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Microwave-assisted graft copolymerization of amino acid based monomers onto starch and their use as drug carriers

Ali Y.A. Alfaifi [a], Mohamed H. El-Newehy [a,b], E.S. Abdel-Halim [a,*], Salem S. Al-Deyab [a]

[a] Petrochemical Research Chair, Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[b] Department of Chemistry, Faculty of Science, Tanta University, Tanta 31527, Egypt

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ABSTRACT

This paper describes the synthesis of two amino acid-based monomer and their graft copolymerization onto starch and utilization of the prepared graft copolymers as drug carriers. The two monomers were synthesized and reacted with acryloyl chloride to get the corresponding acryloylamino acid, which were further grafted onto starch using the microwave-assisted grafting technique. All factors affecting the efficiency of the grafting reaction were studied and the prepared graft copolymers were fully characterized. Atenolol, as a model drug in the form of salt was immobilized onto the graft copolymers by ionic bonds and the loading was confirmed by use of FT-IR, TGA and NMR. The drug release was studied in both acidic and alkaline media and it was found that the release takes place in alkaline medium rather than in acidic medium and this indicates that these polymers can be used as carriers for drugs whose target is the colon.

1. Introduction

The use of microwave irradiation instead of conventional heating offers the advantages of pollution reduction, low cost and high productivity, this is in addition to simple handling and processing (Lidstrom, Tierney, Wathey, & Westman, 2001; Osman, El-Newehy, Al-Deyab, & El-Faham, 2012). Recently, the utilization of microwave irradiation in organic synthesis and polymerization reaction has become a popular technique (Al-Hazimi, El-Faham, Gazzali, & Al-Farhan, 2012; Ghazzal, El-Faham, Abd-Megeed, & Al-Farhan, 2012; Osman et al., 2012). Many researchers reported the use of microwave-assisted synthesis in the preparation of different types of flocculants based on natural polymers, like polyacrylamide grafted inulin (Rahul, Jha, Sen, & Mishra, 2014), polymethylmethacrylate grafted psyllium (Mishra, Sinha, Dey, & Sen, 2014), polyacrylamide grafted agar (Rani, Mishra, Sen, & Jha, 2012) and polyacrylamide grafted Casein (Sinha, Mishra, & Sen, 2013).

The utilization of drug delivery systems based on polymeric materials improves the drug's efficiency, reduces the drug's toxicity, reduces the drug's side effects and improves the recovery percentages. In general, the controlled-release drug delivery systems were found to increase the therapeutic activity of the drug, and on the other hand decrease its side effects and reduces the number of drug required to be admitted to the body during treatment period as well. Also, the use of controlled-release drug delivery system enables the drug to be targeted only to desired organs or tissues (Friend, 2005; George & Abraham, 2006; Kenawy, El-Newehy, Abdel-Hay, & Raphael, 2001; Kenawy, El-Newehy, Abdel-Hay, & Ottenbrite, 2008; Van den Mooter, Weuts, De Ridder, & Blaton, 2006; Yan, Zhuo, & Zheng, 2001).

Moreover, amino acids, as constituents of the peptides, when incorporated into synthetic or natural polymers, by means of for example grafting, they can give totally new biomaterials having a wide variety of properties, which can be modulated by changing the components of the macromolecular backbone during synthesis. Over the past decades, natural polymers such as cellulose, starch and chitosan can be used in designing biomaterials, which can find a wide range of application areas such as in controlled-release drug delivery systems and biocompatible scaffolds in the field of tissue engineering. Moreover, modification of polysaccharide using grafting as a powerful method improves its properties as well as enlargement its use.

In the past decades, polymer–drug conjugates were used to develop highly advanced controlled-release drug delivery systems, which could to great extent improve the drug's therapeutic efficiency (Babazadeh, 2007, 2008; El-Newehy, Elsherbiny, & Mori, 2013; Hoste, Winne, & Schacht, 2004; Kenawy, El-Newehy, et al., 2008; Kenawy, Abdel-Hay, El-Newehy, & Ottenbrite, 2008; Khandare & Minko, 2006; Nichifor, Schacht, & Seymour, 1997). Optimization of the therapeutic properties of drugs, safety and

* Corresponding author.
E-mail address: essamya@yahoo.com (E.S. Abdel-Halim).

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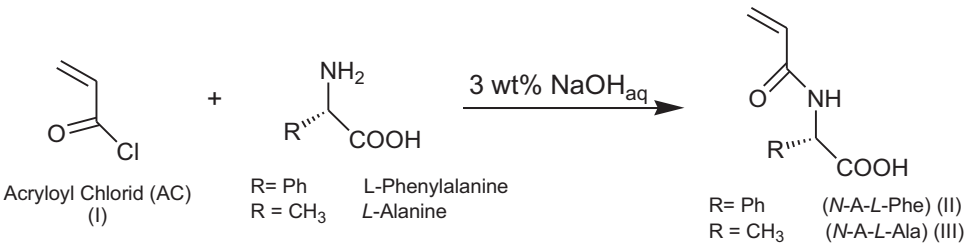


Scheme 1. Monomer synthesis; *N*-acryloyl-*L*-phenylalanine (*N*-A-*L*-Phe) (II) and *N*-acryloyl-*L*-alanine (*N*-A-*L*-Ala) (III).

affectivity can be controlled by the design of polymeric drug delivery systems [10,11]. The drug can be fixed directly onto the polymer backbone or via a spacer in which the carrier is a polymeric material and could be either inert synthetic polymer or natural biodegradable one (Babazadeh, 2008; El-Newehy et al., 2013).

Amino acids, the constitutional components of both peptides and accordingly the higher proteins are capable of constructing highly ordered hierarchical structures, which may scale from few nanometers to several micrometers. Synthetic polymers containing amino acids can be used in different applications such as stationary phases with chiral recognition properties (Babazadeh, Edjlali, & Rashidian, 2007), absorbents for different metal ions (Oishi, Lee, Nakagawa, Onimura, & Tsutsumi, 2002), controlled-release drug delivery systems and biocompatible materials (Barbucci, Casolaro, & Magnani, 1991; Lekchiri, Morcellet, & Morcellet, 1987).

Starch as a natural polymer that can be obtained from agro-sources, is abundant in nature, renewable, and biodegradable polymer (Bentolila et al., 2000; Casolaro & Barbucci, 1991).

In addition, starch is a promising material in drug delivery specifically to the colon, non-food applications as well as the production of biodegradable plastics drug delivery (Çalgeris, Çakmakçı, Ogan, Kahraman, & Kayaman-Apohan, 2012; Casolaro & Barbucci, 1991; Curvelo, de Carvalho, & Agnelli, 2001)

The work in the present study aims at optimizing the grafting conditions for amino acid-based polymers onto starch to prepare amino acid-based polymers-grafted-starch as a carrier for drugs. Grafting process was carried out by use of low concentration of potassium persulfate as an initiator, using microwave (MW) irradiation technique. The effect of grafting time, temperature and the monomer/starch ratio were studied. The structure of the prepared amino acid-based monomers-grafted-starch was confirmed by Fourier transform infrared (FTIR) spectra, elemental microanalysis (as nitrogen percent (N%)) as well as NMR spectra. In addition, the native and grafted starch samples were characterized by TGA. The grafting process was found to be temperature and duration dependent, in addition to its great dependence on the monomer/starch ratio. Atenolol was used as a model drug in this study and its loading and controlled release from the amino acid-grafted-starch were extensively studied.

2. Experimental

2.1. Materials

Starch (Corn starch) (73% amylopectin and 27% amylose), acryloyl chloride and atenolol were purchased from Aldrich. *L*-alanine (98%) and *L*-phenylalanine (98%) were purchased from ACROS. All other chemicals, materials and solvents were of laboratory grade and were used as received without any further purifications.

2.2. Instruments and characterization techniques

Microwave (Monowave 300, Anton paar) maximum filling volumes of 6 mL and 20 mL, for 10 mL vial and 30 mL vial, respectively. Maximum operation pressure 30 bar, Maximum IR temperature 300 °C, Max fiber-optic temperature 300 °C, Maximum power 850 W, Vial material is borosilicate glass and silicon carbide, Cap material is PEEK and Seal material is Teflon-coated silicone.

^{1}H NMR spectra were recorded by use of JEOL JNM-PM X90 Si-NMR Spectroscopy instrument. Thermogravimetric analysis (TGA) was carried out by use of TA-Q500 System. 5–10 mg sample was heated in the temperature range of 30–800 °C at a heating rate of 10 °C min^{-1} under inert nitrogen atmosphere.

Fourier-transformer infrared (FT-IR) spectra were recorded using TENSOR 27, Bruker. UV spectra was recorded by use of PerkinElmer Lambda 35 UV–vis spectrophotometer.

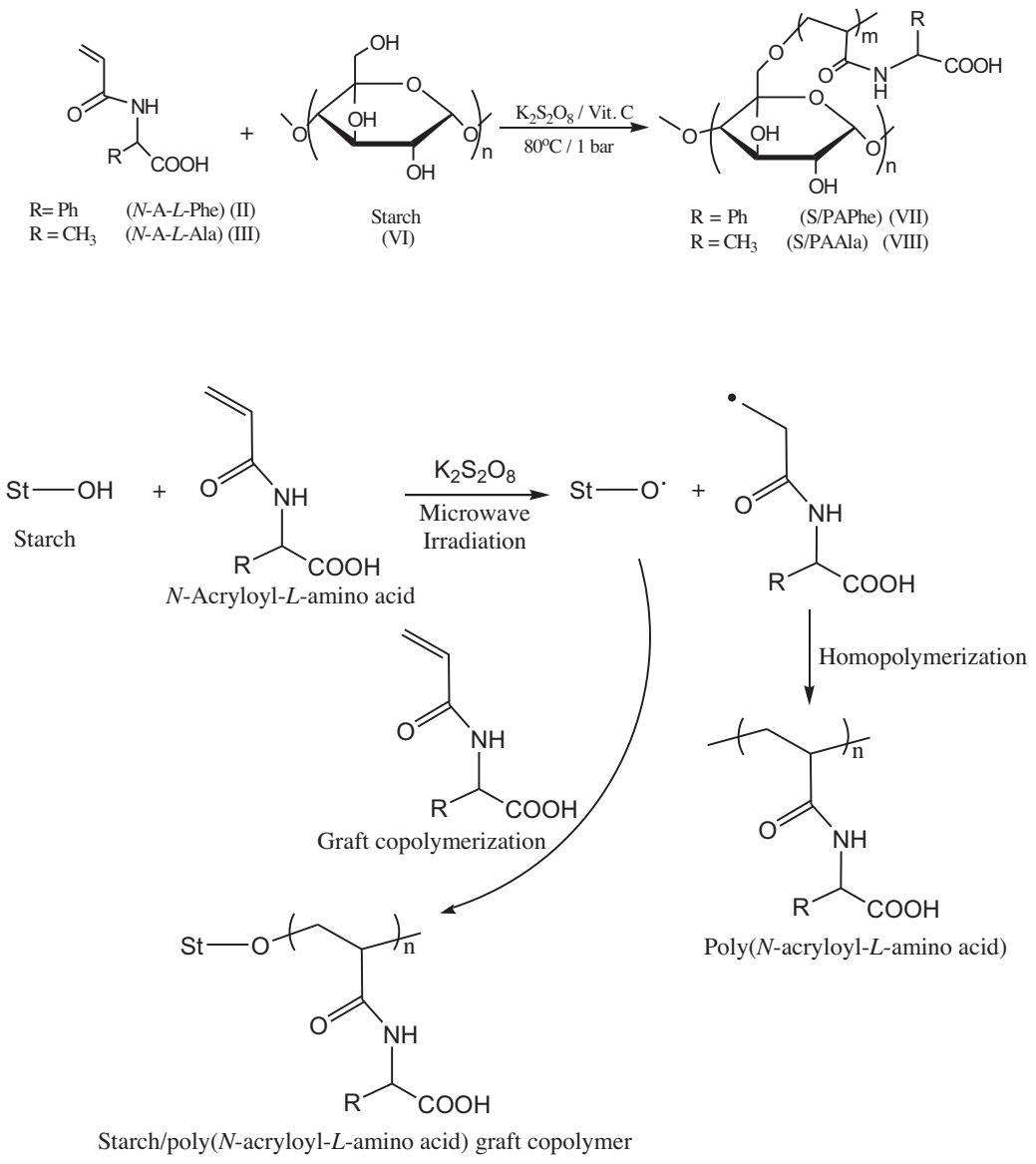
2.3. Monomers synthesis

N-acryloyl-*L*-phenylalanine (*N*-A-*L*-Phe) (II) and *N*-acryloyl-*L*-alanine (*N*-A-*L*-Ala) (III) were synthesized according to a reported method [11], in which *L*-phenylalanine (150 mmol) was dissolved in 100 mL of 3 wt% aqueous NaOH and then was kept at 0 °C in an ice-bath. Acryloyl chloride (160 mmol) was added dropewise and at the end of the addition, the reaction mixture was kept under continuous stirring for 2 h. The mixture was then acidified to pH 1–3 using 6 N HCl, and stirring was continued for an additional 1 h at 25 °C. The resulting mixture was extracted with ethyl acetate (100 mL) for three times and dried over anhydrous manganese sulphate. The solution was filtered and concentrated on rotavapor to one fourth. The residual solution was purified by the column chromatography technique, by use of ethyl acetate as an eluent (Scheme 1).

2.4. Microwave assisted grafting of monomers onto starch

2.4.1. Microwave assisted synthesis of starch/Poly(N-acryloyl-L-phenylalanine) graft copolymer (S/PAPhe) (VI)

Calculated amount of (*N*-A-*L*-Phe) (II), dissolved in water, was added to starch in water slurry. These components were mixed well and then transferred, quantatively to the microwave reaction vessel (30 mL), together with catalytic amount of potassium persulfate (KPS) initiator (0.090 g) and the reaction vessel was claimed on the turntable of the microwave oven. The microwave irradiation time is adjusted to the desired duration. After the irradiation time is vanished, the reaction vessel was removed from the microwave oven and its contents were allowed to cool to the room temperature and the kept as such at room temperature for extra 24 h in order for the grafting reaction to be completed. The graft copolymer in the form of gel-like mass was poured from the microwave reaction vessel into a beacker containing excess amount of acetone in order to precipitate the graft copolymer and separate it



Scheme 2. Microwave-assisted grafting of *N*-acryloyl-*L*-phenylalanine (*N*-A-*L*-Phe) (II) and *N*-acryloyl-L-alanine (*N*-A-*L*-Ala) (III) onto starch (VI); (S/PAPhe) (VII); (S/PAAla) (VIII).

from any homopolymer and other reaction ingredients. The system is filtered and the obtained precipitate was washed several times with acetone and allowed to dry and finally grinded to fine particles.

2.4.2. Microwave assisted synthesis of N-Acryloyl-L-Alanine grafted starch (S/PAAla) (VIII)

The entitled compound was synthesized according to the same procedure described above using the following quantities; (0.33 g) starch (VI) was dissolved in 10 mL of distilled water and different ratios of (*N*-A-*L*-Ala) (III) were dissolved, each in (7 mL) distilled water. Microwave irradiation was performed at 80 °C, for 3 min (Scheme 2).

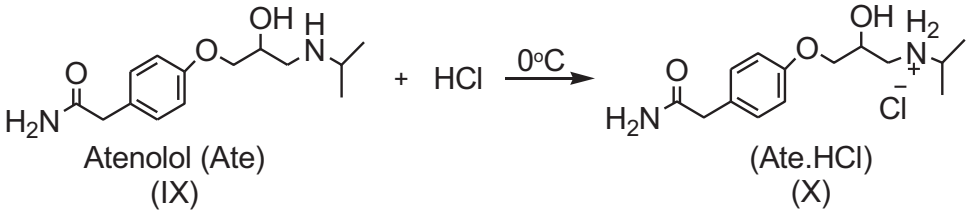
2.5. Synthesis of atenolol hydrochloride (Ate.HCl) (X)

Concentrated hydrochloric acid (4 mL) was added, dropwise to a cold atenolol solution (IX) (3.75 mmol in 15 mL of distilled at 0 °C). The mixture was kept under continuous stirring for 2 h at room temperature. The product was diluted with excess distilled water and then evaporated on Rotavapor till dryness in order to remove the excess hydrochloic acid (Scheme 3).

2.6. Immobilization of drug onto monomer grafted starch

2.6.1. Immobilization of drug onto N-acryloyl-L-phenylalanine grafted starch (S/PAPhe-ate) (XI)

N-acryloyl-*L*-phenylalanine grafted starch (S/PAPhe) (VII) (1.31 mmol) was added to a (5 mL) sodium bicarbonate solution (1.19 mmol in distilled water) and kept under continuous stirring for 1 h. Atenolol hydrochloride (Ate.HCl) (X) (1.38 mmol) was added and the stirring was continued for extra 2 h. The reaction product was separated by filtration, washed thoroughly with an excess amount of distilled water and was finally dried in a vacuum oven at 40 °C overnight to get 91.2% yield (Scheme 4).



Scheme 3. Synthesis of atenolol hydrochloride (Ate.HCl) (X).

2.6.2. Immobilization of drug onto N-Acryloyl-L-Alanine grafted starch (S/PAAla-ate) (XII)

The entitled compound was immobilized in the same way as described above, but using the following quantities; *N*-acryloyl-*L*-alanine grafted starch (S/PAAla) (VIII) (1.97 mmol), sodium bicarbonate (1.72 mmol) and atenolol hydrochloride (Ate.HCl) (X) (2.04 mmol) to get 95% yield (Scheme 4).

2.7. Determination of total atenolol content

2–5 mg of the material was shaken and suspended well in 3 mL of alkaline solution, of pH 9.0 (diluted sodium hydroxide solution). The suspension was kept during the measuring time always at 60 °C, in a thermostatic conditions and the released amount of atenolol was estimated by use of a UV spectrophotometer at λ_{max} = 224 nm.

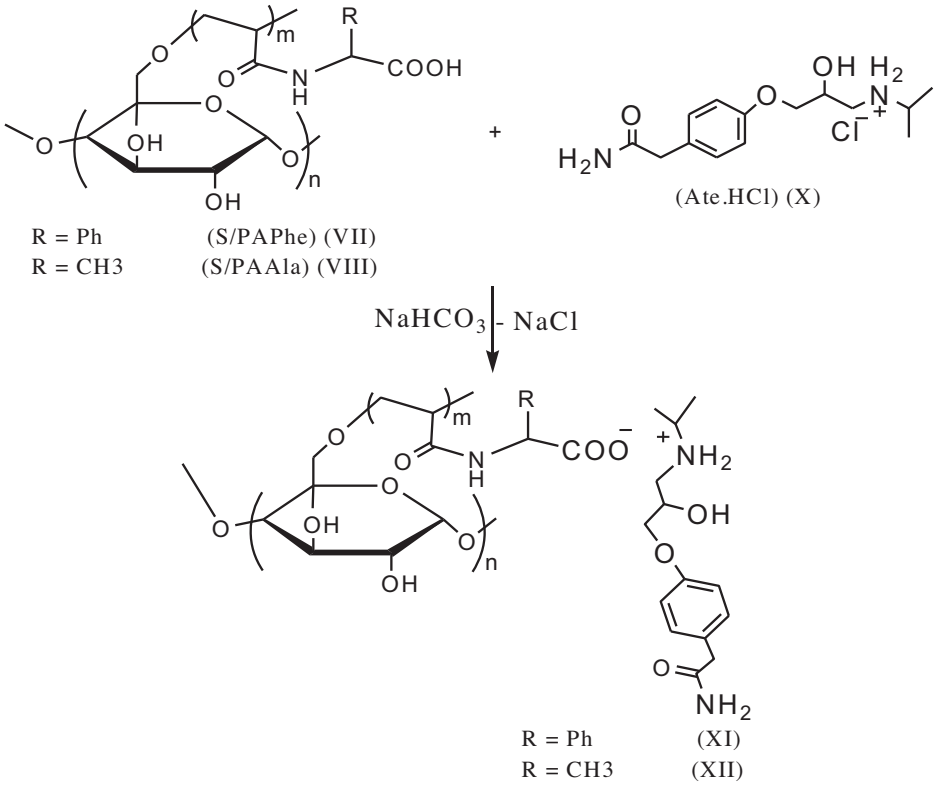
2.8. In vitro drug release

The amount of released atenolol (IX) was estimated, as a function of time using a UV spectrophotometer at (λ_{max} = 224 nm). Known amount of the graft copolymer-atenolol adducts (5 mg) was introduced into a (3 mL) vial, containing phosphate buffer solution at different pH values, namely, 2, 7 and 9 keeping the temperature at 37 °C, like the body temperature. All experiments were done in triplicate and the cumulative amount of released atenolol was recorded, based on calculating the amount of the drug released with time.

3. Results and discussion

3.1. Monomer synthesis

The amino acid-carrying monomers, *N*-acryloyl-*L*-phenylalanine (A-L-Phe-OH, II) and *N*-acryloyl-L-alanine (A-L-Ala-OH, III), were prepared by reacting acryloyl chloride with L-phenylalanine and L-alanine, respectively, according to a previously reported method [1,3]. When acryloyl chloride is reacted with the sodium salt of certain amino acid in aqueous solution, and this is followed by acidification of the reaction medium, the obtained product will be mixture of acrylamide and the corresponding amino acid. The resulting mixture in the form of white precipitate is extracted with ethyl acetate for at leasat three times and the extract is concentrated on rotavapor. The obtained residue is purified by use of column chromatography and ethyl acetate is used as an eluent. The yield of *N*-acryloyl-*L*-phenylalanine (A-L-Phe-OH, **II**) was 51% and its m.p. is 126 °C,



Scheme 4. Immobilization of Ate.HCl (X) onto (S/PAPhe) (XI) and (S/PAAla)(X.

Table 1
Elemental microanalysis of compounds II, III and (VI–XII).

Compound code	C%		H%		N%	
	Calculated	Found	Calculated	Found	Calculated	Found
II	65.72	57.68	5.93	5.29	6.389	5.93
III	50.31	49.76	6.28	6.22	9.78	9.89
VI	44.44	39.88	6.17	6.84	–	–
VII*	56.48	46.94	5.78	5.89	3.68	2.48
VIII*	47.36	35.97	5.92	5.61	4.60	0.42
XI	53.84	46.74	6.80	6.78	6.49	2.73
XII	54.70	41.39	7.01	6.06	7.36	3.91

while the yield of *N*-acryloyl-L-alanine (A-L-Ala-OH, **III**) was 43% and its m.p. 162–164 °C (Scheme 1).

3.1.1. Characterization of N-acryloyl-L-phenylalanine (N-A-L-Phe) (II) and N-Acryloyl-L-Alanine (N-A-L-Ala) (III)

The synthesized monomers were characterized using ^{1}H Nuclear Magnetic Resonance, ^{13}C Nuclear Magnetic Resonance and Fourier Transform Infra-Red Spectroscopy and the results are listed below:

(a) for *N-Acryloyl-L-Phenylalanine (N-A-L-Phe) (II)*: ^{1}H NMR (400 MHz, DMSO-d_6, ppm, from Si$(CH_3)_4$): δ 3.1 (m, 2 H,), 4.5 (m, 1 H), 5.56 (d, J = 10.0 Hz, 1 H), 6.10 (dd, 1 H), 7.24 (m, 5 H), 8.46 (d, 1 H), 8.48 (s, 1 H). ^{13}C NMR (400 MHz, DMSO-d_6, ppm, from Si$(CH_3)_4$): δ 173.5 (—*C*OOH), 165.0 (—*C*O_2NH—), 138.1 (—CH_2—*Ar*), 131.8, 129.6, 126.3 (—*C*H=CH_2, Ar.), 54.1 (>*C*HCO_2H), 37.28 (>*C*HCH_2—) ppm. FT-IR (KBr): Characteristic absorption peaks were observed at 3343 cm^{-1} (N—H, amide), 3028 cm^{-1} (C—H, Aromatic), 2920 and 1651 cm^{-1} (C=C, vinylic), 1818 cm^{-1} (C=O, carboxylic), 1711 cm^{-1} (C=O, amide), 1454 cm^{-1} (C—C), 1438–1065 cm^{-1} (C—N).

(b) *for N-Acryloyl-L-Alanine (N-A-L-Ala) (III)*: ^{1}H NMR (400 MHz, DMSO-d_6, ppm, from Si$(CH_3)_4$): δ 8.4 (1H, —CONH—), 6.28–6.25 (2H, —CH=CH_2), 5.6 (1H, —CH=CH_2), 4.26 (1H, >$CHCO_2$H), 1.3 (3H, —CH_3) ppm. ^{13}C NMR (400 MHz, DMSO-d_6, ppm, from Si$(CH_3)_4$): δ 174.6 (—COOH), 164.8 (—*C*HCONH—), 131.8 (*C*H=CH_2), 126.1 (—CH=CH_2), 48.0 (—*C*HCOOH), 17.7 (—*C*H_3) ppm. FT-IR (KBr): characteristic absorption peaks were observed at 3307 (N—H, amide), 3080 (—CH_3), 2991, 1550 (C=C, vinylic), 1952 (C=O, carboxylic), 1732 (C=O, amide), 1460 (C—C), 1460–1066 (C—N) cm^{-1}.

In addition to the above mentioned characterizations, the structure of the prepared monomers was confirmed by elemental microanalysis and the obtained results are found to be in a good agreement with the calculated ones, as shown in Table 1.

3.2. Microwave assisted synthesis

The aim of this work is to identify the appropriate conditions for grafting a candidate synthesized monomers (*N-Acryloyl-L-Phenylalanine and N-Acryloyl-L-Alanine*) onto starch to prepare amino acid-based polymers-grafted-starch as a drug carrier as shown in Scheme 2.

Grafting process was carried out in the presence of a low concentration of potassium persulfate initiator using microwave (MW) irradiation techniques. The effect of grafting time, temperature and the monomer/starch ratio were studied. The structure of the prepared amino acid-based monomers-grafted-starch was confirmed by Fourier transform infrared (FT-IR) spectra, elemental microanalysis (as nitrogen percent (N%)) as well as NMR spectra. The thermal properties of both native and grafted starch samples were investigated by use of TGA.

3.2.1. Microwave assisted synthesis of N-acryloyl-L-phenylalanine grafted starch (S/PAPhe) (VII)

3.2.1.1. Effect of temperature. The same procedure mentioned above for microwave-assisted grafting was carried out at different temperatures (60, 70, 80 and 100 °C) using the following quantities; starch (VI) (0.33 g), suspended in 10 mL of distilled water and (*N*-A-*L*-Phe) (II) (0.33 g), dissolved in (7 mL) water and the microwave irradiation was performed for 3 min.

The temperature at which the grafting process is conducted has noticeable effect on the grafting process. Different grafting trials were carried out at different temperatures, namely, 60, 70, 80 and 100 °C. The FT-IR spectra displayed in Fig. 1a indicate that 80 °C is the optimum temperature for the microwave-assisted grafting of (APhe) onto starch, that the FT-IR spectra at this temperature show two absorption bands at 1729 and 1657 cm^{-1}, which are corresponding to the carboxylic carbonyl group and amide carbonyl group, respectively. In addition to the appearance of absorption band at 1456 cm^{-1} which is attributed to (C—N).

3.2.1.2. Effect of time. The same procedure mentioned above for microwave-assisted grafting was carried out but for different time intervals (2.0 min, 2.5 min, 3.0 min and 5.0 min) at the same temperature 80 °C using the following quantities; starch (VI) (0.33 g), suspended in 10 mL of distilled water and (*N*-A-*L*-Phe) (II) (0.33 g), dissolved in (7 mL) water.

The grafting process was found to be enhanced with increasing the irradiation time, keeping constant microwave power and temperature. The optimum duration for the said grafting process was found to be 3 min. This is attributed to the availability of more microwave energy, which results in improving the initiation efficiency by generating more free radicals. As shown in Fig. 1b, 3 min represents the optimum exposure time, which is proved by the presence of the two absorption bands at 1664 cm^{-1} and 1648 cm^{-1}, which are corresponding to the carboxylic carbonyl group and amide carbonyl group, respectively.

3.2.1.3. Effect of monomer/starch ratio. The same procedure mentioned above for microwave-assisted grafting was carried out but different monomer/starch ratio (1, 1.5, 2 and 2.5) at the same temperature 80 °C and for the same irradiation period (3 min) using the following quantities, starch (VI) (0.33 g), suspended in 10 mL of

Table 2
Microwave assisted synthesis of Starch/Poly(*N*-acryloyl-*L*-phenylalanine) graft copolymer (S/PAPhe) (VII) and *N*-Acryloyl-*L*-alanine (S/PAAla) grafted starch (VIII) with different starch/monomer ratio.

Code	VI		II or III		Time of irradiation (min)	Temperature (°C)
	Ratio	Wt. (mg)	Ratio	Wt. (mg)		
VII or VIII	1	0.33	1.0 1.5 2.0 2.5	0.330 0.495 0.660 0.825	3	80

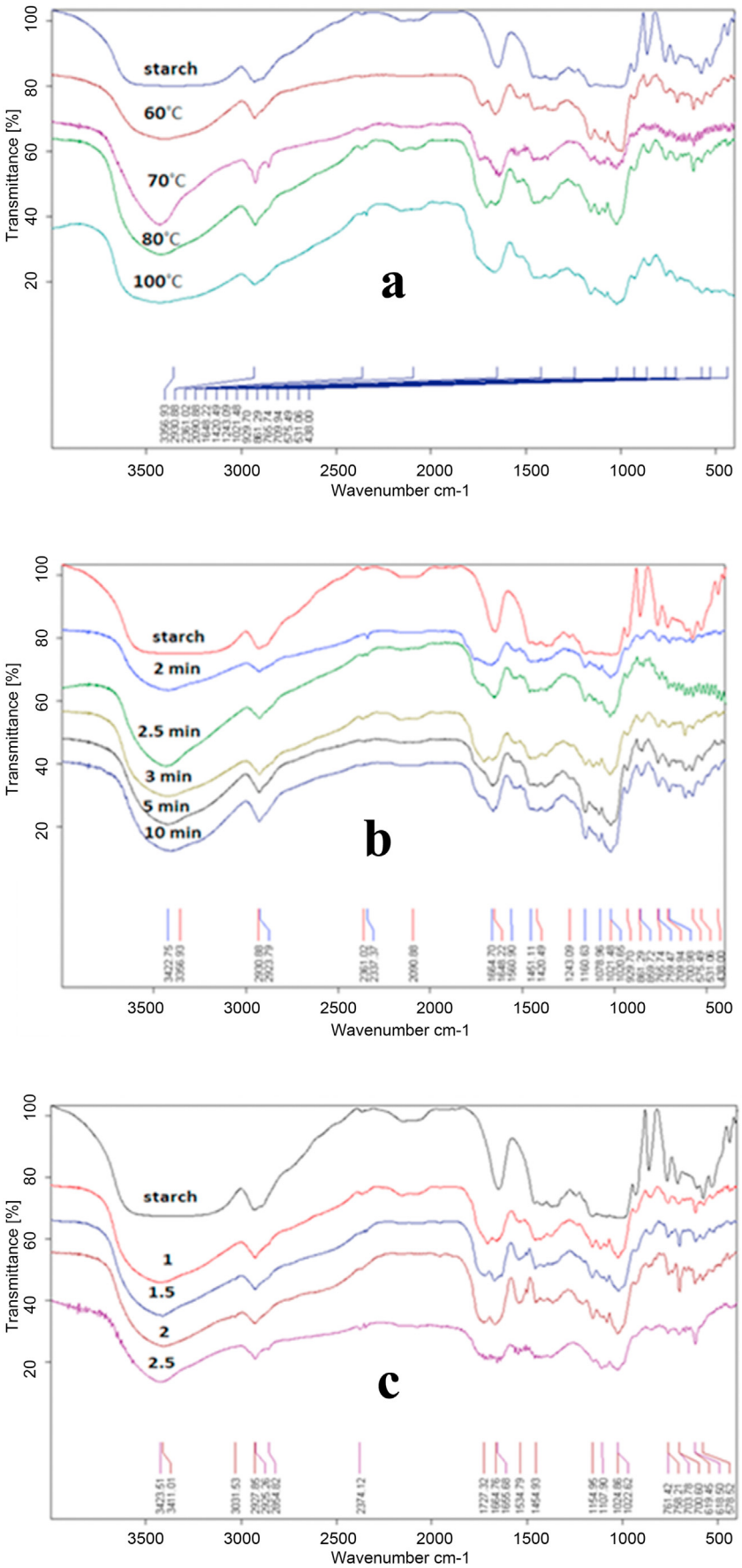


Fig. 1. FT-IR of (a) (S/PAPhe) (VII) at different temperature; (b) (S/PAPhe) (VII) at different time; and (c) (S/PAPhe) (VII) using different monomer ratio.

Table 3
Elemental microanalysis of (S/PAPhe) (VII); and (S/PAAla) (VIII) with different (*M*/*S*) ratio.

Polymer code	*M*/*S* ratio[a]	C%	H%	N%
VII	1.0/1	39.70	6.18	0.20
	1.5/1	43.45	6.11	1.50
	2.0/1	46.94	35.97	2.48
	2.5/1	37.82	5.26	1.18
VIII	1.0/1	40.78	6.47	0.67
	1.5/1	38.00	6.31	0.26
	2.0/1	35.97	35.97	0.42
	2.5/1	34.49	5.40	0.50

[a] *M*/*S* ratio = monomer/starch ratio.

distilled water and different ratios of (*N*-A-*L*-Phe) (II) or (*N*-A-*L*-Ala) (III), dissolved in (7 mL) water as shown in Table 2.

Graft yield was found to increase with each increase in the monomer/starch ratio and the optimum graft yield was obtained on using monomer/starch ratio of 2:1, considering the same microwave irradiation time of 3.0 min and the same power and temperature. The increase in the graft yield by increasing the monomer/starch ratio is attributed to the more availability of monomer units in the vicinity of the grafting site of polysaccharide. Upon increasing the monomer/starch ratio up to 2.5, the graft yield decreases again which may be due to the priority of forming homopolymer at this high monomer/starch ratio. The improvement of the grafting process at monomer/starch ratio of 2:1 is also clear from the FT-IR spectra in Fig. 1c that the spectra show the presence of the two absorption bands at 1727 and 1664 cm^{-1}, which are corresponding to the carboxylic carbonyl group and amide carbonyl group, respectively.

In addition the same conclusion was confirmed by elemental microanalysis (as nitrogen percent (N%)) in which the nitrogen percent changed in the order (N% (*M*/*S* ratio); 0.2(1/1), 1.5(1.5/1), 2.48(2/1), 1.18(2.5/1). The nitrogen percent reached maximum of 2.48% with *M*/*S* ratio of (2/1) which in a good agreement with the FTIR results as shown in Table 3.

3.2.1.4. Thermal analysis of (S/PAPhe) (VII) with different (M/S) ratios. The thermal degradation analysis was done with a heating rate of 10 °C min^{-1} under nitrogen atmosphere. For polymer (IV), the initial weight loss at 30–100 °C is due to the presence of moisture, and the second loss of 7% at 100–223 °C is due to the loss of CO_2 followed by a weight loss of 16.4% at 223–274 °C, which is due to the degradation of the grafted amino acid. The rate of weight loss of 25.7% over 274 °C is due to the degradation of the polymer backbone. It started to decompose at (T_{on}) 234 °C and left a residue of 50.5% at 600 °C. For starch (VI), the initial weight loss of 3.2% at 30–100 °C is due to the presence of moisture, and the second loss of 83.29% at 100–400 °C is due to the degradation of the starch backbone. The rate of weight loss of 6.64% over 400 °C is due to the degradation of the polymer reminder. It started to decompose at (T_{on}) 304 °C and left a residue of 6.54% at 600 °C.

Table 4 summarizes the TGA results of (S/PAPhe) (VII) with different (*M*/*S*) ratios. The TGA of polymer (V) showed a decrease

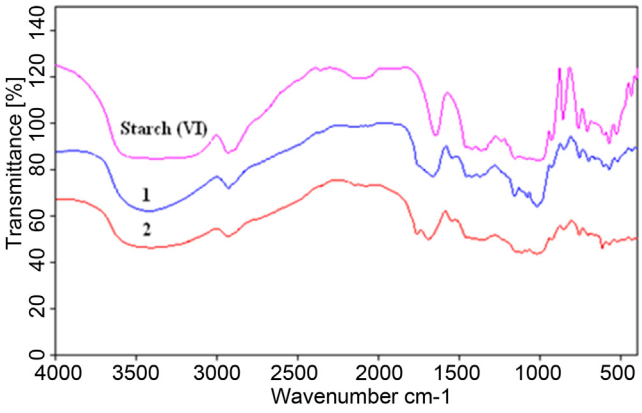


Fig. 2. FTIR of (S/PAAla) (VIII) using different monomer ratio.

in the value of T_{on} as the monomer ratio increase and then increase again with *M*/*S* (2.5/1). This may be due to the grafted polyacrylamide onto starch. The same conclusion was observed with the temperature at which the grafted copolymer losses its 50%.

3.2.2. Microwave assisted synthesis of N-Acryloyl-L-Alanine grafted starch (S/PAAla) (VIII)

N-Acryloyl-*L*-Alanine grafted starch (S/PAAla) (VIII) was prepared as described earlier for *N*-acryloyl-*L*-phenylalanine grafted starch (S/PAPhe) (VII) at 80 °C for three minutes. In a similar way, the effect of the monomer/starch ratio was studied just to show the effect of the bulk group of the immobilized amino acids.

3.2.2.1. Effect of monomer/starch ratio. Graft yield was found to decrease upon increasing the monomer/starch ratio and the optimum ratio was found to be 1:1 for exposure time of 3.0 min at 80 °C. The decrease in the graft yield may be due to the priority of forming homopolymer at this high monomer/starch ratio. The improvement of the grafting process at monomer/starch ratio of 1:1 is also clear from the FT-IR spectra in Fig. 2 that the spectra showed the presence of the two absorption bands at 1763 and 1695 cm^{-1}, which are corresponding to the carboxylic carbonyl group and amide carbonyl group, respectively.

In addition the same conclusion was confirmed by elemental microanalysis (as nitrogen percent (N%)) in which the nitrogen percent changed in the order (N% (*M*/*S* ratio); 0.2(1/1), 1.5(1.5/1), 2.48(2/1), 1.18(2.5/1). The nitrogen percent reached maximum of 0.67% with *M*/*S* ratio of (1/1) which in a good agreement with the FTIR results as shown in Table 3.

3.2.2.2. Thermal analysis of (S/PAAla) (VIII) with different (M/S) ratios. The thermal degradation analyses were done with heating rate of 10 °C min^{-1} under nitrogen atmosphere. For polymer (IV), the initial weight loss of 2% at 30–100 °C is due to the presence of moisture, and the second loss of 4.6% at 100–211 °C is due to the loss of CO_2 followed by a weight loss of 30.6% at 211–320 °C is due

Table 4
TGA results of (S/PAPhe) (VII) with different (*M*/*S*) ratios.

(*M*/*S*) ratio	Distribution of volatile ranges (temperature range) (°C)				Residue (%) at 600 °C	T_{on} (°C)	50% Loss at (°C)
	Moisture 30–150	CO_2 evolution & Grafted group 150–225	Starch 225–450	Remainder 450–600			
(1.0/1)	5.93	72.82	8.83	9.73	338	321	
(1.5/1)	3.85	6.87	57.13	6.29	25.78	287	330
(2.0/1)	3.75	4.61	61.68	4.80	24.90	265	347
(2.5/1)	3.20	2.05	13.64	6.07	34.10	278	349

to the degradation of the grafted amino acid. The rate of weight loss of 46.7% over 320 °C is due to the degradation of the polymer backbone. It started to decompose at (T_{on}) 261 °C and left a residue of 15.4% at 600 °C. For starch (VI), the initial weight loss of 3.2% at 30–100 °C is due to the presence of moisture, and the second loss of 83.29% at 100–400 °C is due to the degradation of the starch backbone. The rate of weight loss of 6.64% over 400 °C is due to the degradation of the polymer reminder. It started to decompose at (T_{on}) 304 °C and left a residue of 6.54% at 600 °C.

Table 5 summarizes the TGA results of (S/PAAla) (VIII) with different (*M*/*S*) ratios. The TGA of polymer (VIII) showed an increase in the value of T_{on} as the monomer ratio increases. This may be due to decrease in the grafted polymer onto starch. The same conclusion was observed with the temperature at which the grafted copolymer losses its 50%.

3.3. Immobilization of drug onto N-acryloyl-L-phenylalanine grafted starch (St-g-A-Ph-ate) (XI) and N-Acryloyl-L-Alanine grafted starch (St-g-A-Ala-ate) (XII)

3.3.1. Characterization of N-acryloyl-L-phenylalanine grafted starch (S/PAPhe) (VII) (M/S ratio: 2/1)

N-acryloyl-*L*-phenylalanine grafted starch (S/PAPhe) (VII) was prepared using microwave with a *M*/*S* ratio of 2/1 at 80 °C for 3 min. The product was characterized by FTIR spectra, ^{1}H & ^{13}C NMR, elemental microanalysis as well as thermogravimetric analysis (TGA).

3.3.1.1. FT-IR spectra. FT-IR spectra of the drug immobilized onto *N-Acryloyl-L-Phenylalanine Grafted Starch* were measured to assign the functional groups introduced to graft copolymer backbone after the immobilization. The IR spectra are presented in Fig. 3 and they showed characteristic absorption peaks at 3411 (N—H, amide), 1727 (C=O, carboxylic), 1657 (C=O, amide), 1447–1024 (C—N) cm^{-1}.

3.3.1.2. Nuclear magnetic resonance spectroscopy (NMR). ^{1}H NMR and ^{13}C NMR characterizations were carried out for the drug immobilized onto *N-Acryloyl-L-Phenylalanine Grafted Starch* and the results were as follow:

(a) for ^{1}H NMR (400 MHz, DMSO-d_6, ppm, from Si(CH$_3$)$_4$): δ 11 (m, 1 H), 8 (k, 1 H), 7.12 (p,q,r,s,t, 5 H), 3.16 (n, 2 H), 4.25 (l, 1 H), 2.73 (j, 1 H), 3.73 (i, 1 H), 3.73 (b,d,1H), 2 (c,e, 2, 1H).
(b) for ^{13}C NMR (400 MHz, DMSO-d_6, ppm, from Si(CH$_3$)$_4$): δ 174.9 (l, —COOH), 175.1 (j, —CO$_2$NH—), 139.5 (n, —CH$_2$—*Ar*), 127.8, 128.6, 126 (o,p,q,r,s, —*C*H=*C*H$_2$, Ar.), 55.9 (k, >*C*HCO$_2$H), 36.7 (m, >CH*C*H$_2$—).

3.3.1.3. Elemental microanalysis. In addition the above characterizations, the structure of (S/PAPhe) (VII) was confirmed by elemental microanalysis and the found results were found to be in a good agreement with the calculated ones as shown in Table 2.

3.3.1.4. Thermal analysis of (S/PAPhe) (VII). The thermal degradation analysis were done with heating rate of 10 °C min^{-1} under nitrogen atmosphere. For (S/PAPhe) (VII) with (*M*/*S* ratio; 2/1), the initial weight loss of 3.75% at 30–150 °C is due to the presence of moisture, and the second loss of 4.61% at 150–225 °C is due to the loss of decarboxylation and degradation of amino acid moiety followed by a weight loss of 61.68% at 225–450 °C is due to the degradation of is due to the degradation of the starch backbone. The rate of weight loss of 4.8% over 450 °C is due to the degradation of the polymer reminder. It started to decompose at (T_{on}) 265 °C and left a residue of 24.9% at 600 °C as shown in Table 4.

3.3.2. Characterization of N-Acryloyl-L-Alanine grafted starch (S/PAAla) (VIII) (M/S ratio: 2/1)

3.3.2.1. FT-IR spectra. FTIR spectra were measured to show the new functional groups introduced onto the structure of (S/PAAla) (VIII), as is presented in Fig. 3a. Characteristic absorption bands were recorded at 3398 cm^{-1} for (N—H, amide), 1763 cm^{-1} for (C=O, carboxylic), 1694 cm^{-1} for (C=O, amide) and 1403–1020 cm^{-1} for (C—N).

3.3.2.2. Nuclear magnetic resonance spectroscopy (NMR). ^{1}H NMR and ^{13}C NMR characterizations were carried out for the drug immobilized onto *N-Acryloyl-L-Alanine Grafted Starch* and the results were as follow

(a) for ^{1}H NMR (400 MHz, DMSO-d_6, ppm, from Si(CH$_3$)$_4$): δ 11 (m, 1 H), 8 (k, 1 H), 1.43 (n, 3 H), 4.64 (l, 1 H), 2.93 (j, 1 H), 3.73 (i, 1 H), 3.73 (b,d, 1H), 2 (c, e, 2, 1H).
(b) for ^{13}C NMR (400 MHz, DMSO-d6, ppm, from Si(CH3)4): δ 174.9 (l, —COOH), 175.1 (j, —CO2NH—), 51.0 (k, >CHCO2H), 16.9, 16.2 (f,m, >—CH3),104.2 (a, >—CHO), 74.1, 68.4,74 (b,c,e, >CH), 76.7, 77.9 (g,h, >CH2).

3.3.2.3. Elemental microanalysis. In addition the above characterizations, the structure of (S/PAAla) (VIII) was confirmed by elemental microanalysis and the found results were found to be in a good agreement with the calculated ones as shown in Table 2.

3.4. Sythesis of atenolol hydrochloride(Ate.HCl) (X)

Atenolol hydrochloride (Ate.HCl) (X) was prepared by addition of concentrated hydrochloric acid 0.01 M to a cold solution of atenolol (Ate) (IX) in water as shown in Scheme 3. The product was obtained via evaporation on Rotavapor till dryness to remove excess hydrochloic acid to get Ate.HCl (m.p. 157 °C; Ate. m.p. 152 °C). In addition to that, the obtained product (X) was confirmed by addition of silver nitrate ($AgNO_3$) to Ate.HCl solution, in which we get white precipitate of AgCl.

The FTIR spectra of the formed product (X) showed characteristic absorption peaks were observed at 3198–3071 cm^{-1} (N—H, amide), 1649 cm^{-1} (H_2N—C=O, amide) and 1403–1020 cm^{-1} (C—N).

3.5. Immobilization of atenolol hydrochloride (Ate.HCl) onto N-acryloyl-L-phenylalanine grafted starch (St-g-A-Ph-Ate) (XI) and N-Acryloyl-L-Alanine grafted starch (St-g-A-Ala-Ate) (XII)

N-acryloyl-*L*-phenylalanine Grafted Starch (St-g-A-Ph-Ate) (XI) and *N*-Acryloyl-*L*-Alanine Grafted Starch (St-g-A-Ala-Ate) (XII) were prepared by reaction of Ate.HCl (X) with (S/PAPhe) (VII) and (S/PAAla) (VIII), respectively at room temperature as indicated in Scheme 4. The reaction was stirred for 3 h, and the product was filtered, washed with excess amount of distilled water and was dried in oven under vacuum at 40 °C overnight (91.2% yield).

The elemental analyses data of (St-g-A-Ph-Ate) (XI) as listed in Table 2 showed that (Found/Calculated (%)), C: 53.84/46.74; H: 6.80/6.78; N: 6.49/2.73.

In similar way, the elemental analyses data of (St-g-A-Ala-Ate) (XII) as listed in Table 2 showed that (Found/Calculated (%)), C: 54.70/41.39; H: 7.01/6.06; N: 7.36/3.91.

Based on the nitrogen analysis, the percent of immobilized atenolol for (St-g-A-Ph-Ate) (XI) and (St-g-A-Ala-Ate) (XII) was found to be 42.1% and 53.1%, respectively.

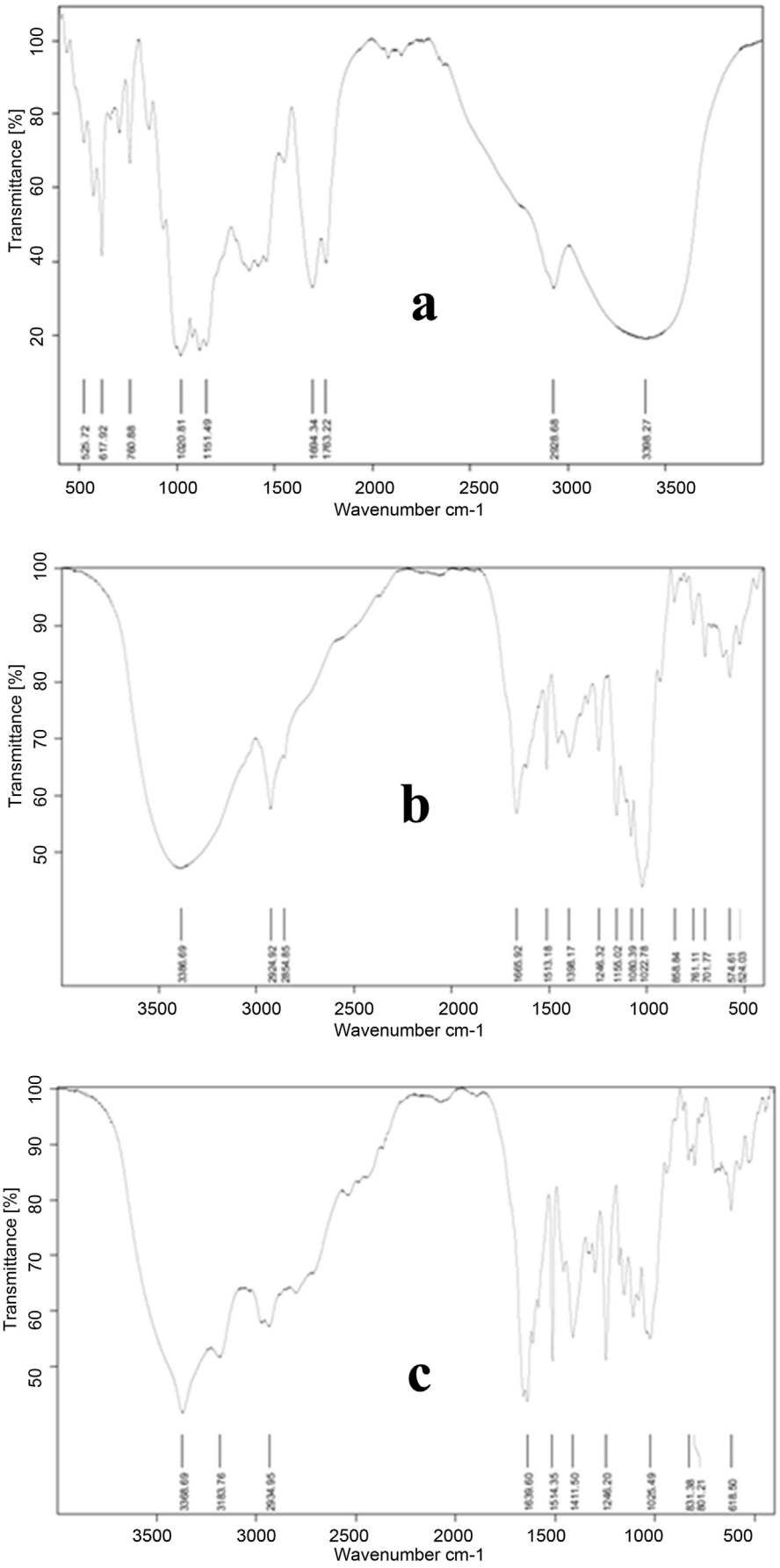


Fig. 3. FTIR spectra of (a) (S/PAAla) (VIII); (b) (S/PAPh-ate) (XI); (c) (S/PAAla-ate) (XII).

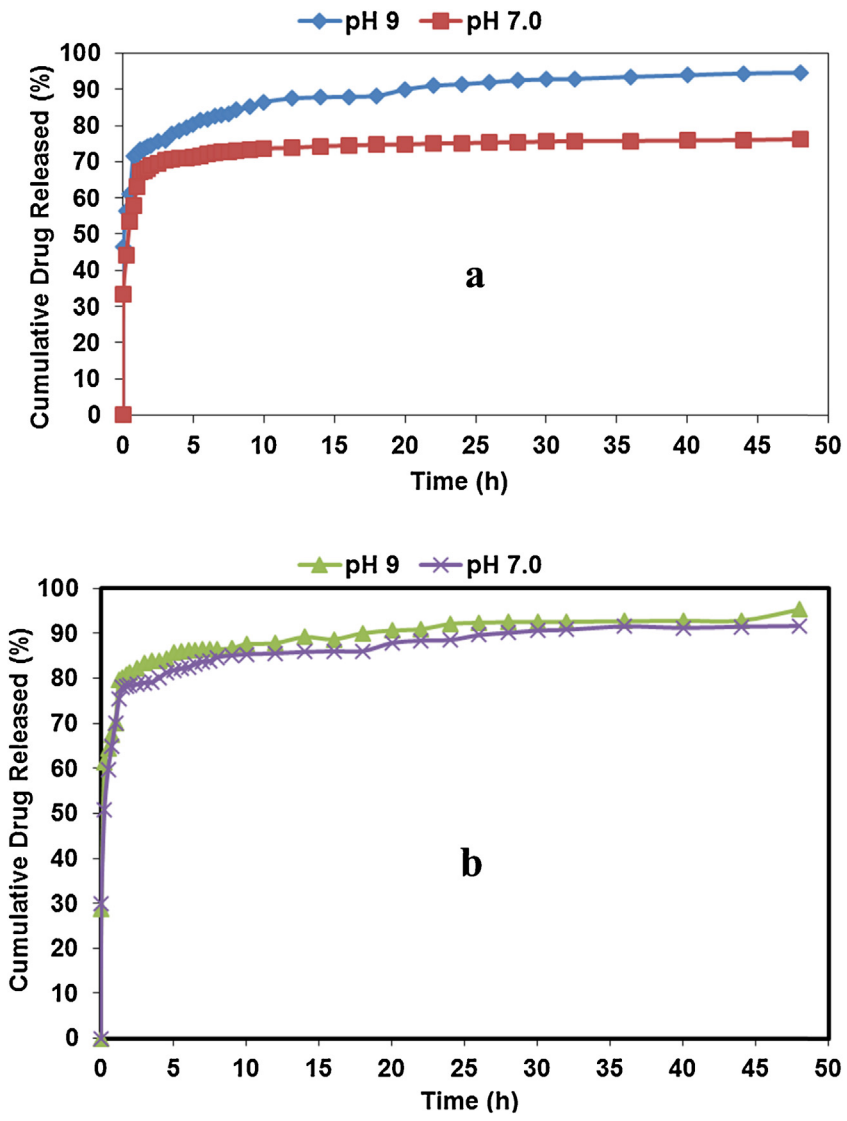


Fig. 4. In vitro release profile of atenolol from (a) (S/PAPhe-ate) (XI); (b) (S/PAAla-ate) (XII) in phosphate buffer with various pH at the body temperature (37 °C).

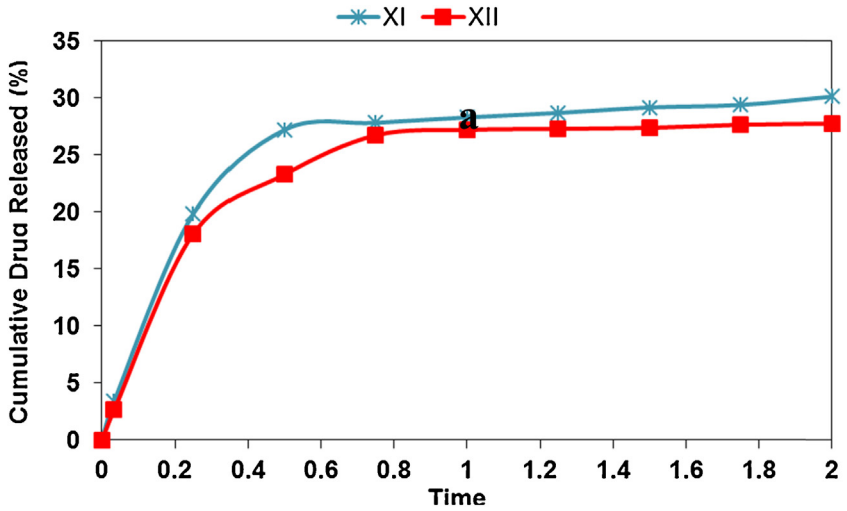


Fig. 5. In vitro release profile of atenolol from (S/PAPhe-ate) (XI) and (S/PAAla-ate) (XII) in phosphate buffer of (a) pH 9; (b) pH 7, at the body temperature (37 °C).

Table 5
TGA results of (S/PAAla) (VIII) with different (*M*/*S*) ratios.

(*M*/*S*) ratio	Distribution of volatile ranges (temperature range) (°C)				Residue (%) at 600 °C	T_{on} (°C)	50% Loss at (°C)
	Moisture 30–150	CO_2 evolution & Grafted group 150–225	Starch 211–400	Remainder 400–600			
(1.0/1)	4.78	9.98	55.26	9.56	19.58	327	314
(1.5/1)	4.19	62.21	10.62	284	306	330	
(2.0/1)	3.88	6.23	51.89	25.24	275	320	347
(2.5/1)	3.52	5.6	49.57	26.00	268	328	349

Generally, for both adducts (XI) and (XII) the percent of N increase in comparison to the started copolymers (VII) and (VIII), respectively as shown in Table 2.

In addition, FTIR spectroscopy was used to investigate the immobilization of Ate.HCl (X) onto (S/PAPhe) (VII) and (S/PAAla) (VIII) as presented in Fig. 3b and c. As shown in Fig. 3b, (St-g-A-Ph-Ate) (XI) showed characteristic absorption peaks at 3198 (N—H, amide), 1666 (C=O, carboxylic), 1649 (H_2N—C=O, amide), 1403–1020 (C—N) cm^{-1}.

As shown in Fig. 3c (St-g-A-Ala-Ate) (XII) showed characteristic absorption peaks at 3183 cm^{-1} (N—H, amide), 1665 cm^{-1} (C=O, carboxylic), 1639 cm^{-1} (H_2N—C=O, amide) and 1398–1022 cm^{-1} (C—N).

3.6. In vitro drug release

The rate of atenolol release from both (S/PAPhe-ate) (XI) and (S/PAAla-ate) (XII) was studied at various pH values, namely, pH 2.0, pH 7.0 and pH 9.0. The rate of drug release is expected to be dependent on the microstructure of the polymer grafted onto starch, how bulky is the amino acid moiety, the hydrophilicity and the pH of the release medium (El-Newehy et al., 2013).

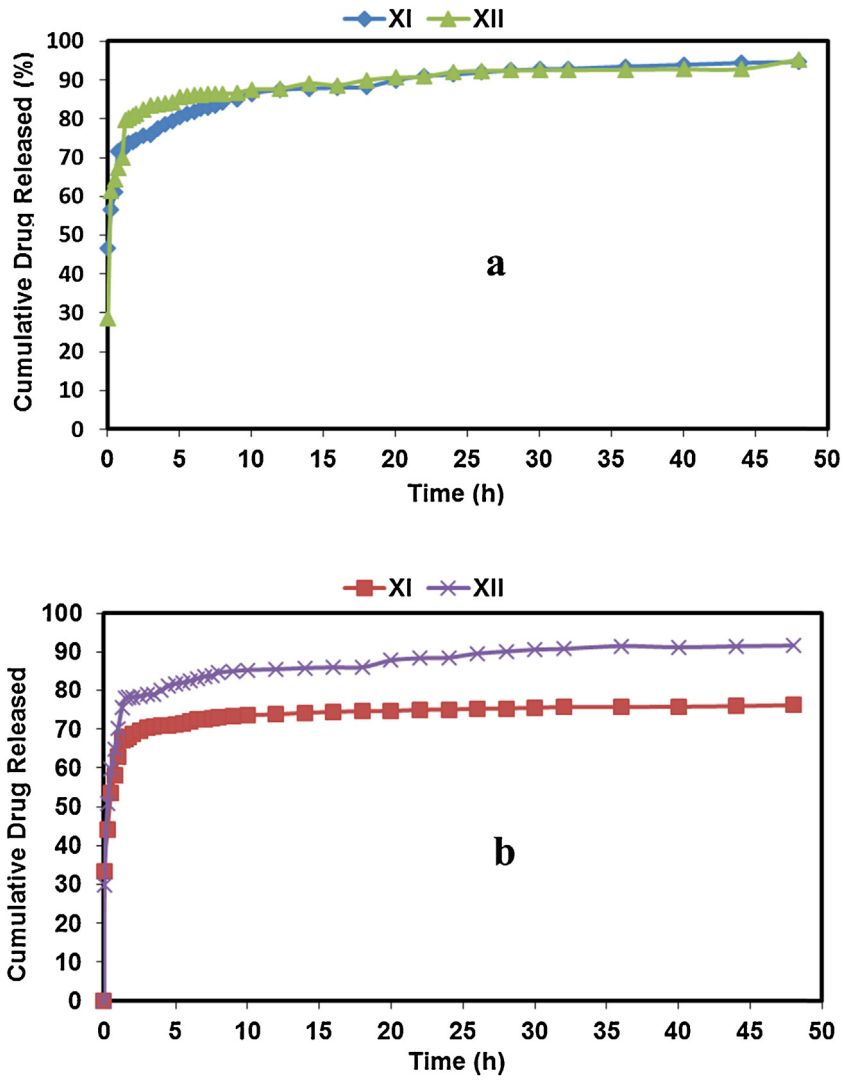


Fig. 6. In vitro release profile of atenolol from (S/PAPhe-ate) (XI) and (S/PAAla-ate) (XII) in phosphate buffer (pH 2) at the body temperature (37 °C).

3.6.1. Effect of pH

The rate of atenolol release was investigated in media having various pH values; pH 2.0 (stomach), pH 7 (physiological fluids) and pH 9.0 (colon). It was found that the rate of atenolol release from (S/PAPhe-ate) (XI) and (S/PAAla-ate) (XII) is low in acid medium, indicating high hydrolytical stability against the strongly acidic medium inside the stomach. On the other hand, the rate of atenolol release was found to reach its maximum value in alkaline medium (inside the colon).

The release study was carried out at fixed temperature 37 °C, for a duration of 48 h. At these conditions, (S/PAPhe-ate) (XI) released 94.6% and 76.2% of their drug contents at pH 9.0 and pH 7, respectively as shown in Fig. 4a and it released 30.1% of its drug content at pH 2.0 after 2 h under the same conditions as shown in Fig. 6.

The total amount of atenolol released from (S/PAAla-ate) (XII) represent 95.3% and 91.6% of its drug content at pH 9.0 and pH 7, respectively, when the release is carried out at 37 °C for 48 h as shown in Fig. 4b. Moreover, (S/PAAla-ate) (XII) released 27.8% of its drug content at pH 2.0 after 2 h under the same conditions as shown in Fig. 6.

3.6.2. Effect of polymer microstructure

The effect of the polymer microstructure on the release of atenolol from (S/PAPhe-ate) (XI) and (S/PAAla-ate) (XII) was studied based on the change in the amino acid moiety from phenylalanine to alanine.

The profiles of drug release from (S/PAPhe-ate) (XI) and (S/PAAla-ate) (XII) at pH 9 (alkaline pH) are illustrated in Fig. 5a and b). The release studies were carried out for 48 h, and in triplicate experiments. The (S/PAPhe-ate) (XI), showed release of 94.6% from its drug content after 48 h at 37 °C. Alanine-based copolymer, (S/PAAla-ate) (XII) showed the same results, in which 95.3% of its drug content was released under the same conditions. Generally, the obtained results indicated that there is no remarkable effect of changing the amino acid structures on the rate of atenolol release, suggesting that the amino acid moiety bulkiness and hydrophilicity have no significant effect of on the extent of drug release.

4. Conclusion

In this study, amino acid-based monomers containing phenylalanine and alanine were synthesized. These amino acid-based monomers were grafted onto starch as a natural polymer using microwave technique. Factors affecting the efficiency of the grafting reaction, like monomer/starch ratio and grafting temperature and duration were studied and the obtained results revealed that optimum temperature for the microwave-assisted grafting of (APhe) is 80 °C and the optimum reaction duration with constant microwave power is 3 min. The optimum monomer/starch ratio for Phe and Ala was found to be 2:1 and 1:1, respectively, for exposure time of 3.0 min at 80 °C.

The obtained graft copolymer was used as stationary phase for immobilization of drugs and finally studying the release of the drug. Based on the nitrogen analysis, the obtained results revealed that the percent of immobilized atenolol for (St-g-A-Ph-Ate) (XI) and (St-g-A-Ala-Ate) (XII) was found to be 42.1% and 53.1%, respectively. The release was found to takes place in alkaline medium rather than in acidic medium and factors like bulkiness and the hydrophilicity of the amino acid moiety were found to have no significant effect on the drug release.

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