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Review article

Synthesis of Pectin Graft Drug to Treatment the Wounds and Inflammations

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ABSTRACT

This idea of this work included preparation new adhesive drug polymers to treatment the wounds and inflammations, new drug polymers were prepared as bio adhesive, which have high viscosity and treatment the wounds by the adhesion of both ends of the wound when it put as well as the speed of the treatment of external inflammation, because it remains inherent to the position of injury fast time, because of the property for it viscosity. A new bio adhesive polymer was prepared by modification of Pectin structure with acrylic acid (P_1) as a spacer by using ceric ammonium nitrate (CAN) as an initiator, and new graft copolymer was substituted with amino drugs such as amoxicilli produced amide polymer. This design carries controlled delivery of therapeutic agents which could release the entrapped drug over an extended period of time due to its biodegradable, nontoxic and slow digesting nature. All prepared adhesive drug polymers were characterized by FTIR, ^{1}H-NMR spectroscopes, thermo gravimetric analysis TGA and DSC were studied. intrinsic viscosities and physical properties of all prepared polymers were measured, biological activity was studied for all adhesive drug polymers this new adhesive drug biological polymers were applied on different infected mice and wounds, It gave outstanding results and compliance mice infected with a full recovery by a short period of time. The prepared drug copolymer was analyzed in different pH values at 37 °C in vitro study and controlled drug release was compared at zero time and after three days .The rate of hydrolysis in basic medium was found higher than acidic medium. It was concluded that modified drug release with extended drug action via slow release and in vivo performance was noted to be promising.

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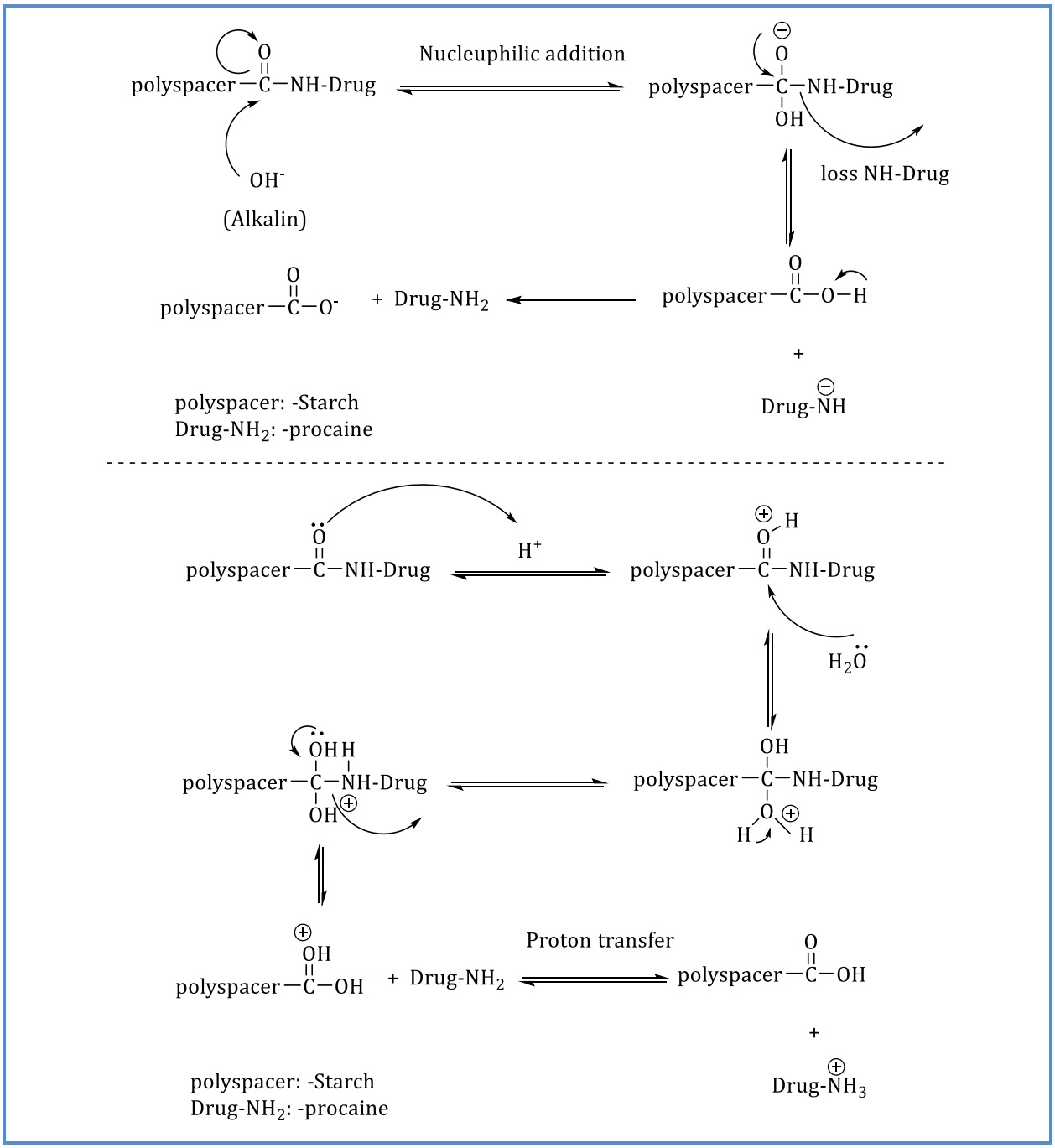
Graphical Abstract



Introduction

Pectin is important structural components of cell walls of the soft, non woody parts of fruit, vegetables and terrestrial plants. Within a living plant it is an important structural polysaccharide with functions in plant growth, morphology and development [1]. Fruit ripening involves Pectin.

breakdown induced by the enzymes pectinase and pectinerase leading to cell separation [2]. When extracted, the major commercial use for pectin is as a gelling or thickening agent and as a stabiliser in food, for example in jams and yoghurt drinks. The most important source of commercial pectin today is waste from the juice industry in the form of citrus peel, mainly from lemon and lime. Other commercial pectin are sourced from orange peel and apple pomace, and an emerging new source is from sugar beet from the sugar industry. Pectin are conceivably the most complicated of the natural plant carbohydrates, both in terms of their chemical composition and their physical chemical structure. They contain a number of defined structural units [3] homo galacturonans (HG), rhamnogalacturonan I (RGI), and substituted galacturonans such as rhamnogalacturonan II (RGII). Other substituted galacturonans (apiogalacturonans and xylogalacturonans) have been identified, but only in extracts from certain specific plant species. The familiar components of interest in discussing the bioactivity of pectin as an anti-cancer agent are HG and RGI. The most predominant region of pectin is HG, composed principally of a homo polymer of linked α-D-galacturonic acid (GalA) partially methylated at C-6 (Figure 3) [4]. The degree of methylation (DE) refers to the ratio between methylated and non-methylated GalA. Pectin with high DE is known as HM Pectin and generally refers to Pectin with 50% or more methyl ester groups on the HG backbone, and low DE Pectin (LM Pectin) with fewer than 50%. The methyl-ester content is particularly important in Pectin research as it strongly determines the physical properties of Pectin. The GalA residues at O-2 and O-3 may also be partially esterified with acetic acid in certain plant species such as sugar beet Again [5], the ratio between acetylated and non-acetylated GalA is referred to as the degree of acetylation (DAc).

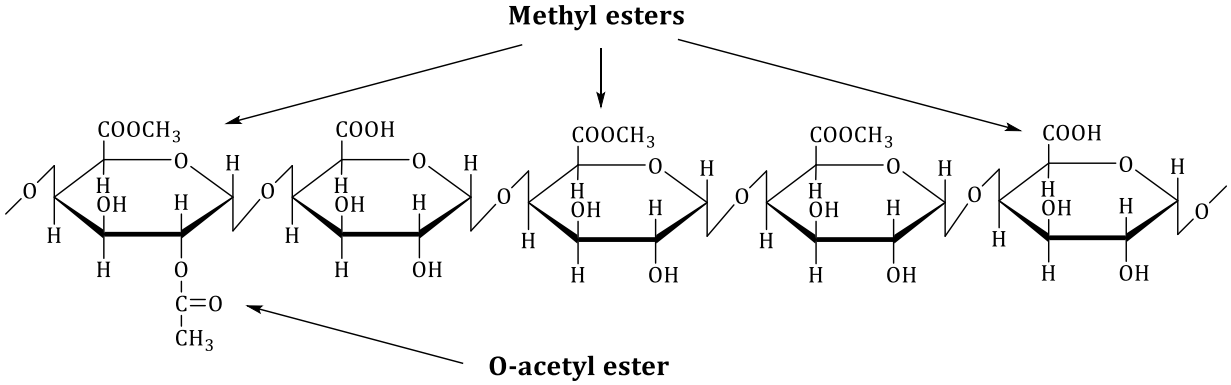


Figure 1. Catechin

Primary structure of the polygalacturonic acid backbone of Pectin

well-characterised component constitutes the 'hairy' regions of Pectin or rhamnogalacturonan I (RGI) regions. RGI consists of a backbone composed of a repeating disaccharide of GalA and rhamnose (Rha) residues [-4)-*α*-D-GalA-(1,2)-*α*-L-Rha-(1-]n [6, 7]. They are highly branched structures with neutral sugar side chains of varying degrees of polymerisation attached to O-4 or O-3 position on the α-L-rhamnose residues (Figure 2) [8, 9]. These side chains consist mainly of α-L-arabinose and/or *β*-D-galactose residues. The major types of side chain present are: (i) Arabinan (Ara), comprising (1→5)-*α*-L-Ara units and often ramified with short (1→3)-*α*-L-Ara or single *α*-L-Ara non- reducing units at O-2, O-3 or O-5 positions (Figure 4b); (ii) galactan (Gal) comprising linear, type I (1→4)-*β*-D-Gal (Figure 4a) or branched, type II (1→3,6)-*β*-D-Gal, depending on the plant source; (iii) Arabinogalactan I (AGI) consisting of a basal chain of (1→4)-*β*-D-Gal substituted at O-3 with short (1→2)/(1→3)-*α*-L-Ara or single *α*-L-Ara non-reducing units (Figure 2c); (iv) type II arabinogalactan (AGII) which has a backbone of (1→3)-*β*- D-Gal heavily substituted at position 6 by mono and oligosaccharide Ara and Gal side chains. Recent studies on the bioactivity of Pectin are beginning to emphasise the potential importance of these neutral sugar chain-containing regions. RGIs, as with whole pectins themselves are ,depending on the source the side chains may contain minor amounts of other sugars such fucose, xylose, mannose, glucose [10] glucuronic acid and methyl esterified glucuronic acid and, in some, phenolic [11]. Arabinogalactan II; (c) branched (1→3, 5)-*β*-L-Arabinan; (d) arabinogalactan extraction with hot acid. The peel or pulp is suspended in 70-90 °C water with nitric acid to pH 1-3 for 3-12 hours. This is then filtered and the fluid that has been leached from the plant material is concentrated and mixed with alcohol to precipitate the pectin, after which it can be dried and milled [12].

For research purposes, pectin extraction in the laboratory tends to be under milder conditions and to have more complex steps [13]. Extraction may be optimized to preserve or isolate parts of the pectin depending on what is being researched. An alternative method of pectin extraction is microwave-assisted flash extraction. As hot acid extracted pectin undergoes a relatively long period of heating, it experiences thermal degradation, whereas microwave. Extracted pectin can take just 15 minutes of heating, therefore producing a higher yield and higher molecular weight pectin in a fraction of the time [14-18].

To create pectins for different functions, the pectin has to be modified. This is easily achieved as pectins are unstable and susceptible to changes in pH and temperature. Pectin has good stability in aqueous solution at around pH 3-4. At acidic conditions lower than pH 3 glycosidic bonds and methyl-ester linkages may undergo hydrolysis. The rate of hydrolysis increases with higher temperature and lower pH [19-20].

Hydrolysis of the sensitive neutral sugar side chains may lead to an increase in GalA content and decrease in neutral sugar content. Studies have shown that mild acid hydrolysis causes the progressive release of sugars accompanied by their rapid degradation [21-24].

Material

Pectin was purchased from Fluka and dried at 110 °C for about ٢ h to remove absorbed moisture. Cerium ammonium nitrate (CAN), acrylic acid amoxicillin and procaein were purchased from Sigma Chemicals, All other solvents and reagents were of analytical grade.

Instrumentation

Melting point was measured using thermal microscope (Kofler-method), and reichert thermovar, Stuart SMP 30. Infrared spectrophotometer measurements were performed using Shimadzu FT-IR 8400 series Fourier transform, ^{1}H-NMR spectra were measured with a Bruker spectrophotometer model ultra-shield at 300.13 MHz in DMSO-d6. U.V-visible double beam scanning spectrophotometer VARIAN (UV-vis)-100 Conc, at room temperature. Industrial Pectin have particular specifications, confirmed by the Food and agriculture organisation that includes no less than 65% GalA, as well as various other stipulations to fulfil the specification of E440 as a food additive.

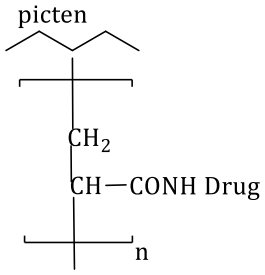
(A) Preparation of pectin graft acrylic acid (P2)

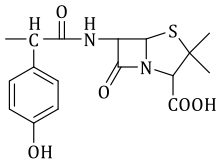
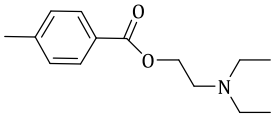
(1 Gm) of pectin was dissolved in (10 mL) of dioxin, (0.5 mL 0.2% soln.) of ceric ammonium nitrate (CAN), (1 Gm) of acrylic acid was added, the mixture was introduced in polymerization bottle, and heated about 2 hour at (50 °C). The yellow color product was produced (90%) conversion ratio. S.P (115-125 °C).

(B) Substitution of (P2) with amino drugs (P2A-P2B)

(0.30 Gm) of Pectin g-acrylic acid P1 was dispersed in (10 mL) of dioxin, (0.30 Gm) of amoxicillin dissolved in (5 mL) of dioxin, (0.3 mL) of DMF was added to the mixture. then was refluxed with stirring about 1 hour at (٦0 °C), the colored solution was filtered, the filtrate was isolated and the solvent was evaporated, the brown product (P2A) of pectin-g-[*N*-Amoxicllinyl acrylic acid] was washed with ether two times and dried at (50 °C) in a vacuum, conversion ratio (93%), S.P (125-150 °C). Similar procedure was used for preparation with other, amino drugs such as procaine, amoxicillin. All physical properties were listed in Table 1.

Table 1. Physical properties of remylops prepare



Pol.	-Drugs	Color	Softening point 0C	Conversion ratio %
P2A	 Amoxicillin	Nutty	125-150	93
P2B	Procaine 	Black	110-120	75

Controlled drug release

Release of P2A was studied. 100 mg was added continuously in (100 mL) buffer solution at (37 °C). The wavelength of λ_{max} was measured at different periods and different pH values (1.1–7.4) by using UV spectrometer. The sample was analyzed by UV-spectroscopes periodically withdrawn the sustained release was measured by the mole fraction constructed from UV.

Determination of median lethal (LD_{50})

In this experiment 6 mice (three male, 3 female) were administered with 3 % P2A. The mice were watched for 72 hours, the LD_{50} value revealed that P2A has no toxic effect on mice [25].

Results and discussion

Chemical modification of Pectin by grafting with acrylic acid. Pectin can be grafted as polymerized and initiated by ceric (Ce^{+4}) salts ion, offers many advantages because of its high grafting efficiency. When such as cerium ammonium nitrate (CAN) is used as initiator in the grafting of vinyl monomers onto pectin, at first a ceric ion–pectin complex occurs, and then it decomposes to (Ce^{+3}) ion and pectin radicals created by hydrogen abstraction from pectin. Thus, the radical formation on the pectin backbone occurs on the backbone of pectin polymer acts as the active sites for the graft

copolymerization. The mechanism of grafting monomer onto pectin [26, 27]. Mordanting of cotton with Harda (Myrobalan) as natural biomordant.

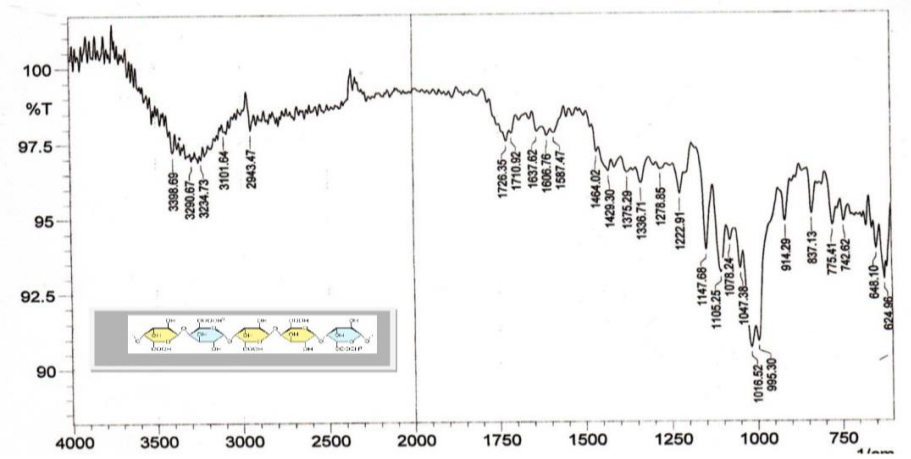


Figure 1. FTIR spectrum of pectin

Figure 1. FTIR spectrum of natural polymer (pectin) showed absorption bands at (3250 cm^{-1}) of υ (O-H) group and υ (C-O-C) ether absorption bands at (1016 -1147 cm^{-1}), bands at (2943 cm^{-1}) are due to υ(C-H aliphatic) stretching respectively.

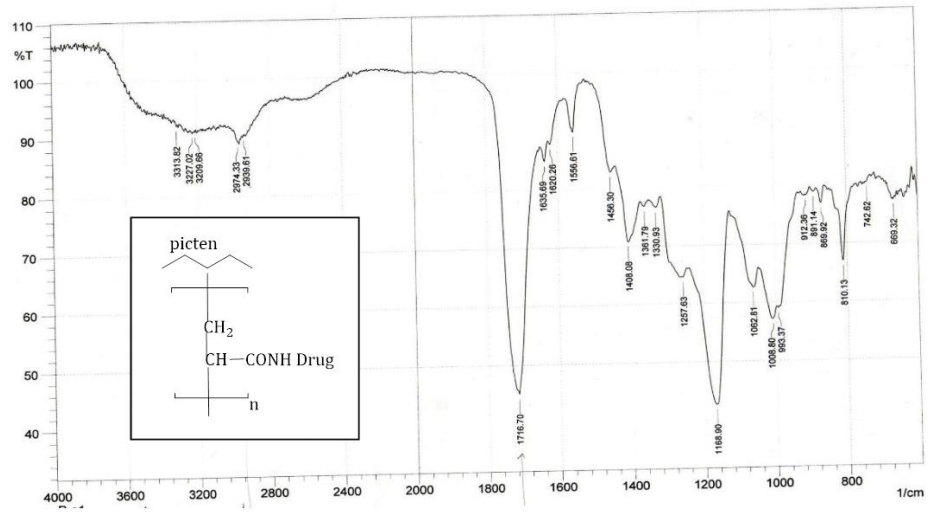


Figure 2. FTIR spectrum of (P2) pectin grafted acrylic acid

Figure 2. FTIR spectrum of (P2) pectin grafted Acrylic acid showed the appearance of absorption at (3227) cm^{-1} broad band assigned to (–OH) stretching carboxylic group of poly acrylic acid, band at (2974) cm^{-1} due to the stretching of C-H aliphatic, (1716) cm^{-1} due to υ(C=O) of carboxylic group of acrylic acid.

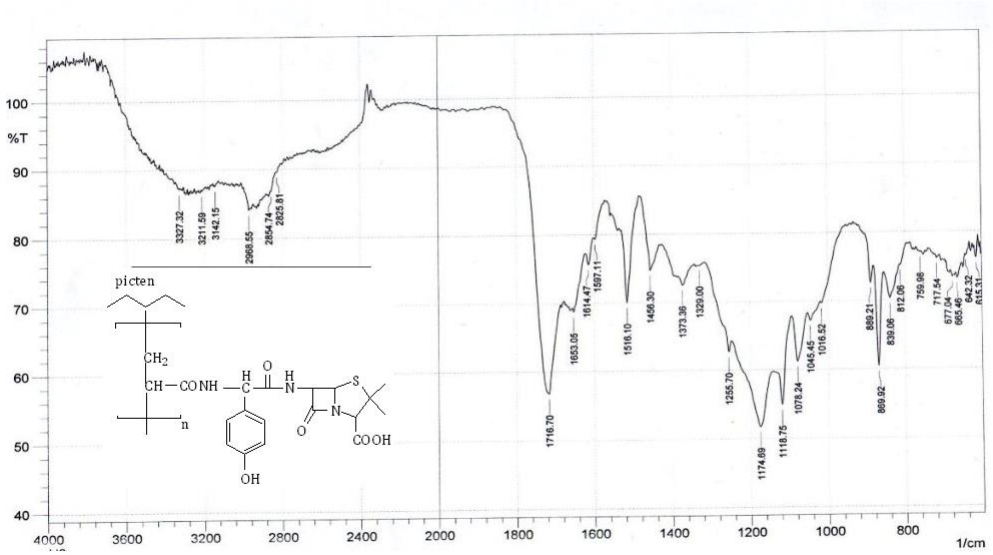


Figure 3. FTIR spectrum of (P2A) pectin-g-[N-amoxillinyl acrylic acid]

Copolymer containing hydroxyl group as characteristic absorption was appeared at (3338 cm^{-1}) in addition (-NH) at (3220 cm^{-1}), absorption of amide (CONH) appeared at (1633 cm^{-1}), band at (1706) cm^{-1} due to (C=O) stretching vibration of acid.

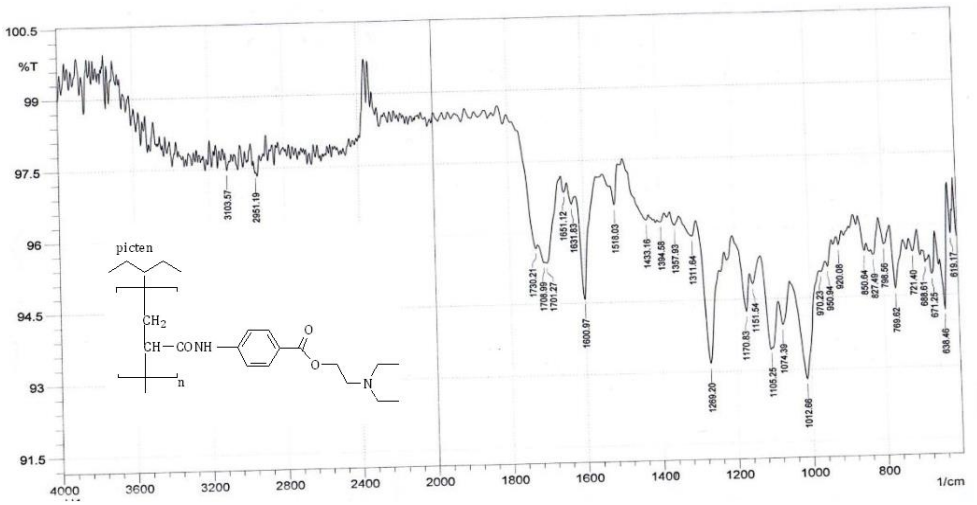


Figure 4. FTIR spectrum of (P2B) pectin-g-[*N*-procainyl acrylic acid]

Figure (٤). FTIR spectrum of polymer P2B showed the appearance of absorption bands at (2851) cm^{-1} are due to υ(C-H aliphatic) and (3105) cm-1of (N-H)and (1600) cm-1of c=c aromatic, (1631-1651) cm^{-1} c=c aliphatic (1701) cm^{-1} due to υ(C=O) of carboxylic group of acrylic acid. Other bands of the compounds are listed in Table 2.

Table 2. FT-IR absorptions of grafted Natural polymers (Pectin) with acrylic acids and substituted with drug compound (Procaine) [P2B]

Comp No.	υ (O-H) cm $^{-1}$ alcoho	υ(N-H) cm $^{-1}$ amide	υ (C=O) cm $^{-1}$ amide	υ (C=C) cm $^{-1}$ Aromatic	υ (C-O) cm $^{-1}$ acid	υ (C=O) cm $^{-1}$ carboxylic	υ (O-H) cm $^{-1}$ carboxylic	υ (C-N) cm $^{-1}$	υ (C-O-C) cm $^{-1}$ Ether	υ (C-H) cm $^{-1}$ aliphatic	υ other band cm $^{-1}$
pectin	3290 broad	3101	1637	1587	1222 strong	1710	2400-3500 very broad	1336 strong	1012-1219 strong	2961-2852	1606-NH bending
P2	3227	-	1620	1558	1257	1716 strong	2400-3500 very broad	1330 weak	1004-1253 strong	2852-2962	1620-NH bending
P2A	3327	3142	1653	1516-1597	1255	1716 strong	2400-3500 very broad	1329 weak	1074-1174 strong	2825-2966	-
P2B	3220	3105	1631	1518	1209 strong	1701	2400-3500 Very broad	1311 medium	1014-1170 strong	2851-2933	1601 –NH bending

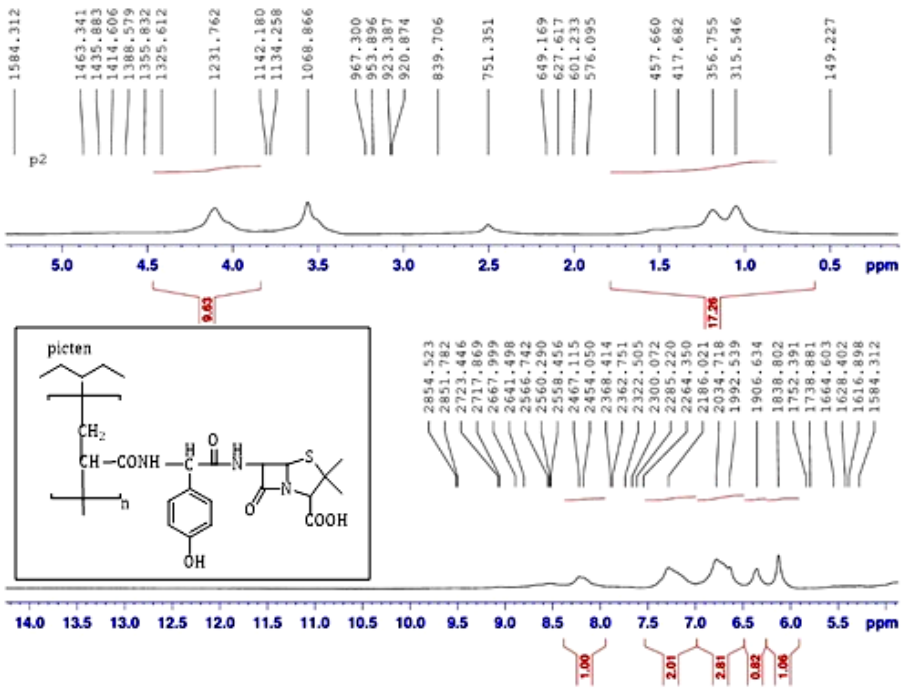


Figure 5. ^{1}H-NMR spectrum of prepared polymer (P2A)

Figure 5. ^{1}H-NMR spectrum of prepared polymer (P2A) was showed in Figure (5), which showed the following signals 1.25 ppm (Triplet, 3H, CH_3), 2.34 ppm (Triplet, 2H, CH_2), 3.6 ppm (Triplet, 2H, CH_2), 6.7 ppm (Singlet,1H, CO-NH amide), 6.8 ppm (Singlet,1H, CO-NH imide), 7.1-7.7 ppm (4H, aromatic ring).

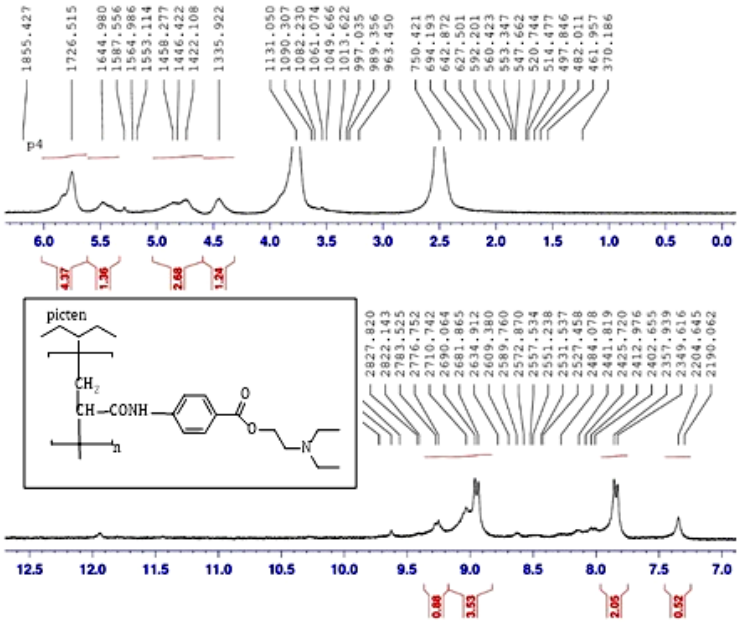
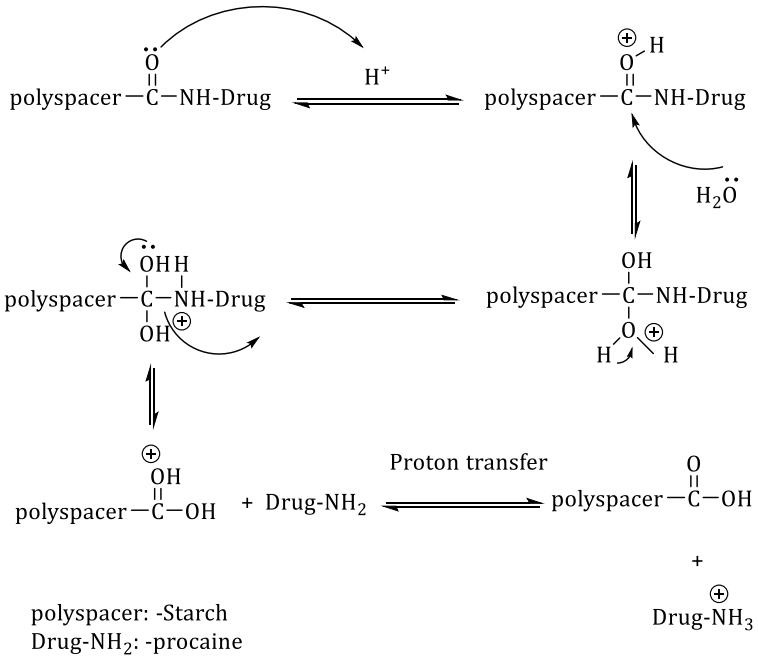
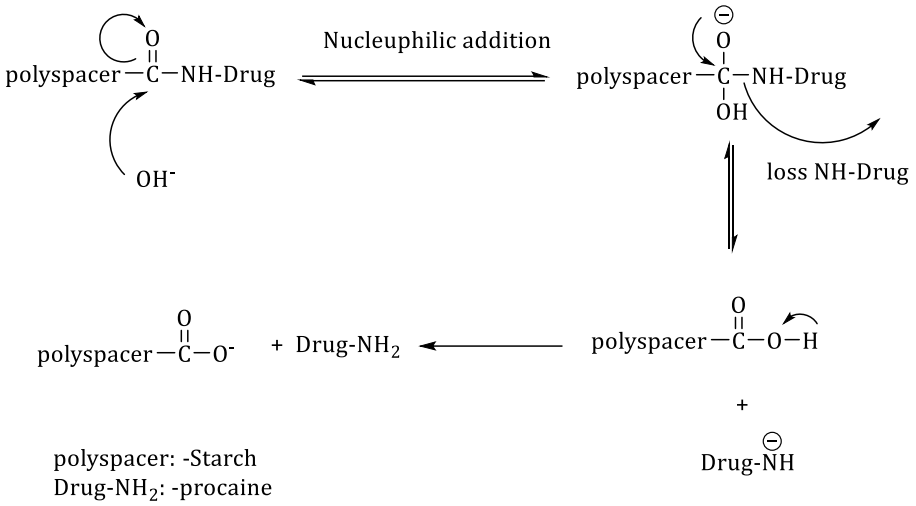


Figure 6. ^{1}H-NMR Spectrum of prepared polymer pectin-g-[*N*-procaine acrylic acid]

Figure 6. ^{1}H-NMR Spectrum of prepared polymer pectin-g-[*N*-procaine acrylic acid] was which showed the following signals 1.2 ppm (Triplet, 3H, CH_3), 6.2 ppm (Singlet, 1H, CO-NH amide), 7.8–7.9 ppm (4H, aromatic ring), 4.5 ppm (Singlet, OH for pectin), 12.0 ppm (Singlet, 1H, COOH). Mechanisms of drug illustrated as shown in the Scheme (4).



Scheme 4. Mechanism of Hydrolysis drug polymer in acidic medium



Scheme 5. Mechanism of Hydrolysis drug polymer in basic medium

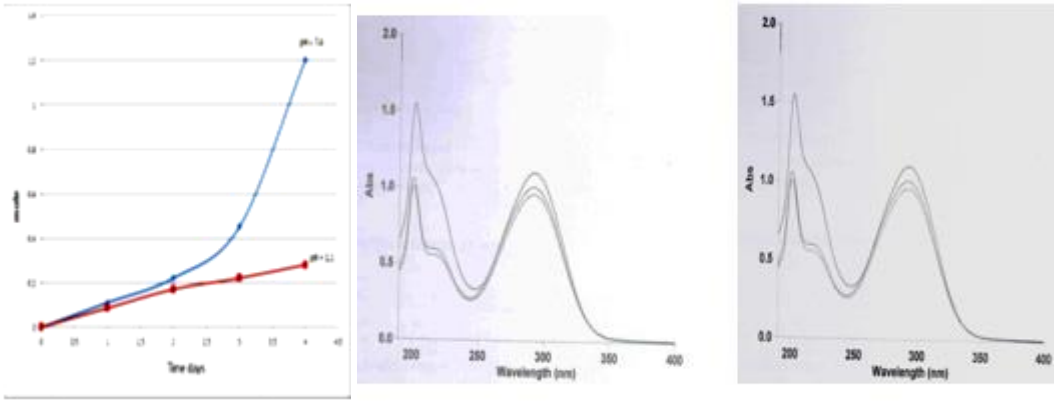


Figure 7. UV spectra hydrolysis of P2A in pH 7.4 and pH 1.1

Thermal stability of prepared polymers were investigated by (TGA and DSC) Table 3. TGA showed the results of some prepared drug polymers which indicated the high thermal resistance and showed their steps of weight loss-temperature. This high thermal resistance indicated the high interaction between amide hydrogen bonding through the polymer chains and led to best sustain drug release. Several thermal stability parameters were determined from TGA and DSC curves as shown in Table 3.

Thermal stability of some selective compounds were investigated by thermo gravimetric analysis (TGA). The change in weight was measured as a function of temperature which gave valuable information about the thermal stability of the prepared compounds. Several thermal stability parameters were determined from TGA and DSC curves as following:

1-Decomposition temperature (DT). Two type of DT were determined initial decomposition temperature (T_{endo}) and the optimum decomposition temperature (T_{exo}).

2-Weight loss temperature (Ts), which was determined from the TG curve, which represents the temperature at which the sample lost of its total weight in this study (17-22) mg, was taken from the prepared polymers under a programmed heating rate of 10 °C/mint. Under inert atmosphere, (N_2 gas 50 mL/mint). Thus, the weight-loss vs. temperature thermo grams were recorded and analyzed. The above parameters which were determined for some of the prepared compounds, were explained and listed in the Table 3.

Table 3a. TGA analysis of some drug polymers

No. drug polymer	Temperature	Losses weight%
P2	121 .281	43, 38
P2A	448	66

Table 3b. DSC analysis of some drug polymers

No. drug Polymer	Onset Temp. °C	End set Temp. °C	Peak Temp. °C	ΔH (J/g)
P2	51.5	111.1	59.7	46.61
P2A	100.8 129.6	. 1287 167.1	109.1 133.3	4.86 10

Showed the results of some prepared drug polymers which indicated the high thermal resistance and showed their steps of weight loss-temperature. This high thermal resistance indicated the high molecular weight of the prepared polymers with high interaction between amide hydrogen bonding through the polymer chains and led to best expire date to protect the drug.

Conclusions

A new bio adhesive polymer was prepared by modification of Pectin structure with acrylic acid ($_{P1}$) as a spacer by using ceric ammonium nitrate (CAN) as an initiator, and grafted copolymer was substituted with amino drugs such as amoxicillin produced amide polymer P1A. This design carries controlled delivery of therapeutic agents which could release the entrapped drug over an extended period of time due to its biodegradable, nontoxic and slow digesting nature. The new drug copolymer was investigated The prepared drug copolymer was analyzed in different pH values at 37 °C in vitro study and controlled drug release was compared at zero time and after many days. The rate of hydrolysis in basic medium was found higher than acidic medium. It was concluded that modified drug release with extended drug action via slow release and *in vivo* performance was noted to be promising. This new adhesive drug biological polymers were applied on different

infected mice and wounds, It gave outstanding results and compliance mice infected with a full recovery by a short period.

Acknowledgement

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