

Release Behavior of Bovine Serum Albumin Loaded on Hydrogels of Natural Polymer Blend Poly (Vinyl Alcohol) and Analyze Their Compositions

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To cite this article:

Roua'a Kassim Al-Ojar, Fawzi Habeeb Jabrail. Release Behavior of Bovine Serum Albumin Loaded on Hydrogels of Natural Polymer Blend Poly (Vinyl Alcohol) and Analyze Their Compositions. *American Journal of Polymer Science and Technology*.

Vol. 5, No. 2, 2019, pp. 40-54. doi: 10.11648/j.ajpst.20190502.12

Received: March 16, 2019; Accepted: April 26, 2019; Published: June 4, 2019

Abstract: Amylopectin AP, shellac SH, starch ST and ethyl cellulose EC, the natural polymers of multi functional groups have been blended with poly (vinyl alcohol). The new hydrogels were cross-linked chemically and physically using glutaraldehyde and sodium hexametaphosphate respectively. The prepared hydrogels and according to their different functional groups were studied for their degree of swelling in (pH4, pH7 and pH9) swelling medium and in saline solution of 0.1 N NaCl. The wt% of blend composite of the final hydrogels beside their degree of cross-link was manipulated for maximum loading and suitable release of BSA protein. FT-IR studies were used to improve blending of the mixed polymers in prepared hydrogels from their distinctive functional groups in the final hydrogels structures, as well as the emphasis on BSA protein loaded on prepared hydrogel. The XRD patterns have shown low crystalline structure of the prepared hydrogels after blending, with some elevation in degree of crystallinity for hydrogels cross-linked physically in comparison with hydrogels chemically cross-linked. The DTA thermograms have shown blending of polymers would change the thermal stability of the final hydrogels, and according to their T_g , T_c and ΔH_f the hydrogels were thermally more stable in chemically cross-linked structures than cross-linked physically which because of their ionic interactions and their competition with hydrogen bonds. SEM micrographs have shown the homogeneous structures of the hydrogels after blending beside the irregular and fold surface for chemical cross-linked hydrogels which increase the surface area and increase the loading efficiency of some prepared hydrogels. Whereas physical cross-linked hydrogels have shown surface of smooth and uniform character with high porosity which increase the loading percentage too. The BSA protein model was depended for loading on prepared hydrogels, where the pH, time of loading and BSA concentrations have been shown a significant effects on maximum loading percentages. Finally, the cumulative release percentages R_{cum} of BSA protein from the prepared hydrogels were examined in different pH and temperatures of the release medium. The hydrogels after release the protein have shown morphological surface from SEM images filled with holes and remain stable where they can be used again.

Keywords: Natural Polymers, Sustainable Release, Blend Polymers, Hydrogels, Bovine Serum Albumin

1. Introduction

Hydrogels are three dimensional polymers have hydrophilic and insoluble groups with cross-linked networks. A large amount of water can uptake inside the hydrogel network [1, 2]. Hydrogels may swell up to thousands of times of their dry weight in water [3]. Dispersion of water in

hydrogels will changes to colloidal gels [4]. The hydrophilic groups such as $-OH$, $-CONH$, $-COOH$, $-SO_3H$, and $-NH_2$ are increase the ability of the hydrogels to absorb water [5]. Integrity, biocompatibility and flexibility are the most important factors which help the hydrogels of hydrophilic

three dimensional matrices to be used as carrier materials. The industrial prepared hydrogels from different type of monomers have shown many uses especially in tissue-engineering scaffolds, Sustained release or for carry of implantable devices [6, 7]. The hydrogels are often cross-linked either chemically or physically [1]. Most of the hydrogels and because of their functional groups are responding to the external stimuli where their volume or wetting characteristics change under slide change in temperature, pH, pressure, solvent, electric fields and ionic strength.

Polyvinyl alcohol (PVA) the hydrophilic polymer with semi-crystalline structure made of linear synthetic polymer where PVA is produced commercially from polyvinyl acetate [8-12]. It has water soluble nature and insoluble in organic solvent except slightly soluble in ethanol [11]. It has good mechanical, biodegradable and biocompatible properties and it can form good films [12].

Natural or semi-natural polymers are used in the preparation of hydrogels with PVA (more often through blending) such as amylopectin the polysaccharide with its short linear chains and have a branched structure [13-15]. The high viscosity of amylopectin [14] is due to it is composed of clusters which are connected by long chains. In addition, Shellac the animal origin resin. It is a natural polymer and similar chemically to synthetic polymers with plastic nature [15, 16]. Shellac has a poor mechanical property and instability and it gives a brittle and impervious coating [17].

However, the starch with its biodegradability and its availability and low cost, it is considered one of the most important. Starch, in its natural state exists in a granular and has unique properties [18]. Therefore, combining natural polymers with starch can provide individual advantages like starch-based biodegradable polymers in the form of microsphere or hydrogel can be used for drug delivery [19]. Finally, Ethyl cellulose is the derivative of cellulose which is slightly hygroscopic, odorless and tasteless powder. It is insoluble in water, but soluble in certain organic solvents depending upon the ethoxyl content. Ethyl cellulose is a stabilizer and thickener for foods [20] and because it is soluble in gastric juice and swell so it can be used in drug release [20, 21].

In the present work, poly (vinyl alcohol) PVA was used for preparation of new hydrogels through blending with the following natural polymers; amylopectin AP, shellac SH, and starch ST and semi-natural polymer ethyl cellulose EC. For comparison glutaraldehyde GLU the chemical cross linker and sodium hexametaphosphate SHMP the physical cross linker were used. The degree of swelling of the prepared hydrogels was measured in different pH medium. Moreover, loading of BSA protein on different hydrogels with different concentrations and in different time intervals were investigated, and the cumulative release of the loaded BSA from the hydrogels under different release medium conditions of pH and temperatures were analyzed.

2. Experimental

2.1. Materials

Poly (vinyl alcohol) PVA granular ($\bar{M}_v=101,000$ g.mol⁻¹) was obtained from Qualiquens, India and used without purification. Amylopectin AP ($\bar{M}_v=280,000$ g.mol⁻¹), starch ST ($\bar{M}_v=105,700$ g.mol⁻¹), ethyl cellulose EC ($\bar{M}_v=310,000$ g.mol⁻¹) were obtained from BDH, UK and shellac SH ($\bar{M}_v=1,500$ g.mol⁻¹) was obtained from Sigma-Aldrich, UK. The cross-linkers glutaraldehyde GLU (50wt %) and sodium hexametaphosphate SHMP were obtained from Fluka SW and BDH, respectively. Bovine serum albumin BSA and other chemicals were analytical grade reagents were received from Fluka.

2.2. Hydrogels Preparation

In separate beakers, 10 ml of 10% w/v aqueous solutions of PVA polymer were prepared. The average molecular weights of the used polymers were depended to prepare blending in equal mole concentration of 10^{-3} M from natural polymers with PVA. Where in separate beakers, aqueous solution of 5 ml of 28% w/v of AP, hot aqueous solution of 10 ml of 11% w/v of ST, ethanol solution of 10 ml of 0.1% w/v of SH and ethanol solution of 5 ml of 31% w/v of EC, were prepared and then has been added individually into PVA solution beakers [22]. For each mixture, 10 ml of 5% w/v of the initiator ammonium persulfate APS was added. Subsequently, the final collected solutions were divided into two portions and for each, either 5 ml of 30 % w/v aqueous solution of SHMP as physical cross-linker or 0.5 ml of 50 wt% GLU as chemical cross-linker was added quickly and with vigorous mixing to avoid the fast agglomeration of the solution. For homogenize products one hour extra mixing was continued. Finally, the beakers were left in the oven at less than 60°C until dried.

2.3. Characterizations of the Prepared Hydrogels

2.3.1. FT-IR Spectroscopy

Fourier Transformer Infrared spectrophotometer type Shimadzu IR- Affinity/ Japan instrument was used for characterization of the prepared hydrogels in the spectral region 500-4000cm⁻¹. Where the absorption frequencies of the chemically cross linked hydrogels are shown in Table 1, and the absorption frequencies of the physically cross linked hydrogels are shown in Table 2, beside their absorption frequencies after loading with the BSA protein.

2.3.2. X-ray Diffraction

XRD-P analytical type, made of Netherland at 2013 was used for measuring of X-ray diffraction for the pristine hydrogels for characterization of their crystal structures. Where the measurements are recorded up to 2 θ scale in an angle range of 5°-90° at a scan speed of 1°/min using copper/Indium (0.9/0.1) 100% radiation target and nickel filter at a current of around 20 μ A a voltage of 35kv. The crystallinity percentages and other XRD intensity scans

values of the prepared hydrogels were given in Table 3.

The crystallinity percentage ($\%X_c$) was calculated according to:

$$\% X_c = \frac{A_c}{A_a + A_c} \times 100 \quad (1)$$

Where, A_c and A_a are the area of crystalline and amorphous phases, respectively.

2.3.3. Thermal Analysis

Differential thermal analysis DTA of the prepared hydrogels were investigated using DTA-60/ Simultaneous DTA-TG, Apparatus, Shimadzu/ Japan instrument with heating rate of $10^\circ\text{C}/\text{min}$ in nitrogen atmosphere. The BSA loaded and unloaded hydrogels were investigated and their results of DTA, maximum temperature decomposition T_{max} and glass transition temperature T_g and other thermal parameters were given in Table 4 and 5.

2.3.4. SEM Analysis

The SEM micrographs of the prepared hydrogels were measured using TESCAN, Vega, III, 2011, Czech Republic instrument. The SEM images of some pristine hydrogels and their images after release of BSA were studied. Where double adhesive taps have been fixed on aluminum studs and the samples were mounted on it and then coated with cold ion under vacuum using beam sputter.

2.4. Degree of Swelling DS Measurements

The degree of swelling (DS) of the studied hydrogels prepared in small size pieces were measured. Dry hydrogel pieces with a fixed weight were immersed in distilled water and each 3 hrs their precise weight has been measured after removal of all un-adsorbed water and even tissue paper for more drying has been used. The following equation for measuring the degree of swelling DS was depended [23].

$$DS\% = \frac{W_t - W_o}{W_o} \times 100 \quad (2)$$

Where, W_t & W_o are the weight of swell hydrogel at time t , and the weight of dry hydrogel respectively. The effects of the pH swelling medium of pH4, pH6 and pH8 were investigated which represent acidic, neutral and basic swelling medium, respectively.

2.5. Loading of BSA on Prepared Hydrogel

The loading of the prepared hydrogels with BSA model has been investigated in different loading conditions. However, the time of loading and the pH of loading medium, beside the concentrations of BSA protein used for loading have been investigated to reach maximum loading. 100 mg of the hydrogels were immersed in beaker contain 50 ml of 2.0g/L BSA concentration and the maximum loading was examined in pH4, pH6 and pH8 for 12hrs at room temperature. Each 1.5 hrs the hydrogel sample were removed and the remain BSA solution was measured using UV-visible spectrophotometer (Jasco V-630 spectrophotometer/ Japan),

where the instrument was fixed at λ_{max} 279 nm and the absorbance (A) was measured. The calibration curve was used for determination of the concentration of remain BSA solution. However, the favorable loading pH solution was fixed and the time of loading also measured at (1.5, 3.0, 4.5 and 6.0) hrs beside the BSA loading concentrations was changed between (0.5, 1.0, 1.5 and 2.0) g/L for optimum loading conditions. The following equations were applied for determination of BSA maximum loading (L_{max}) and efficiency of loading (EL) of the examined hydrogels [24].

$$\% L_{\text{max}} = \frac{\text{Amount of BSA protien loaded on hydrogel}}{\text{Amount of hydrogel taken for loading}} \times 100 \quad (3)$$

$$\% \text{EL} = \frac{\text{Amount of BSA protien loaded on hydrogel}}{\text{Amount of BSA protien taken for loading}} \times 100 \quad (4)$$

2.6. Cumulative Release of Loaded BSA Protein from Hydrogels

Hydrogels of different blends and cross linked and with BSA maximum loaded have been depended in their cumulative release. However, 100 mg of BSA maximum loaded hydrogel were kept in 200 ml of physiological saline (PS) solution prepared from 0.9%w/v aqueous solution of NaCl. The cumulative release of BSA from the hydrogel was measured each 3hrs Using UV-Visible spectrophotometer and for time intervals of 12hrs by recording the absorbance A at λ_{max} 279 nm (the maximum wave length of BSA protein) for the solution of the release medium and with restitution with new PS solution. The cumulative release (R_{cum}) of BSA protein was calculated by the following equation [25].

$$\text{Percent Cumulative Release } (\%R_{\text{cum}}) = \frac{W_t}{W_o} \times 100 \quad (5)$$

Where, W_t & W_o are the amount of BSA protein released at time t , and total amount of BSA protein released finally.

The cumulative release R_{cum} of the studied hydrogels were investigated under two variables is temperature of the release medium at (15, 25 and 40°C) and pH of the medium (pH4, pH6 and pH8) respectively.

3. Results and Discussion

Prepared hydrogels by blending are usually retain the unique properties of their individual components, and the final hydrogels are combination of two or more different and unusual properties in one polymer structure.

Smart or intelligent polymers are recently prepared also called hydrogels that are responsive to external stimuli such as temperature, pH and have many applications in bio-releasing or bio-processing of many important biomaterials like protein.

3.1. Hydrogels Characterization

A new hydrogels were prepared from blending of Poly (vinyl alcohol) PVA with natural polymers AP, SH, ST, and semi natural polymer EC. The prepared hydrogels were cross linked physically and chemically. However, new hydrogels

need characterizations to their chemical structures, crystalline structure, thermal behavior and morphological surface using different types of techniques. The goal of preparing new hydrogels is to applied in bioseparation and bioprocessing of some sensitive biomaterials like proteins. Therefore, the prepared hydrogels were used for loading and releasing of BSA as modern protein and examined under different conditions in order to reach the maximum loading percentages and efficient cumulative release.

Accordingly, the prepared pristine hydrogels are

characterized depending on FT-IR spectroscopy, besides the X-ray diffraction XRD, thermal analyses and SEM images for selected hydrogels.

3.1.1. FT-IR Studies

The FT-IR spectra of PVA blend hydrogels have been studied and their absorption frequencies with their featured functional groups are gradate in Tables 1 and 2, and some distinct FT-IR Figures 1-4,

Table 1. FT-IR wavenumbers of the characteristic bands of hydrogels cross-linked chemically.

Examined Sample	Wavenumber of characteristic band, cm^{-1}					
	$\gamma(\text{O-H})_{\text{str}}$	$\gamma(\text{C-H})_{\text{str}}$	$\gamma(\text{C=O})_{\text{str}}$	$\gamma(\text{C-O})_{\text{str}}$	$\gamma(\text{C-O-C})_{\text{str}}$	$\gamma(\text{C-H})_{\text{def}}$
AP/P-b-CH	3524, 1373	2920, 3080	1744, 1603	1450	1140	787
ST/P-b-CH	3462	2950	1650, 1603	1100	1140	793
SH/P-b-CH	3505	3100	1726, 1402	1148	1148	793
EC/P-b-CH	3458	3080	1603	----	1150	779

Table 2. FT-IR wavenumbers of the characteristic bands of hydrogels cross-linked physically.

Examined Sample	Wavenumber of characteristic band, cm^{-1}						
	$\gamma(\text{O-H})_{\text{str}}$	$\gamma(\text{C-H})_{\text{str}}$	$\gamma(\text{C=O})_{\text{str}}$	$\gamma(\text{C-O})_{\text{str}}$	$\gamma(\text{C-O-C})_{\text{str}}$	$\gamma(\text{C-H})_{\text{def}}$	$\gamma(\text{P-O-P})_{\text{str}}$
AP/P-b-PH	3495, 1350	3080	1747, 1603	1404	1150	795	720,1150, 1100,1260
ST/P-b-PH	3499	2960	1650, 1603	1404	1157	795	710,1145, 1100,1255
SH/P-b-PH	3547	2839, 2910	1713, 1650	1458	1150	797	720, 1150, 1100,1280
EC/P-b-PH	3503	2920	1746, 1650	1450	1150	777	720, 1150, 1100,1260

Where shows the characteristic peaks represent the more characteristic functional groups of the different polymers blend PVA hydrogels which cross-linked chemically with GLU or physically with SHMP.

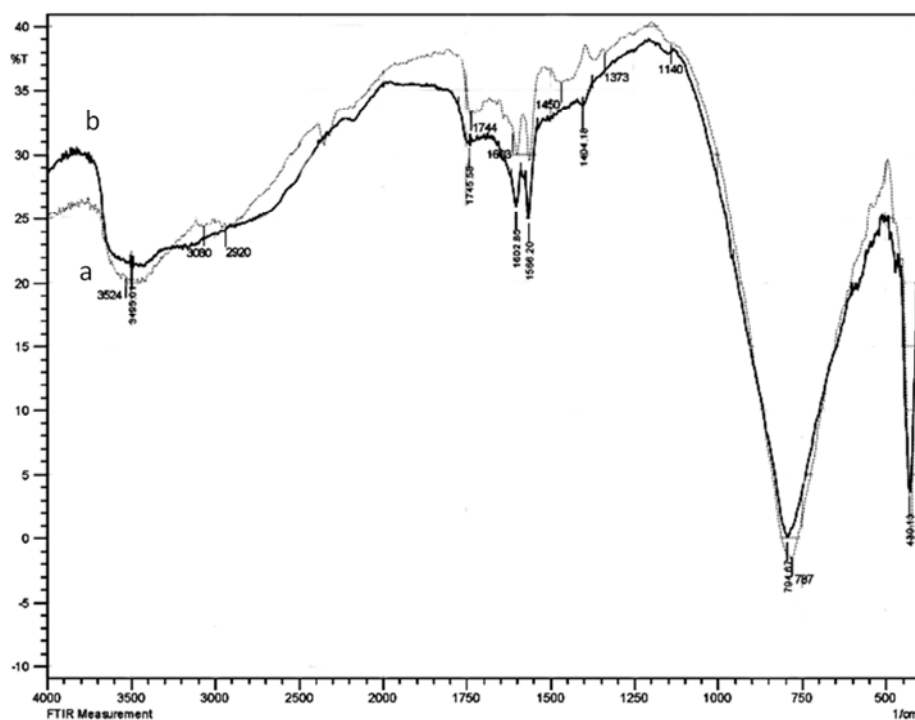


Figure 1. FT-IR spectra of a- AP/P-b-CH hydrogel and b- AP/P-b-PH hydrogels.

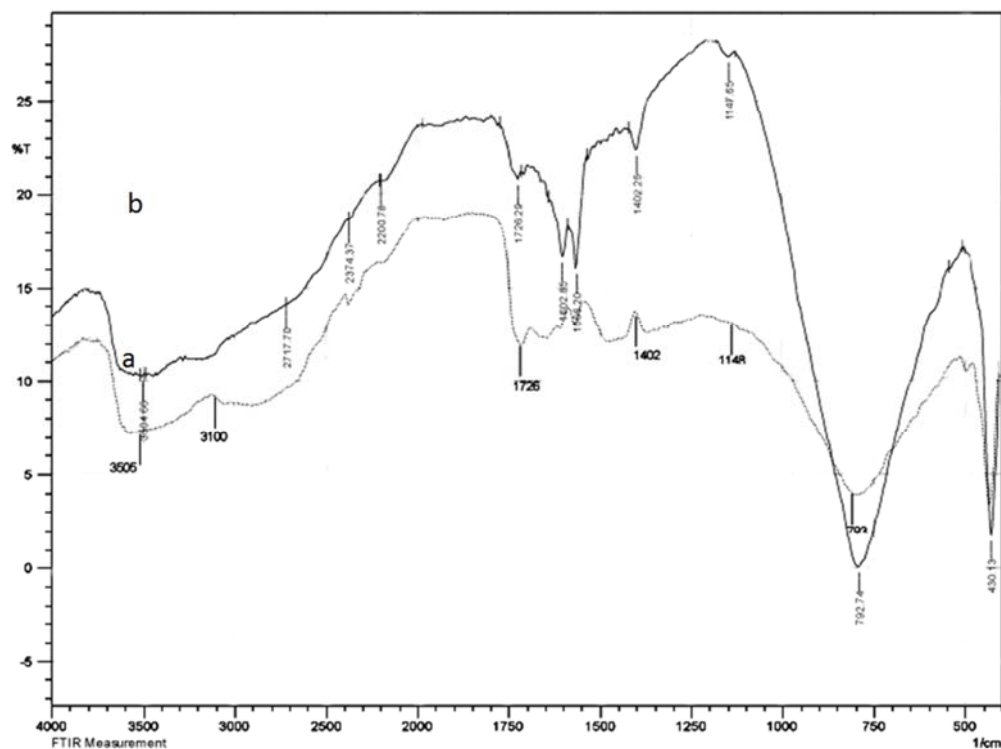


Figure 2. T-IR spectra of a- SH/P-b-CH hydrogel and b- SH/P-b-PH hydrogels.

Whereas, the characteristic bands of AP/P-b-CH represent amylopectin blend PVA and cross linked chemically, Figure 1 and Table 1, have shown wavenumbers at (3524 and 1373) cm^{-1} represent $\gamma(\text{O-H})$ str, and at (2920 and 3080) cm^{-1} represent $\gamma(\text{C-H})$ str, and at (1744 and 1603) cm^{-1} represent $\gamma(\text{C=O})$ str, and at 1450 cm^{-1} represent $\gamma(\text{C-O})$ str, and at 1140 cm^{-1} represent $\gamma(\text{C-O-C})$ str. Finally, the band at 787 cm^{-1} represent $\gamma(\text{C-H})$ def. The previous mentioned characteristic

bands indicate that sometimes interferences occur in the characteristic peaks of both blend polymers and even groups of cross-linker. The other characteristic bands mentioned in Table 1, Figures 2, 3 and 4, are belong to ST/P-b-CH which represent starch blend PVA, and SH/P-b-CH which represent shellac blend PVA, and EC/P-b-CH which represent ethyl cellulose blend PVA and all are cross linked chemically, respectively [2, 4-7, 26].

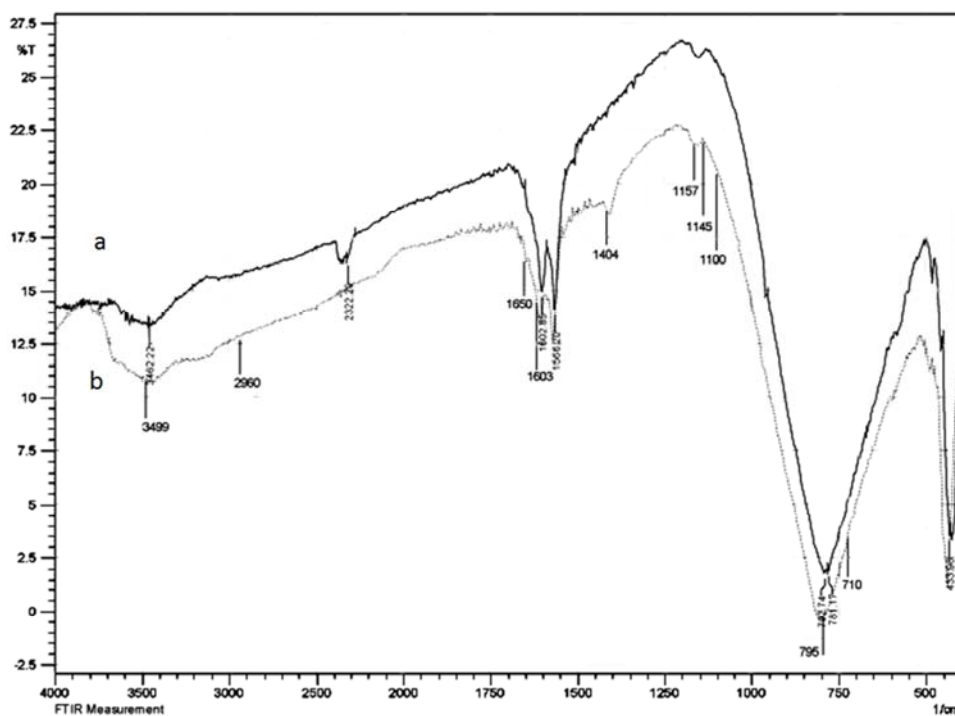


Figure 3. FT-IR spectra of a- ST/P-b-CH hydrogel and b- ST/P-b-PH hydrogels.

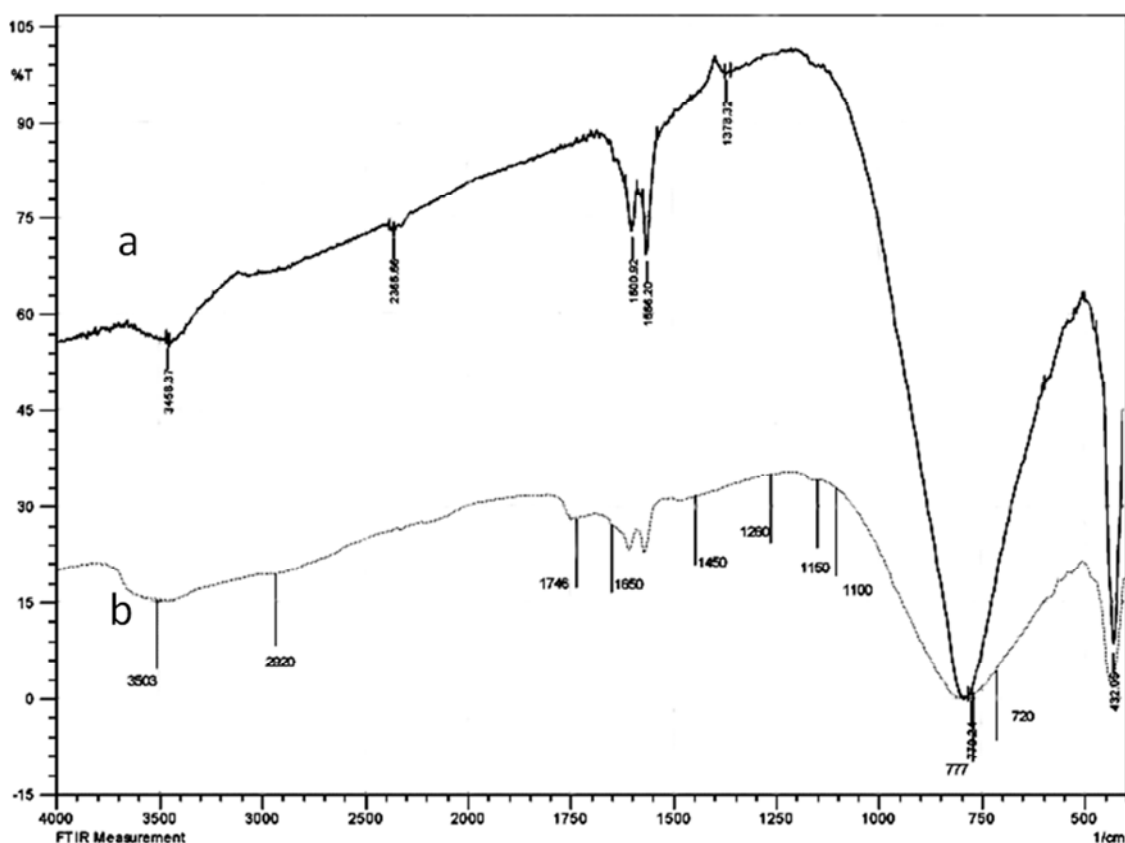


Figure 4. FT-IR spectra of a- EC/P-b-CH hydrogel and b- EC/P-b-PH hydrogels.

The previous blend hydrogels are also cross linked physically and gradate in Table 2 and their characteristic peaks are shows in Figures 1, 2, 3 and 4 and are as follows AP/P-b-PH, ST/P-b-PH, SH/P-b-PH and EC/P-b-PH which are shows almost same wavenumbers of characteristic bands except those of the physical cross linker SHMP which their frequencies appear at (722, 1150, 1100 and 1280) cm^{-1} which represent the $\gamma(\text{P-O-P})$ str band of SHMP appeared [27].

3.1.2. Degree of Swelling DS Investigations

The degree of swelling DS of the prepared hydrogels in different pH swelling medium and in 0.1 N saline solution were studied in order to reach the best degree of swelling conditions.

However, the degrees of swelling of AP/P-b-CH and AP/P-b-PH hydrogels were measured according to the Equation (2) using swelling medium of pH4, pH7 and pH9 in addition to 0.1 N NaCl. Figure 5 shows significant effects of pH or ionic solution of swelling medium on DS [28].

The amylopectin blend PVA hydrogel Figure 5 has shown two points, where the degree of swelling percentage DS% of AP/P-b-PH hydrogel in pH9 swelling medium reach about 28 hundred times higher than its original volume. In addition the AP/P-b-PH hydrogels in all examined swelling solutions have higher DS% in comparison with chemical cross-linked hydrogels Figure 5, because amylopectin has only hydroxyl groups and when hydrolyses will produce anions [29] would be in repulsion with pH9 anions of the medium in addition to the anions of the cross linker SHMP, and the final result was

spacing of the hydrogel chains.

Similarly, in pH7 and pH4 the repulsion between the hydrogel anions and of SHMP will let the physical cross linked hydrogels swell higher than those of chemically cross-linked Figure 5. However, in both pH7 and pH4 the hydrogels were swelling less than pH9 due to the absence of the swelling medium anions and less more in pH4 Figure 5, because of the pH4 cations which decrease the space between chains.

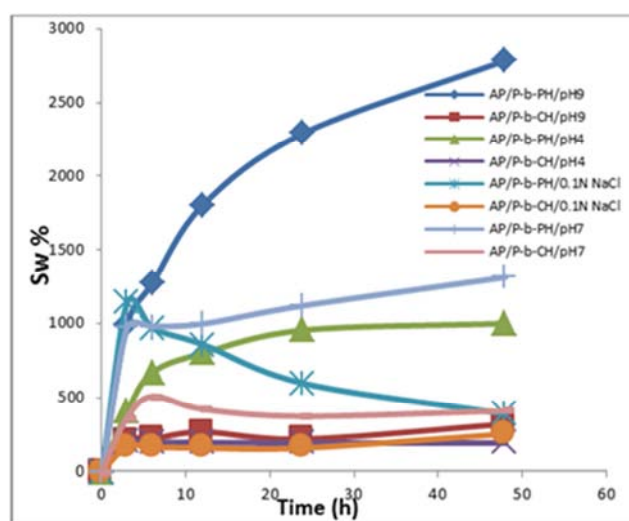


Figure 5. Effects of pH & saline solution on $S_w\%$ of AP/P-b-CH and AP/P-b-PH hydrogels.

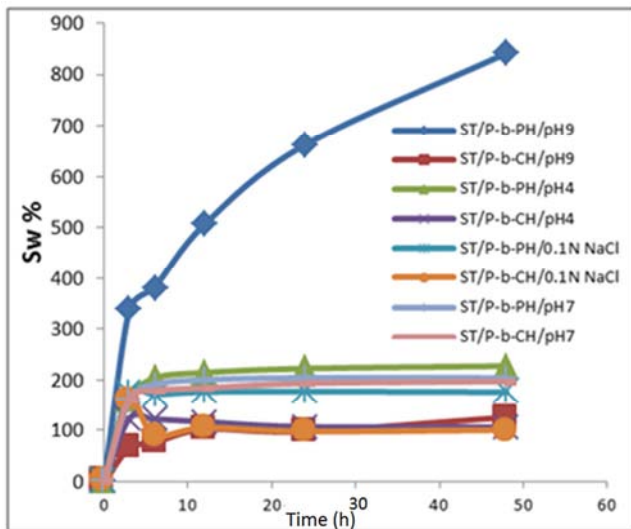


Figure 6. Effects of pH & saline solution on $S_w\%$ of ST/P-b-CH and ST/P-b-PH hydrogels.

Similarly, hydrogels made of starch blend PVA Figure 6 have shown almost the same swelling behavior as amylopectin blend PVA hydrogels Figure 5 with less degree of swelling DS% in comparison with amylopectin hydrogels, and this is because starch is consist of amylose and amylopectin molecules [30] and because amylopectin is branched whereas amylose is linear and helical in its structure and the aforesaid has no three dimensional structure for increasing the volume of the hydrogel on swelling like amylopectin structure.

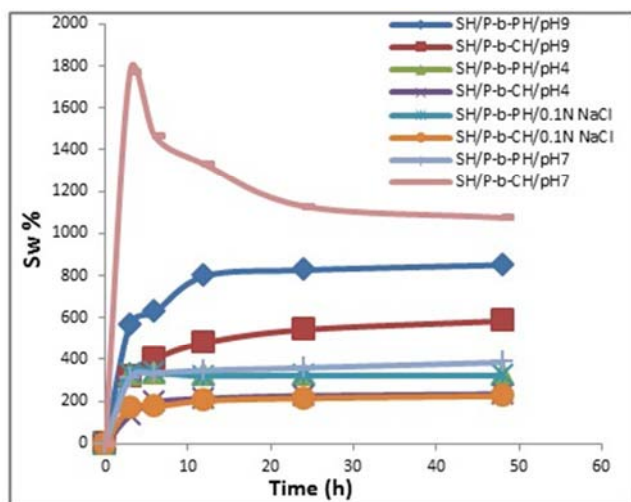


Figure 7. Effects of pH & saline solution on $S_w\%$ of SH/P-b-CH and SH/P-b-PH hydrogels.

Shellac SH, the natural polymer has carboxylic acid groups in its structure which can easily hydrolyze in swelling medium [31]. Therefore in pH9 medium neutralization will occurred and effect on DS% of the hydrogel. Therefore, SH/P-b-CH hydrogel in pH7 medium has shown the highest

degree of swelling Figure 7, because no ions are there in the swelling medium. But however a sharp decrease in DS% in pH7 would occurred after some hours of swelling Figure 7, is because the hydrogel would lose some of its weight due to the hydrolysis of the shellac polymer inside the swelling medium.

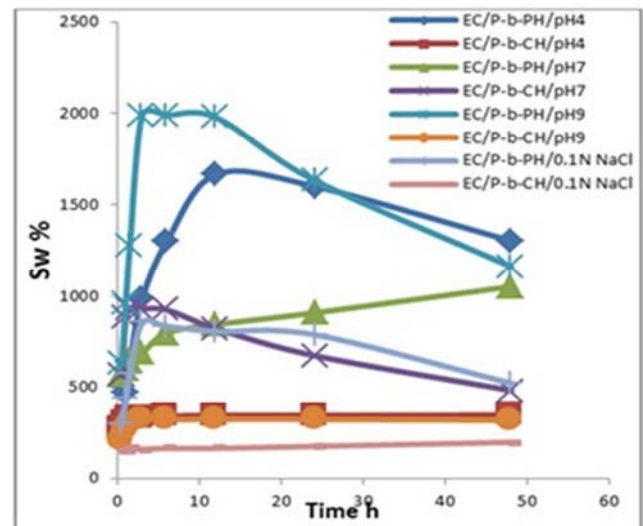


Figure 8. Effects of pH & saline solution on $S_w\%$ of EC/P-b-CH and EC/P-b-PH hydrogels.

Ethyl cellulose EC, the derivative of cellulose when swell, some of its hydroxyl groups are converting into ethyl ether groups. Blending of EC with PVA will produce hydrogel have folded morphological structure with high porous. Therefore, the degree of swelling DS% of EC blend PVA hydrogel is high [32]. Moreover, and because of the hydroxyl groups of the ethyl cellulose, therefore its hydrogel inside swelling medium act as anionic polymer and for that its DS% is high especially for physically cross linked hydrogels because of its anions and the pH9 anions which together will push the hydrogel chains for repulsion and finally its DS% will increase Figure 8.

3.1.3. X-ray Diffraction Studies

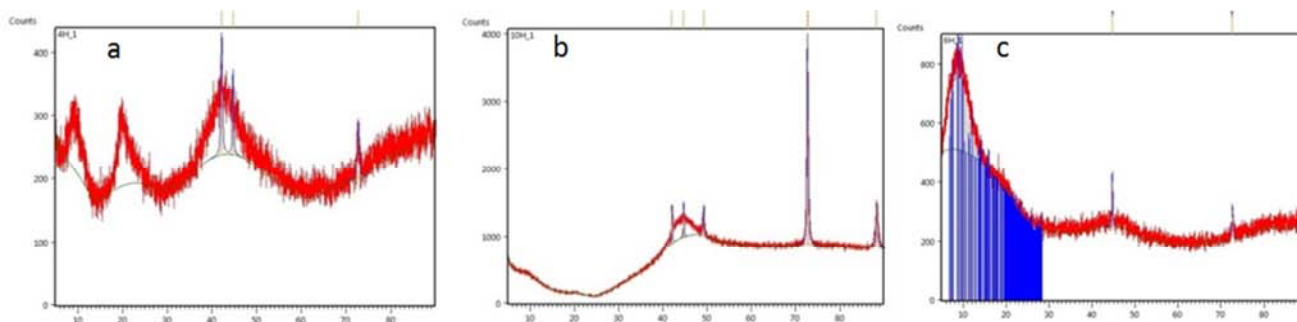
The X-ray diffraction analysis means scattering or diffraction of X-ray radiation by electrons present in the tested material. Therefore, the regular structure means crystalline material which scattered in maxima, but the diffracted intensity is low and in broad for amorphous material. Generally, the crystallographic structure of the material and its atomic composition can be determined from x-ray diffraction pattern from the position and intensity of the maxima [33].

The XRD pattern data shown in Table 3 represent some of the studied chemical and physical cross linked hydrogels.

Table 3. XRD intensity scans values of chemically and physically cross-linked hydrogels.

Examined Sample	Peaks at $2\theta^\circ$	Interplanar distance "d" (Å)	FWHM (Å $^\circ$)	Crystalline phase A_c (Å $^\circ$)	Amorphousness phase A_a (Å $^\circ$)	Cryst. Percent. % X_c , Equate1
AP/P-b-CH	42.139	2.144	0.409	55.57	243.54	18.58
	44.698	2.027	0.807	30.86	243.82	11.23
	72.636	1.302	0.512	36.11	204.29	15.02
	5.227	16.907	0.614	17.75	520.03	3.30
	20.815	4.268	0.921	418.04	544.68	43.42
SH/P-b-PH	42.064	2.148	0.154	79.62	1056.86	7.01
	49.237	1.851	0.205	50.57	984.57	4.88
	72.662	1.301	0.230	599.98	911.69	39.69
	88.317	1.107	0.461	289.52	911.07	24.11
	44.691	2.028	0.204	31.11	254.96	10.87
ST/P-b-CH	44.691	2.028	0.204	31.11	254.96	10.87
	72.576	1.303	0.256	25.35	210.94	10.73

Whereas the data are calculate precisely from Figure 9.

**Figure 9.** XRD pattern a- AP/P-b-CH, b- SH/P-b-PH, c- ST/P-b-CH hydrogels.

However, the XRD patterns give good events about the components of the blend hydrogels and excellent view about their crystallinity and crystal structure. Where the XRD diffractograms and the data entered in Table 3 are clear and with no doubt that the pure PVA is highly crystalline but blending it with other polymer would produce new hydrogel and do depression in its crystallinity. This depression depends to a certain degree on crystalline structure of the added natural polymer, beside the type of the cross-linker material. Where the data have shown that physical cross-linker such as SHMP has significant effects on the elevation of the crystallinity of the hydrogel in comparison with the chemical cross-linker GLU. As a result, the degree of crystallinity of the PVA will reduce due to the blending that will collapse the regularity formed by the hydrogen bonding between the PVA chains [33].

The XRD pattern of AP/P-b-CH hydrogel Table 3, Figure 9a were shown five peaks some are intense and other are broad. The three intense peaks at $2\theta^\circ$ of (42.139, 44.698 and 72.636) were shown crystallinity percentage of (18.58, 11.23 and 15.02)% respectively, means low crystalline structure after blending of the hydrogel due to the changes in its composite structure and low crystallinity is more suitable for the hydrogel for loading and releasing [34].

The presence of sodium ions in the physically cross-linked hydrogel comes from SHMP will elevate its degree of crystallinity, although the hydrogel is blends with the natural polymer of low crystalline structure [35].

Therefore, SH/P-b-PH hydrogel has shown XRD pattern with peaks appeared with sharp and narrow shape Table 3 and Figure 9b, and at $2\theta^\circ$ of (20.815 and 72.66) which have shown high

crystallinity percentage of (43.42 and 39.69) % and even the crystallinity percentage of SHMP characteristic peak was increased and record at 24.11%, and as a result, the final crystallinity of the whole hydrogel structure would elevate.

Finally, the XRD pattern of ST/P-b-CH hydrogel has shown also low crystalline structure even in comparison with AP/P-b-CH. Where broad shape peaks with less number are appeared as shown in Table 3 and Figure 9c, where ST/P-b-CH hydrogel has recorded its peaks at $2\theta^\circ$ of (44.691 and 72.576) and gave low crystallinity percentage of (10.87 and 10.73)%, means the hydrogel is tend to the amorphous structure after bending.

3.1.4. Thermal Studies

Thermal studies of some prepared hydrogels were examined and the differential thermal analysis DTA was depended for studying the pristine hydrogels before loading. The DTA thermograms studies supplies thermal information includes thermal stability and some transitions such as endothermic and exothermic transitions as a function of temperature.

The thermal data recorded in Table 4 give thermal characterizations of some studied hydrogels.

Table 4. DTA thermal data of some hydrogels cross-linked chemically and physically.

Examined sample	T_g (°C)	T_{max} (°C)	T_{cr} (°C)	ΔH_f (J.g $^{-1}$)
AP/P-b-CH	67.7	293	255	+5.0; -59.9
ST/P-b-CH	73.8	433	404	+62.3; -38.0
EC/P-b-CH	110.2	425	392	+36.2; -16.4
EC/P-b-PH	95.7	430	395	+4.7; -3.5
SH/P-b-PH	104.5	397	341	+15.1; -34.4

Where the AP/P-b-CH hydrogel has shown Table 4, Figure 10a glass transition temperature T_g at 67.7°C means the hydrogel is thermally stable, and its crystalline temperature T_{cr} and its maximum decomposition temperature T_{max} at 255°C and 293°C respectively, means the hydrogel at 255°C start loss its crystalline structure and be ready for

decomposition at 293°C and loss its structure. The heat of fusion ΔH_f of the hydrogel gives two important transitions Table 4, Figure 10a, one represent endothermic transition of $+5.0\text{J.g}^{-1}$, give indications that the hydrogel has some crystalline portions in its morphology therefore it need some energy for decomposition.

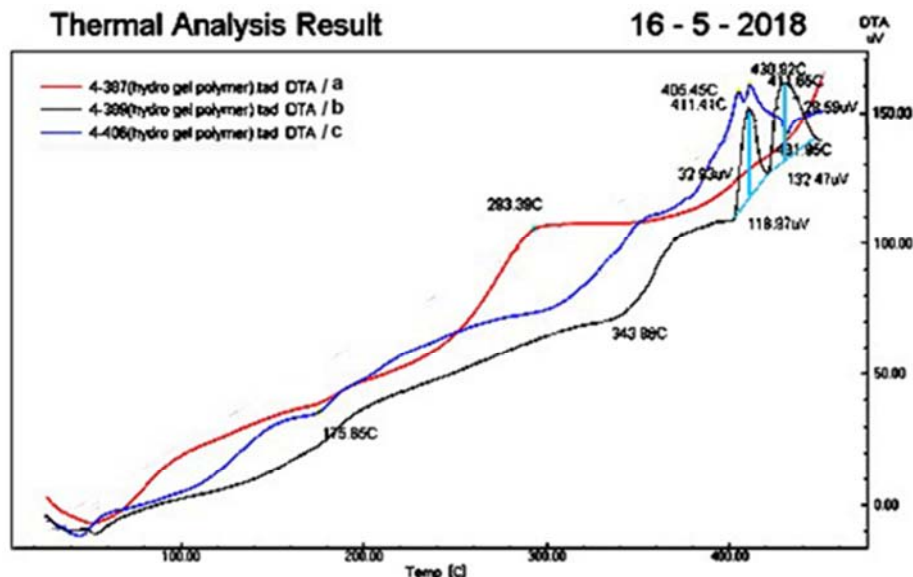


Figure 10. DTA of a- AP/P-b-CH, b- ST/P-b-CH, and c- EC/P-b-CH hydrogels.

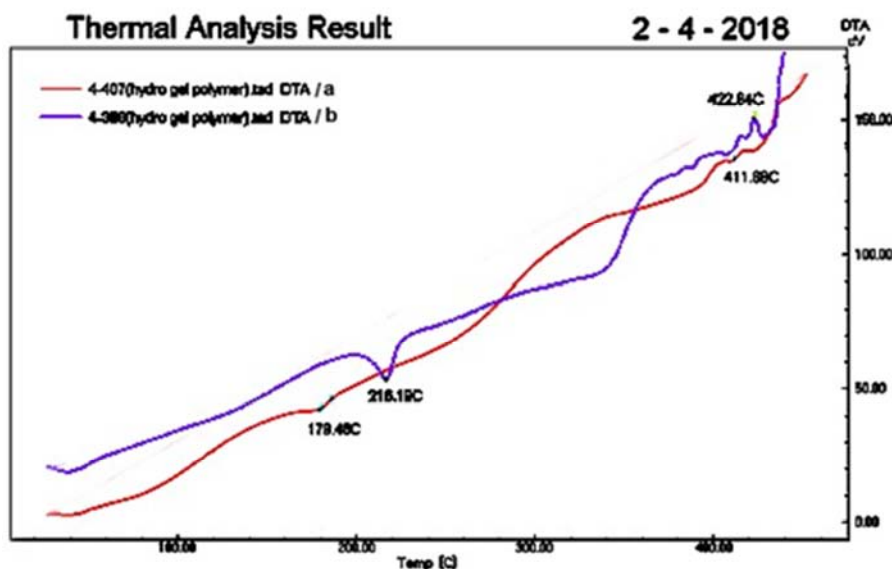


Figure 11. DTA of a- EC/P-b-PH, and b- SH/P-b-PH hydrogels.

Whereas the second transition of -59.9J.g^{-1} means the hydrogel has amorphous portions and on decomposition will liberate energy of 59.9J.g^{-1} of the hydrogel [36]. In comparison, the ST/P-b-CH and even EC/P-b-CH hydrogel have shown Figure 10b, c and Table 4, thermally stable hydrogels as it is clear from their high T_g , T_{max} and T_{cr} . In addition the ΔH_f for both hydrogels are shows high endothermic transitions means thermally stable with low exothermic transitions [37].

On the other hand, the physical cross-linking hydrogels

Table 4 and Figure 11 have shown completely different DTA thermograms. The EC/P-b-PH hydrogel has shown Figure 11a little depression in its thermal parameters represent T_g and ΔH_f in comparison with EC/P-b-CH, means ethyl cellulose blend PVA polymer loss some of its thermal stability with physical cross link where ionic interactions compete with hydrogen bonding leads to loss some. Whereas SH/P-b-PH hydrogel has shown higher T_g and ΔH_f Table 4, Figure 11b means thermally is stable [38].

3.1.5. SEM Micrograph Studies

SEM micrographs and their surface morphologies of some blend PVA hydrogels cross-linked chemically and physically were studied. Where the SEM image of AP/P-b-CH hydrogel has shows Figure 12a, the hydrogel surface morphology be

appear smooth with cracks, which indicate that the hydrogel blend materials are mixed homogeneously but the hydrogel surface seems to be brittle due to the high degree of bonding [30].

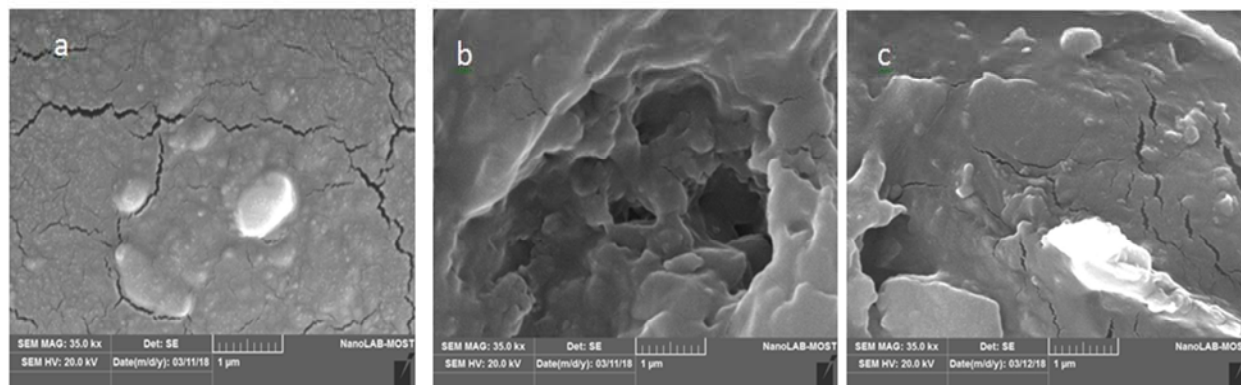


Figure 12. SEM micrograph of a- AP/P-b-CH, b- ST/P-b-CH hydrogel and c- EC/P-b-CH.

Whereas the SEM image of ST/P-b-CH hydrogel Figure 12b, has shown irregular and fold surface with a lot of cavities and high degree of homogeneous blend polymers [37] which lead to wide surface area and high efficient for loading. Similarly, the SEM image of EC/P-b-CH hydrogel Figure 12c has shown non undulant surface with few uneven folds. Pores are spread inside and in between the folds which means the hydrogel could be present in elastic form and could be efficient in loading [39].

Whereas, the SEM images of physically cross linked PVA blend hydrogels has shown different surface morphologies, where the SEM micrograph of AP/P-b-PH hydrogel Figure 13a, has shown almost smooth and uniform surface with high number of pores and salience which would be help in high loadings. Likewise, the SEM micrograph of SH/P-b-PH hydrogel Figure 13b, has shown smooth and uniform surface contain some big holes could be useful also in loading [40].

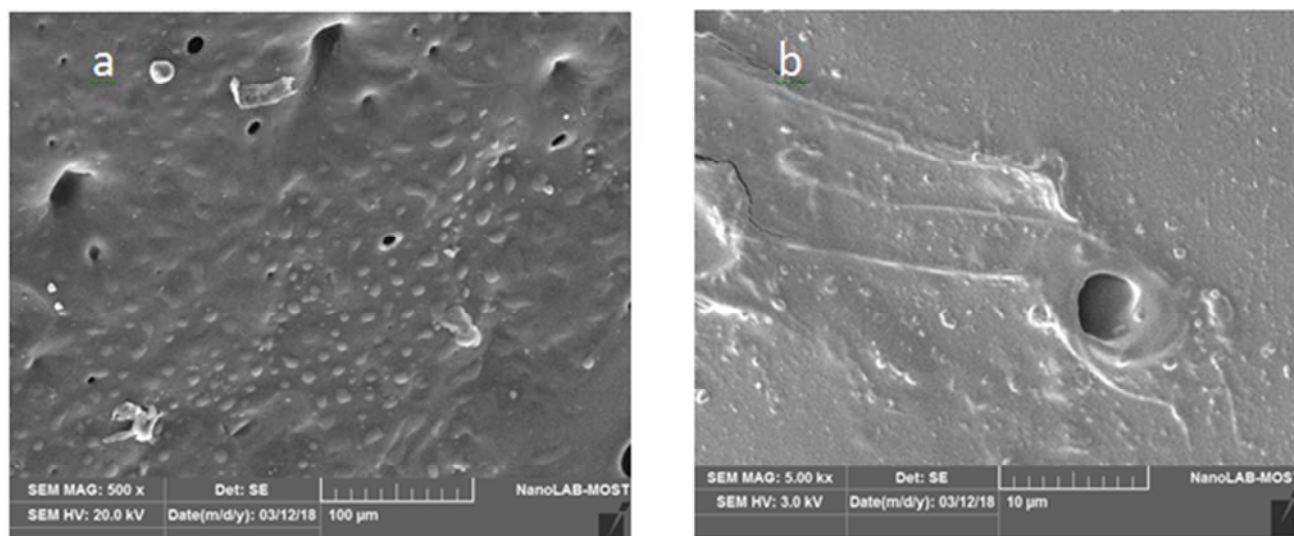


Figure 13. SEM micrograph of a- AP/P-b-PH, b- SH/P-b-PH hydrogels.

3.2. Loading of Hydrogels with BSA Protein

The prepared hydrogels were loaded with BSA as model protein and for both chemically and physically cross linked. The BSA loading studies include investigation of pH of the loaded solution and different concentrations of loaded BSA. Some of the BSA loaded hydrogels were characterized using FT-IR spectroscopy and differential thermal analysis DTA, in order to confirm the loading of the BSA protein on the

hydrogels.

3.2.1. Characterization of BSA Loaded Hydrogels

The BSA loaded hydrogels were characterized using FT-IR spectroscopy technique and the examined samples were shown appearing of new characteristic frequencies represent the absorption bands of BSA loaded protein on studied hydrogels. The absorption bands which characterized in the examined spectra are of amide-I ($\gamma(\text{C=O})_{\text{str}}$) represent the

carbonyl functional group of BSA protein, beside absorption band of amide-II ($\gamma(\text{N-H})_{\text{str}}$) represent the amine functional group of the protein.

The other common absorption frequencies are representing the characteristic bands of both blend polymers of the

hydrogel components which have been already characterized in Table 1&2.

Similarly, the differential thermal analysis DTA of some loaded samples was studied and their final results are recorded in Table 5, Figure 14.

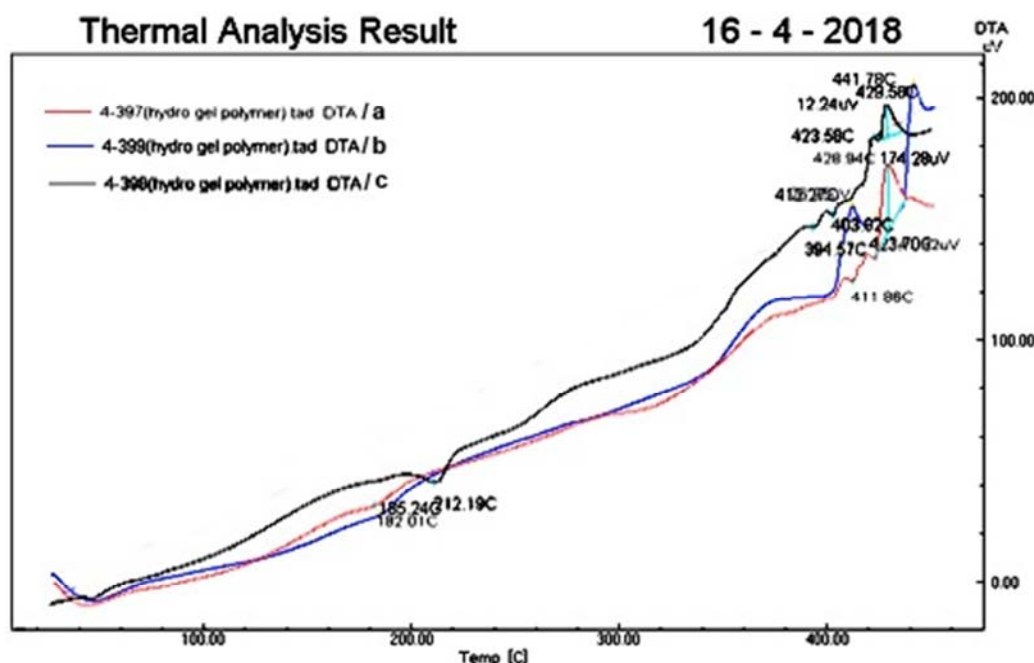


Figure 14. DTA thermogram of a- AP/P-b-CH, b- ST/P-b-CH, and c- SH/P-b-PH hydrogels loaded with BSA protein.

Table 5. Thermal analysis parameters of some blend hydrogels loaded with BSA protein.

Examine sample	T_g (°C)	T_{max} (°C)	T_{cr} (°C)	ΔH_f (J.g ⁻¹)
AP/P-b-CH	107.6	440	422	+13.5; -7.7
ST/P-b-CH	100.2	422	402	+16.0; -3.6
SH/P-b-PH	96.2	438	426	+8.8; -1.9

The thermal parameters of BSA loaded AP/P-b-CH hydrogel have shown complete changes after loading Table 5, Figure 14a in comparison with its parameters before loading Table 4, Figure 10a Where its T_g , T_{max} and T_{cr} are elevate into high degrees due to the formation of hydrogen bonding between the amide groups of BSA protein and the hydroxyl groups of the amylopectin and poly (vinyl alcohol) and form high crystalline structure for the final hydrogel and therefore, ΔH_f increase in its endothermic transitions from (+5.0 to +13.3) J.g⁻¹ and decrease highly in its exothermic transitions from (-59.9 to -7.7) J.g⁻¹, Figure 14a and Table 5, means the hydrogel became more crystalline after loading. Whereas the thermal parameters of BSA loaded ST/P-b-CH have shown Figure 14b, Table 5 increase in its T_g from (73.8 to 100.2)°C, while both the endothermic and exothermic transitions are decreased from (+47.7 to +16.0) and (-38.0 to -3.6)°C respectively Figure 14b and Table 5, the elevation in its T_g and the highly decrease in its exothermic transitions means ST/P-b-CH hydrogel get crystalline structure after loading but not stable like AP/P-b-CH hydrogel.

Whereas the SH/P-b-PH hydrogel of highly crystalline structure after be loaded with BSA protein has been lost and this is clear from its DTA thermal parameters, especially in its glass transition temperature T_g and heat of fusion ΔH_f Figure 14c and Table 5 and this is because the functional groups of the BSA protein have no opportunity to form hydrogen bonding with functional groups of the hydrogel because recently mentioned groups are engaged with SHMP anions.

3.2.2. Loading Procedure of Hydrogels with BSA Protein

In order to reach maximum loading and to increase its efficiency, different loading conditions are considered. The pH of the loading medium, its time and the BSA concentrations were depended for loading. The UV-Visible technique at λ_{max} 279 nm were depended for measuring the absorbance A of BSA loaded protein on prepared hydrogels and Equations (3 and 4) are considered for determination of maximum loading percentage (% L_{max}) and the efficiency loading percentage (%EL).

However, loading on hydrogel depends mainly on its degree of swelling [41]. Accordingly, and because degree of swelling is affected significantly with pH, the loading on hydrogels was studied in different pH loading medium and Figure 15a has shown the efficiency of BSA loading (EL) versus different pH solutions in order to reach the maximum efficient loading.

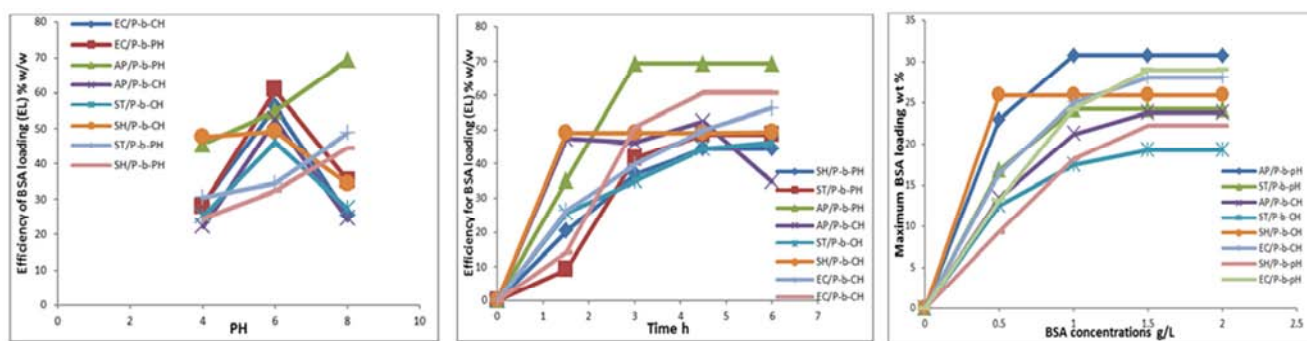


Figure 15. Efficiency of BSA loading for a- pH, b- Time and for c- BSA concentrations optimization.

The degree of swelling also has been found depending on time of loading, which is in the end will effect on efficiency of loading [42]. Therefore the time of loading Figure 15b also has shown a significant effect on efficiency of loading of BSA on different hydrogels.

Finally, Different BSA concentrations have been investigated in the loading of the studied hydrogels in order to achieve maximum loading percentages. Therefore, Figure 15c has shown the effect of different BSA concentrations on the maximum loading of BSA in order to optimize the economical concentration of BSA used for loading. The degree of swelling of the hydrogels cross-linked chemically or physically is shows significant effect on maximum loading [43]. But because the physically cross-linked hydrogels have ionic interactions between their chains, they show more effects in previous studied loading conditions than those cross linked chemically.

3.3. BSA Releasing Investigations from Hydrogels

Cumulative release (R_{cum}) percentages of BSA from different loaded hydrogels were examined and for continued 12 hrs and for each time intervals of 3hrs. Physiological saline (PS) solution of 0.9% w/v was used as release medium because BSA hardly diffuse in D. W., Whereas the Saline ions prevent non-ionic interaction by ordering the structure of water [44] So that the BSA protein molecules absorbed on hydrophobic groups of the hydrogel could release easily in saline solution [45-47].

The release process was examined under two variable release medium conditions, where the release medium was fixed at temperatures of 15, 25 and 40°C and its PH was fixed at pH4, pH6 and pH8. The Equation 5 has been depended for calculations of cumulative release (R_{cum}) percentages of BSA, and new calibration curve using UV-Visible spectrophotometer was prepared in PS solution for the calculation of unknown BSA concentrations released.

The degree of swelling of the hydrogels will also effects on its degree of release but because its release was in PS and not in distilled water therefore they have been shows less degree of swelling in saline solution [48].

The chemically cross linked hydrogels have shown total different behaviors in their cumulative release percentages ($\%R_{cum}$) beside the release conditions in comparison with the physically cross linked hydrogels.

Table 6. Maximum cumulative release wt% of BSA from different blend hydrogels with optimize release conditions of pH and time.

Examined sample	Maximum BSA loading wt%	pH	Temperature (°C)	Maximum Cumulative Release wt%
AP/P-b-CH	23.8	6	40	19.6
AP/P-b-PH	30.8	8	15	27.2
ST/P-b-CH	19.3	6	25	16.2
ST/P-b-PH	24.3	8	25	19.6
SH/P-b-CH	25.9	6	15	20.7
SH/P-b-PH	22.3	8	25	19.4
EC/P-b-CH	28.1	6	25	21.4
EC/P-b-PH	29.0	8	25	26.6

Therefore, AP/P-b-CH, ST/P-b-CH, SH/P-b-CH and EC/P-b-CH hydrogel have shown Figure 16 and Table 6, almost both maximum loading and maximum cumulative release of BSA except SH/P-b-CH hydrogel lower than physically cross linked hydrogels, beside showing their maximum release in neutral release medium of pH6 and need almost high temperature for release because the hydrogels and according to their morphological structures, their surface appear salient with a lot of folds and holes Figure 12 which help BSA to remain loaded inside the hydrogels.

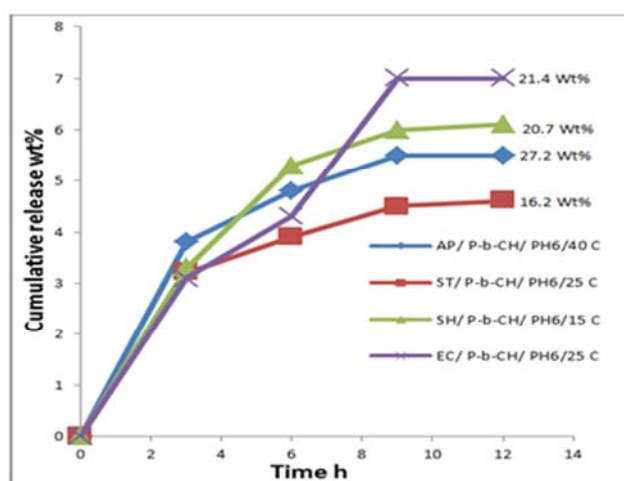


Figure 16. Maximum cumulative release of BSA from different chemically cross linked hydrogels.

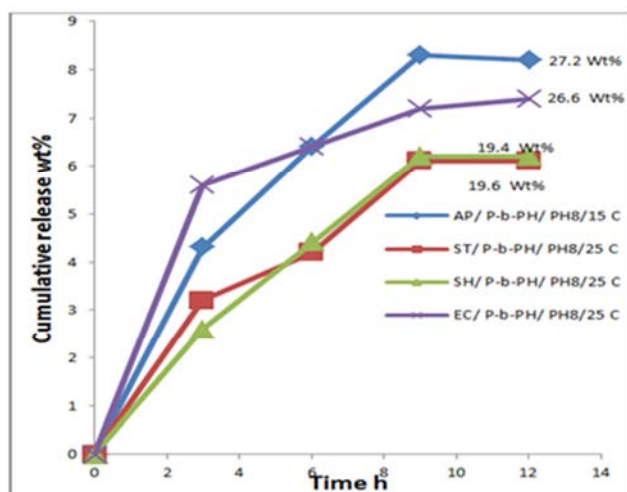


Figure 17. Maximum cumulative release of BSA from different physically cross linked hydrogels.

Whereas, the physically cross linked hydrogels have shown Figure 17 and Table 6, almost both maximum loading and maximum cumulative release of BSA higher except SH/P-b-CH hydrogel in comparison with physically cross linked

hydrogels, beside showing their maximum release in basic release medium of pH8 and need almost lower temperature because of the ionic interactions in physically cross linked hydrogels represented with SHMP anions and PS release medium anions which help for more repulsions between the hydrogel chains and increase the opportunity of the burst release and especially if those hydrogels are showing smooth and uniform surface with high number of pores and salience Figure 13 which could help also for more loading Figure 17 and Table 6.

The hydrogels and after release their loaded BSA should be characterized by SEM technique in order to evaluation used again as carrier for biomaterials. Therefore, studies the structures of the hydrogels after release and keep their form compact and retains with folds, holes and porosity are important signals to keep use again. The morphological surface of some studied hydrogels after release Figure 18 have shows polymers agglomerates distributed on the hydrogel surface represent cavities or holes are opened, empty and remained stable after release.

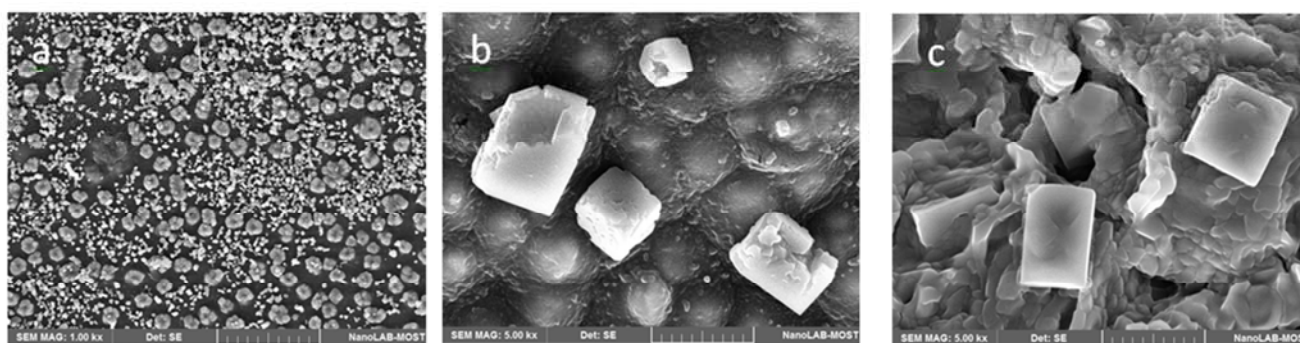


Figure 18. SEM image of a- AP/P-b-CH, b- ST/P-b-CH, c- SH/P-b-PH hydrogels after release.

Moreover, the hydrogel after release remain intact and there are some salt crystals belong to the PS solution Figure 18 need remove by washing with water, then the hydrogel could be used for carrying biomaterials many times.

4. Conclusion

PVA blend natural polymers such as AP, ST, SH and EC could produce hydrogels have shown homogeneous blends, compact form with elastic nature and could swell easily and to high extent in water and physical saline solution. Moreover, their structures contain folds, holes with undulant and coarse surfaces that could help for loading biomaterials and then release in high efficiency. The blend hydrogels were cross linked for improve their three dimensional structures, glutaraldehyde GLU and SHMP salt have been used as chemical and physical cross linker respectively. Their natures after cross linking have shows different character according to the types of cross linker due to the ionic nature of the prepared hydrogels. Generally, all the equipments used for

diagnosing, such as FTIR, XRD, DTA and SEM have shows the hydrogels are not similar either because of the types of blend natural polymer used or due to the types of the applied cross linker.

Bovine serum albumin was used as model protein for loading on different blend hydrogels. Different maximum loading percentages $%L_{max}$ have been recorded on different prepared hydrogels depending on the ionic nature of the hydrogels and their surface morphology beside the effects of loading conditions as pH, time and BSA concentrations of the loading medium. The release pattern of the loaded BSA from different hydrogels has been investigated in different pH release solution and at different temperatures. High cumulative release percentages of BSA from different hydrogels were recorded and in different competencies according to their degree of swelling which in turn affected by both natures of the hydrogels and release conditions. Generally, all the examined hydrogels are suitable and with great efficiency could carry the BSA protein safely with no mutants.

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