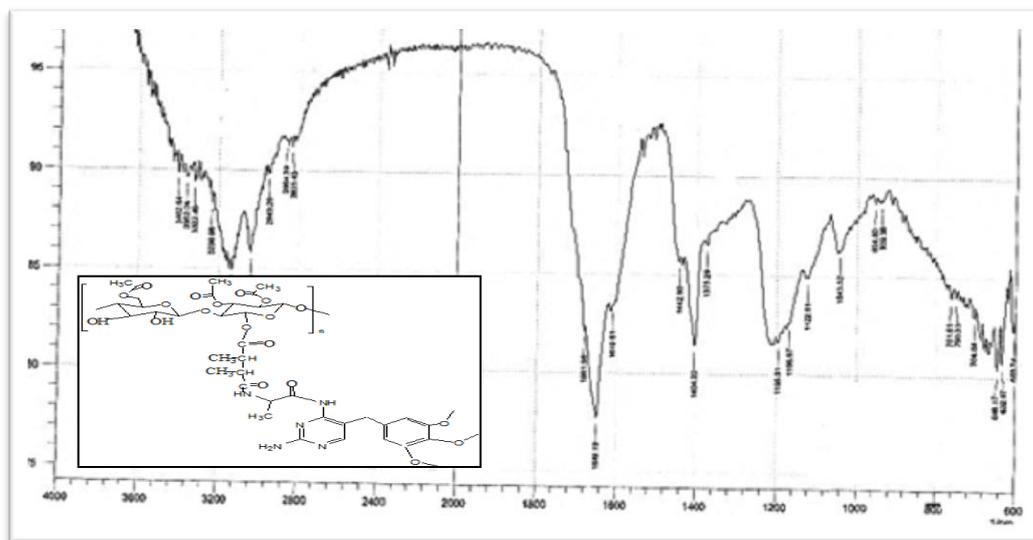


Figure (6) FT-IR spectrum of B4

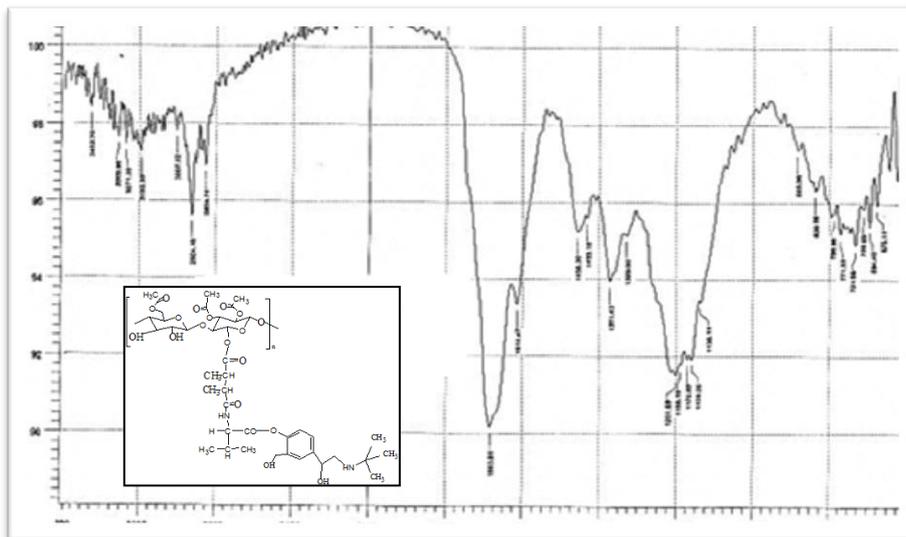


Figure (7) FT-IR spectrum of B5

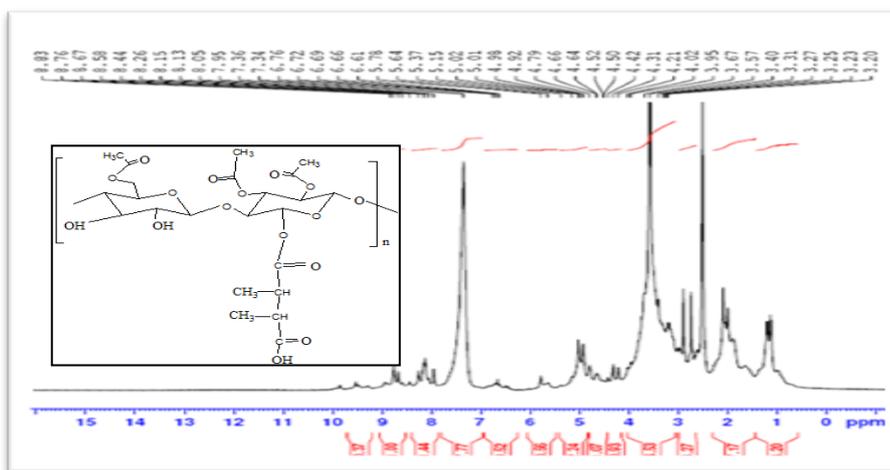


Figure (8) NMR of B1

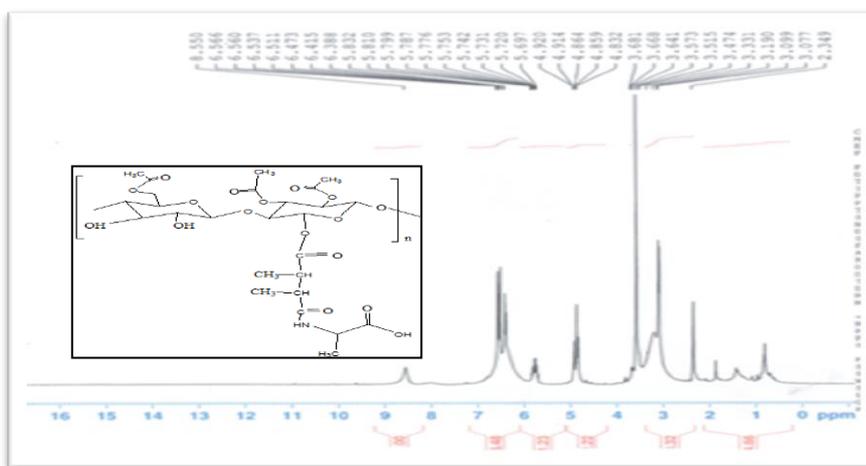


Figure (9) NMR of B2

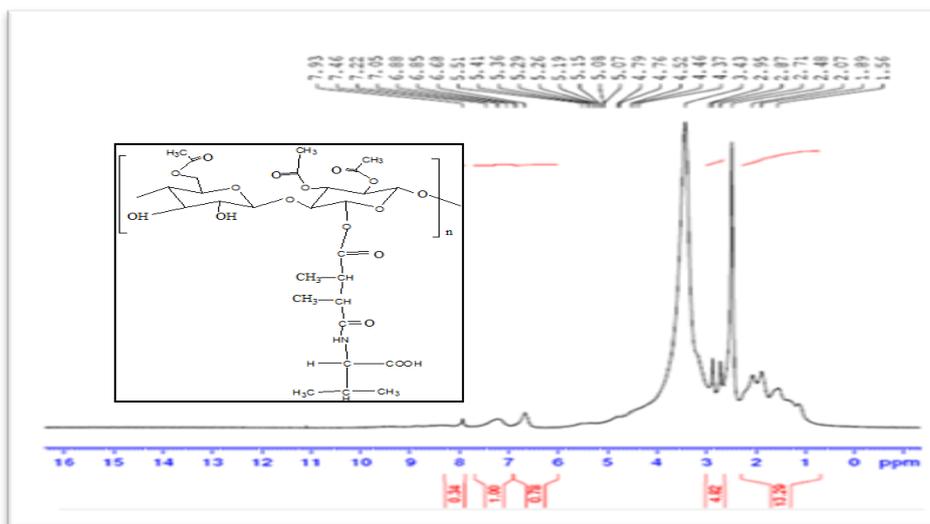


Figure (10)NMR of B3

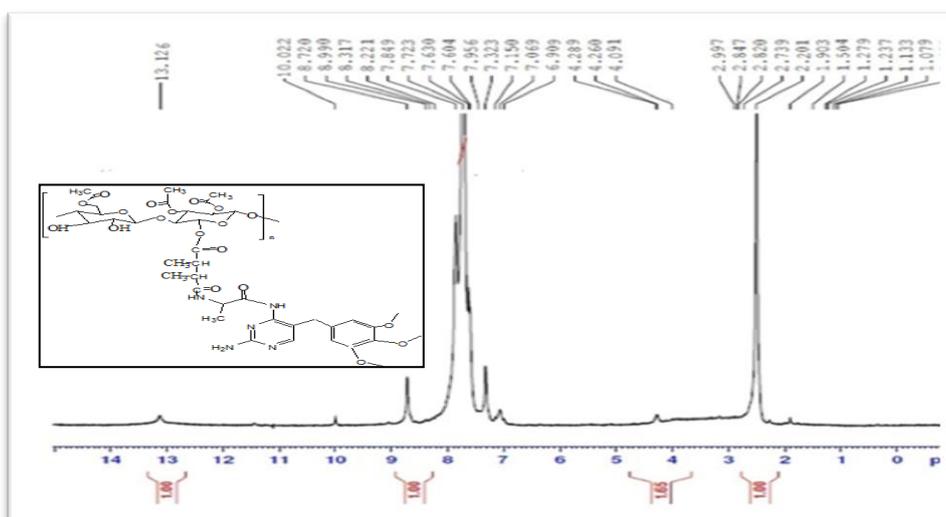


Figure (11)NMR of B4

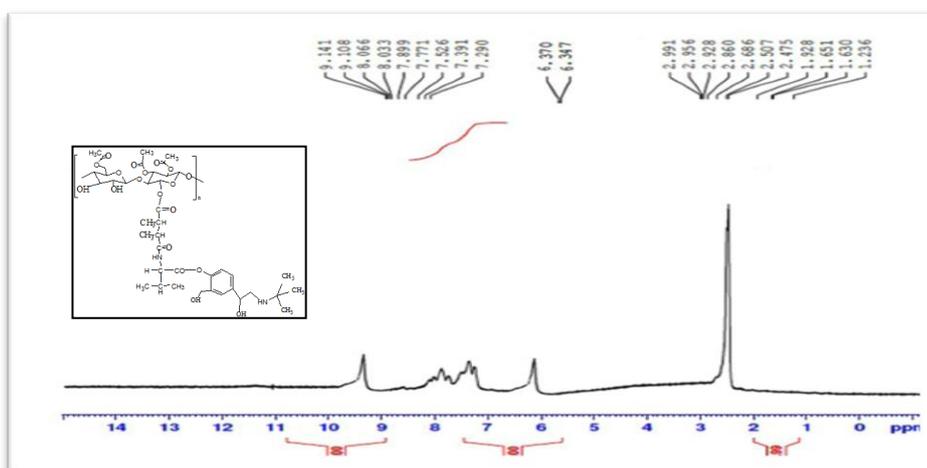


Figure (12)NMR of B5

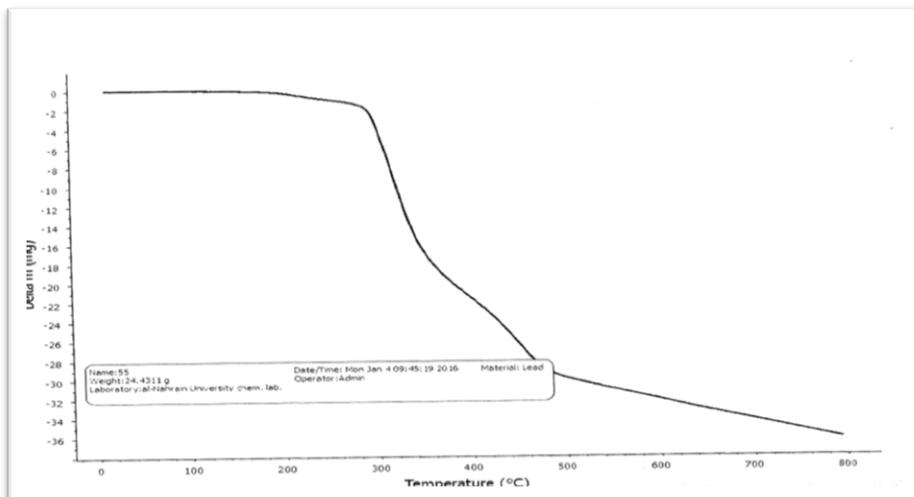


Figure (13)DSC of B1

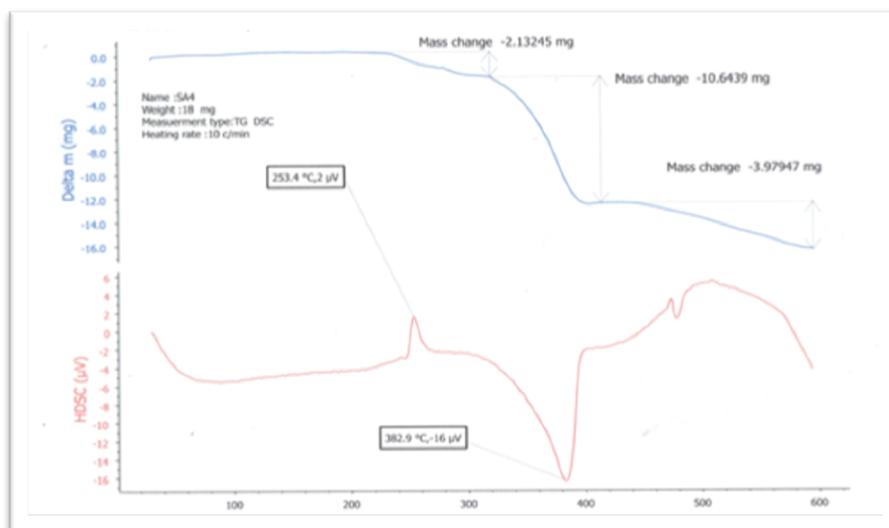


Figure (14)DSC and TGA of B4

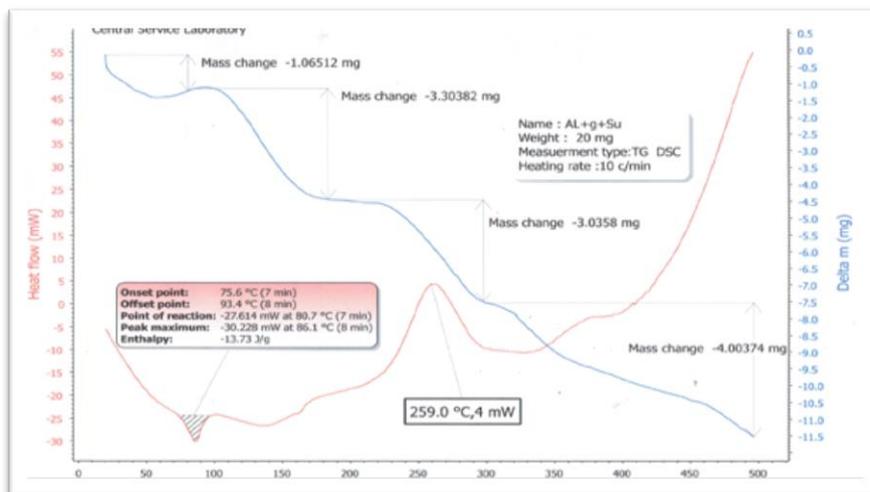


Figure (15) DSC and TGA of B5

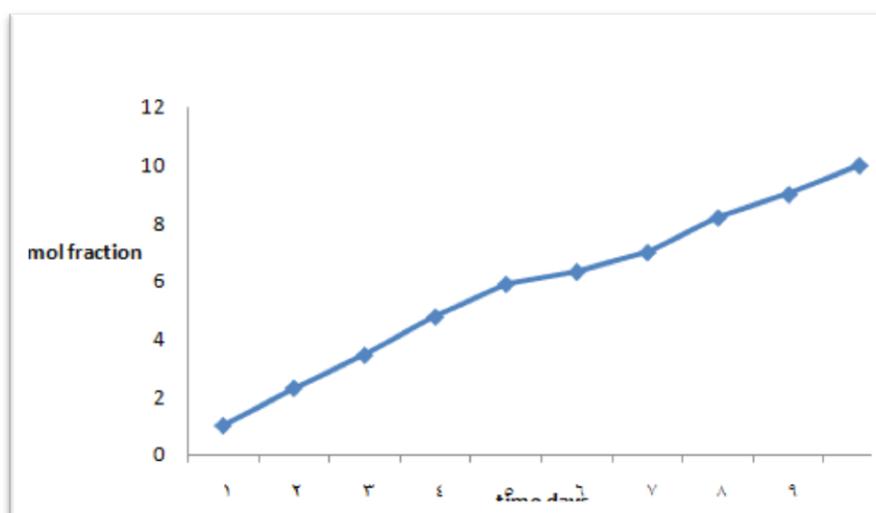


Figure (16) Control drug release of B4 in PH=7.5

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