

Studies on Preparation and Characterization of Blend Polymers for Hydrogels Synthesis and Use for Protein Release

Roua'a K. Al-Ojar

Ministry of Science and Technology

Fawzi H. Jabrail

*Department of Chemistry/ College of Science/
University of Mosul*

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ABSTRACT

Carboxy methyl cellulose, pectin, chitosan or acrylic acid– poly (vinyl alcohol) blend hydrogels were prepared using Glutaraldehyde (GLU) as chemical or sodium hexameta phosphate (SHMP) as physical cross-linking agent. The prepared hydrogels have carried the advantage properties of both blend materials and can be used for loading and releasing of BSA protein. The degree of swelling of the prepared hydrogels was measured due to their different functional groups in different pHs of (pH4, pH7 and pH9) in addition to the electrolyte solution of 0.1 N NaCl. The blend hydrogels are characterized for their chemical structure using FT-IR. The XRD analysis was investigated for determination of the hydrogels crystallographic structure. The differential thermal analysis DTA and scanning electron microscope SEM were depended to study the thermal stability and surface morphology of the hydrogel respectively. The hydrogels were loaded with BSA protein model, and time of loading at (1.5, 3.0, 4.5 and 6.0) hrs were measured, beside the pH of the loading solution of pH4, pH6 and pH8 were considered and the BSA concentrations of (0.5, 1.0, 1.5 and 2.0) g/L have been investigated for maximum loading percentages %L_{max} of BSA on the hydrogels. Finally the loaded BSA was released under different release conditions in 0.9% w/v NaCl solution and pH4, pH6 and pH8 were depended and the BSA was tested to release at 15, 25 and 40°C releasing medium temperatures.

Keywords: Poly (vinyl alcohol) hydrogel; Bovine serum albumin release; Natural occurring polymers; Chitosan; Pectin; Carboxy methyl cellulose; Acrylic acid.

PE CMC)
PVA () AA (CS
.SHMP GLU
.BSA
pH7, pH4)
.0.1N NaCl (pH9
. XRD FTIR
SEM DTA
BSA ()
pH6 pH4) (1.5, 3.0, 4.5 and 6.0) hrs

(0.5, 1.0, 1.5 and 2.0) g/L BSA (pH8
 BSA %L_{max}
 pH8 pH6 pH4 NaCl0.9% w/v
 .(15, 25 and 40)°C
 :

INTRODUCTION

Hydrogels are polymers have the ability to imbibing huge amount of water or any bio-fluids due to their dimension structure and the presence of a lot of hydrolysable groups (Mallikarjuna *et al.*, 2014; Maitra and Shukla, 2014). Hydrogels are cross-linked chemically or through physical interactions (Ahmed, 2015); the interaction occurs between polymer and liquid as water is called polymer- liquid interaction and if the polymer is hydrophilic in its nature at that the product is called hydrosol (McKenzie *et al.*, 2015) which depend on many elements present in the polymer's structure or in the solution, such as functional groups of the polymer beside the ions present in the polymer structure, their types and amounts, in addition to the pH and temperature of the polymer solution (Ghobashy *et al.*, 2017). The chemical cross-linked hydrogel are permanent and hardly dissociated, whereas physical cross-linked hydrogel and because are formed by secondary forces like hydrogen bonding or ionic interactions and recent are reversible and may disrupted through the change in physical forces or under stress (Kitsara and Ducr'ee, 2013).

Poly(vinyl alcohol) PVA is hydrophilic and semi- crystalline polymer, it is linear and easily soluble in water (Gomez *et al.*, 2017). PVA has ability to blend with synthetic and natural monomers or polymers (Marin, *et al.*, 2014). The formed hydrogels of PVA are biomaterials have desirable properties and could improve their thermal, mechanical, physical and chemical properties. The blend hydrogels of the final improvement properties are depended fundamentally on the embedded materials properties (Marin *et al.*, 2014). Natural occurring polymers with their convenient properties like compatibility, high hydrophilicity, biodegradability and low production cost are very important materials to share synthetic polymers in preparation of superabsorbent polymer (Pourjavadi *et al.*, 2010). Carboxy methyl cellulose CMC, the important cellulose derivatives, is a linear, long chain with anionic water soluble polysaccharide material (Gupta and Jabrail, 2007). Whereas chitosan CS, the cationic amino polysaccharide, which produced from chitin is biocompatible, non-toxic and insoluble in water and organic solvent. although it is a hydrophilic polymer, its dissolution in water only takes place in dilute acidic solution like acetic acid (Mallakpour and Ezhieh, 2017; Del Valle *et al.*, 2017; Fan *et al.*, 2016).

Pectin is a natural biopolymer has a hetero structure polysaccharide mainly extracted from citrus fruits and used as a gelling agents, thickening agent and stabilizer and have several applications (Mongkolkitikul *et al.*, 2018).

In the present work, hydrogels are prepared from blending of carboxymethylcellulose (CMC), pectin (PE), and chitosan (CS), as a natural occurring polymers and acrylic acid (AA) as a synthetic monomer with poly (vinyl alcohol) (PVA). The prepared hydrogels were cross-linked for comparison chemically and physically using Glutaraldehyde and sodium hexameta phosphate (SHMP) respectively. The cross-linked hydrogels were used after loading for releasing of bovine serum albumin (BSA) protein in physiological saline (PS) solution.

EXPERIMENTAL

Materials

Poly (vinyl alcohol) (PVA: average mol. Wt. (MW) 101,000 $g.mol^{-1}$) was obtained from Qualiquens, India. Pectin (PE: MW:20,000 $g.mol^{-1}$), Carboxy methyl cellulose (CMC: MW:250,000

$g.mol^{-1}$), Chitosan (CS: MW:200,000 $g.mol^{-1}$) were obtained from BDH, UK; Fluka, SW; Sigma-Aldrich, UK, respectively. Acrylic acid (AA) was obtained from CAS, India. The cross-linkers Glutaraldehyde (GLU: 50 wt%) and Sodium hexameta phosphate SHMP were obtained from Fluka, SW and BDH, UK respectively. bovine serum albumin (BSA) was obtained from Fluka, SW.

Preparation of PVA Blend Hydrogels

10ml of 10% w/v PVA aqueous solution were prepared separately in different beakers. In other beakers, solution of 5 ml of 25% w/v of CMC in aqueous solution, 10 ml of 2% w/v of PE in aqueous solution, 5 ml of 20% w/v in 5% v/v of CS in acetic acid solution and 8 ml of 85% w/v of AA in 30% w/v NaOH alkaline solution were prepared. All previous solutions were prepared in equimoles with PVA solution according to their average molecular weights. Where $10^{-3}M$ of natural polymer solution or synthetic monomer, has been added into PVA solution (Wang and Gunasekaran, 2006) 10ml of 5% w/v of the initiator ammonium persulfate APS was added, then the final collected solutions were divided into two portions before the addition of cross-linker.

Subsequently, for preparation of natural polymer (CMC, PE and CS) blend PVA hydrogel, 5ml of 30 % w/v aqueous solution of SHMP as physical cross-linker was added for one portion and 0.5 ml of 50wt%GLU as chemical cross-linker was added for the second portion.

Quick addition with vigorous mixing of the cross-linkers has been done in order to avoid fast agglomeration and un-homogenized products. The blend content was continued mixing for extra one hour and finally, the beakers were left in the oven less than $60C^{\circ}$ until dried.

Similarly, the preparation of AA blend PVA hydrogel was prepared with same procedure only the difference is in the amounts of cross-linker added, where 3.5 ml of 10% w/v aqueous solution of SHMP or 0.25 ml of 50 wt.%GLU were added.

Characterizations of the hydrogels

FT-IR Spectroscopy

Fourier Transformer Infrared spectrophotometer type Shimadzu IR- Affinity/ Japan instrument was used for characterization of the prepared hydrogels. Where the wavenumbers of the hydrogel characterization bands are recorded in Table 1 for hydrogels cross-linked chemically and Table 2 for hydrogels cross-linked physically and after the hydrogels are loaded with the BSA model protein.

X-ray diffraction

X-ray diffraction type XRD-P analytical, 2013, Netherland, was used for characterization of the crystal structure of the pristine hydrogels were recorded up to 20 scale in an angle range of 5° - 90° at a scan speed of $1^{\circ}/min$ using copper/Indium (0.9/0.1) 100% radiation target and nickel filter at a current of around $20\mu A$ a voltage of 35kv.

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.

Thermal analysis

Differential thermal analysis DTA, beside maximum temperature decomposition T_{max} and glass transition temperature T_g of the BSA loaded and unloaded hydrogels were investigated using Thermal analysis, DTA-60/ Simultaneous DTA-TG, Apparatus, Shimadzu/ Japan instrument, with heating rate of $10^{\circ}C/min$ in nitrogen atmosphere.

SEM analysis

The SEM micrographs of pristine, BSA loaded and after release hydrogels were studied, and TESCAN, Vega, III, 2011, Czech Republic instrument was used. 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.

Degree of swelling measurements

The degree of swelling (S_w) of prepared hydrogels were measured after cut off the hydrogel into 1-3mm size pieces. Fixed weight of dry hydrogel pieces were immersed in distilled water and

their precise weight each 3hrs has been measured after removal of all un-adsorbed water drops, even tissue paper were used for more accuracy. The following equation was depended for S_w measuring (Sing *et al.*, 2008).

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

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

Loading of hydrogels with BSA Protein

Three loading effective variables were studied on BSA loading hydrogels are time, pH of loading medium and the concentration of BSA protein used for 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 1.5hrs the hydrogel pieces 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} 279nm and the absorbance (A) was measured and calibration curve was used for determination of remain BSA solution concentration. 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 maximum loading (L_{max}) and efficiency of loading (EL) of the hydrogels (Krishna *et al.*, 2008).

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

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

Cumulative release of BSA protein from hydrogel

100 mg of BSA maximum loaded hydrogels were kept in 200ml of physiological saline PS solution prepared from 0.9%w/v aqueous solution of NaCl. The cumulative release of BSA from the hydrogels was measured each 3hrs and for time intervals of 12hrs by recording the absorbance A at λ_{max} 279nm of release medium and with replacement using UV-Visible spectrophotometer. The cumulative release (R_{cum}) of BSA protein was calculated by the following equation (Saputra *et al.*, 2014).

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

Where, W_t is the amount of BSA protein released at time t, and W_o is represent total amount of BSA protein release finally.

Two variables are considered for measuring R_{cum} are temperature of the release medium and its pH as pH4, pH6 and pH8 were tested for calculation of R_{cum} .

RESULTS AND DISCUSSION

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

Smart or intelligent polymers that are responsive to external stimuli such as temperature, pH for applications in bioseparation and bioprocessing of biomaterials like protein.

Hydrogels Characterization

Poly (vinyl alcohol) PVA was used for preparation of blend hydrogels and the following abbreviation codes were depended:

- 1- CMC blend PVA and cross-linked chemically is **CMC/ P-b-CH**
- 2- CMC blend PVA and cross-linked physically is **CMC/ P-b-PH**
- 3- PE blend PVA and cross-linked chemically is **PE/ P-b-CH**
- 4- PE blend PVA and cross-linked physically is **PE/ P-b-PH**
- 5- CS blend PVA and cross-linked chemically is **CS/ P-b-CH**
- 6- CS blend PVA and cross-linked physically is **CS/ P-b-PH**
- 7- AA blend PVA and cross-linked chemically is **AA/ P-b-CH**
- 8- AA blend PVA and cross-linked physically is **AA/ P-b-PH**

The pristine hydrogels are characterized depending on their FT-IR, X-ray diffraction, thermal and SEM analysis.

FT-IR Spectroscopy

FT-IR spectra of the hydrogels were measured and the absorption frequencies of their features functional groups were studied for fixing the hydrogel chemical composition, nature of bonding and the type of the conjugations.

The important characteristic bands of the investigated hydrogels are almost same especially for the same hydrogel and the only differences are the type of cross-linker, Where either acetyl linkages formed by Glutaraldehyde cross-linker or phosphate linkages formed by SHMP cross-linker. The characteristic bands of hydroxyl group $\gamma(\text{O-H})$ of PVA beside the methyl carboxylate anions in CMC polymer occurred in CMC/ P-b-CH and CMC/ P-b-PH hydrogels Fig.(1) and Tables (1 and 2) have absorption frequencies almost same and only the frequencies appear at (722, 1150, 1100 and 1280) cm^{-1} which represent the $\gamma(\text{P-O-P})_{\text{str}}$ band of SHMP (Saputra *et al.*, 2014).

Table 1: FT-IR Wavenumbers of the characteristic bands of chemical cross-linked hydrogels

Examined Sample	Wavenumber of characteristic band, cm^{-1}							
	$\gamma(\text{O-H})_{\text{str}}$	$\gamma(\text{C-H})_{\text{str}}$	$\gamma(\text{C=O})_{\text{str}}$	$\gamma(\text{C-O})_{\text{str}}$	$\gamma(\text{C-O-C})_{\text{str}}$	$\gamma(\text{N-H})_{\text{str}}$	$\gamma(\text{C-N})_{\text{str}}$	$\gamma(\text{C-H})_{\text{def}}$
CMC/P-b-CH	3493,1340	2950	1620,1738	1440	1150	-----	-----	781,802
PE/P-b-CH	3435,1320	----	1650,1390	1450	1150	-----	-----	760,804
CS/P-b-CH	3470,1320	2860	1744	1075	1146	3210	1566	795
AA/P-b-CH	3505,1315	2918	1750	1440	-----	-----	-----	783

Table 2: FT-IR wavenumbers of the characteristic bands of physical cross-linked hydrogels

Examined Sample	Wavenumber of characteristic band, cm^{-1}								
	$\gamma(\text{O-H})_{\text{str}}$	$\gamma(\text{C-H})_{\text{str}}$	$\gamma(\text{C=O})_{\text{str}}$	$\gamma(\text{C-O})_{\text{str}}$	$\gamma(\text{C-O-C})_{\text{str}}$	$\gamma(\text{N-H})_{\text{str}}$	$\gamma(\text{C-N})_{\text{str}}$	$\gamma(\text{C-H})_{\text{def}}$	$\gamma(\text{P-O-P})_{\text{str}}$
CMC/P-b-PH	3476, 1315	2936	1650, 1740	1443	1150	-----	-----	792	722,1150, 1100,1280
PE/P-b-PH	3550, 1319	2950	1738, 1603	1435	1148	-----	-----	789	710,1148, 1095, 1290
CS/P-b-PH	3482, 1320	2920	1750, 1650	1440	1150	3280	1540	727	727,1150, 1090,1270
AA/P-b-PH	3506	3090	1742,1603	1402	-----	-----	-----	799	720,1153

Similarly in PE/P-b-CH and PE/P-b-PH hydrogels also have shown characteristic bands Fig. (2) and Tables (1 and 2) almost same for carboxyl and hydroxyl groups of pectin beside the hydroxyl group of PVA (Baum *et al.*, 2017) and only diverse in their functional groups of cross-link type. The FT-IR spectra of chitosan has shown amid-I and amid-II beside carbonyl functional groups (Bisen *et al.*, 2007) which are characteristic bands Fig. (3) and Tables (1 and 2) all are

shown in both CS/P-b-CH and CS/P-b-PH hydrogels and the cross-linker was the only the difference between them. Finally carboxylic acid groups of acrylic acid beside the hydroxyl group of PVA were characteristic bands of AA/P-b-CH and AA/P-b-PH hydrogels and the only differences in their FTIR spectra Tables (1 and 2) and Fig. (4) were the functional groups of the cross-linker.

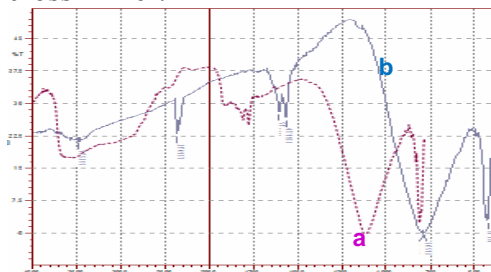


Fig. 1: FT-IR spectra of a. CMC/P-b-CH and b. CMC/P-b-PH hydrogels

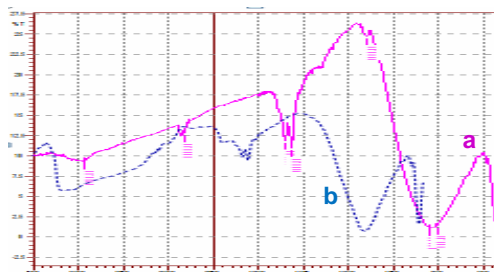


Fig. 2: FT-IR spectra of a. PE/P-b-CH and b. PE/P-b-PH hydrogels

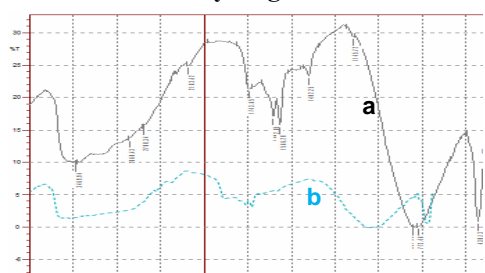


Fig. 3: FT-IR spectra of a. CS/P-b-CH and b. CS/P-b-PH hydrogels

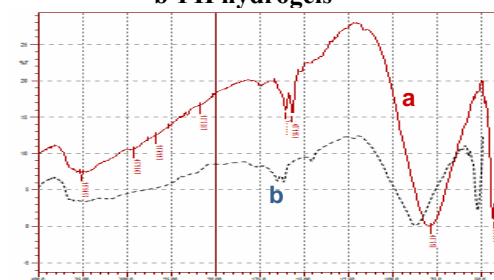


Fig. 4: FT-IR spectra of a. AA/P-b-CH and b. AA/P-b-PH hydrogels

X-ray diffraction

The XRD pattern data recorded in (Table 3) are shown five peaks some are intense and other are broad for CMC/P-b-CH hydrogel. The peaks positions at 2θ axis have shown almost three different crystalline material in the hydrogel. The full width at half maximum (FWHM) of the broadened line along of CMC polymer in the hydrogel was depended for calculation of the area under peaks. Therefore, the percent crystallinity of CMC polymer in the hydrogel, (Table 3) is shown broad peak which give indication that CMC is non crystallizable and will decrease the crystallinity of the hydrogel necessarily.

Table 3: XRD intensity scan values of chemically cross-linked hydrogels

Examined sample	Peaks at $2\theta^{\circ}$	Interplanar distance "d" (Å)	FWHM (Å)	Crystalline phase (A _c) (Å)	Amorphousness phase (A _a) (Å)	Crystallinity percentage (X _c) (%)
CMC/P-b-CH	42.095	2.146	0.230	126.90	962.80	11.65
	44.693	2.027	0.307	111.38	1013.28	9.90
	49.149	1.853	0.256	73.44	973.00	7.02
	72.630	1.302	0.231	671.98	823.61	44.93
	88.217	1.107	0.512	328.14	897.53	26.77
PE/P-b-CH	8.227	10.747	0.819	210.17	558.90	27.33
	72.777	1.299	0.409	17.20	217.78	7.32
CS/P-b-CH	42.152	2.144	0.256	30.44	236.17	11.42
	49.167	1.853	0.307	26.51	255.23	9.41
	72.664	1.301	0.179	51.06	154.17	24.88
	88.351	1.106	0.614	30.47	120.63	20.17
AA/P-b-CH	44.657	2.0293	0.154	16.29	709.32	2.25
	72.650	1.301	0.358	135.59	426.43	24.13
	88.411	1.106	0.614	67.92	404.40	14.38

Whereas, the sharp and narrow peaks of PVA alone before blending give high crystallinity, but its blend in the CMC/P-b-CH hydrogel beside the cross-linker, PVA polymer start loss part of its crystallinity (Table 3). Finally, the CMC/P-b-CH hydrogel loss its crystallinity because crystal degree of PVA chains is reduce through breaking down the inter chain hydrogel bonding along the PVA chains (Deshpande *et al.*, 2011).

The XRD patterns of PE/P-b-CH, CS/P-b-CH and AA/P-b-CH hydrogels are tend to non-crystallizable especially in case of pectin hydrogel because of its high amorphousness of the polymer, but their amorphousness would reduce when sodium hydroxide was added because of the high crystallinity of its sodium ions (Hermans and Weidinger, 1951). As it is clear, in CMC/P-b-CH hydrogel where it is in sodium form. Even in AA/P-b-CH hydrogel, the percent crystallinity is depressed inside the hydrogel because blending will decay the regularity of PVA chains (Deshpande *et al.*, 2012).

The physical cross-linked hydrogels in presence of SHMP with its huge quantities of sodium ion will elevate the degree of the blend hydrogels, where CMC/P-b-PH hydrogel (Table 4), shows PVA peaks appeared with atypical sharp and narrow shape with high intensity, which will elevate the whole crystallinity of the hydrogel structure.

Similarly, PE/P-b-PH hydrogel has reach high percent crystallinity and it is expected that the CS/P-b-PH hydrogel and AA/P-b-PH hydrogel also their percent crystallinity were elevate due to the presence of crystalline SHMP cross-linker.

Table 4: XRD intensity scan value of some physically cross- linked hydrogels

Examined Sample	Peaks at $2\theta^{\circ}$	Interplanar distance "d" (Å)	FWHM (Å ^o)	Crystalline phase (A _c) (Å ^o)	Amorphousness phase (A _a) (Å ^o)	Crystallinity percentage (X _c) (%)
CMC/P-b-PH	19.845	4.474	0.921	563.36	594.18	48.67
	42.116	2.146	0.307	73.41	731.83	9.12
	44.667	2.029	0.154	32.54	729.75	4.27
	49.132	1.854	0.409	48.81	665.71	6.83
	72.664	1.301	0.102	122.77	617.83	16.58
	88.257	1.107	0.409	137.40	686.31	16.68
PE/P-b-PH	24.314	3.661	0.818	19.54	339.02	5.45
	42.131	2.145	0.154	69.69	910.66	7.11
	44.711	2.027	0.307	89.93	928.00	8.56
	49.158	1.853	0.256	54.07	869.38	5.86
	72.643	1.302	0.205	310.52	673.98	31.54
	88.373	1.106	0.461	247.85	784.13	24.02

Thermal Studies

Differential thermal analysis DTA of the pristine hydrogel were investigated, DTA thermograms gives thermal information about the studies hydrogel such as glass transition temperature T_g , maximum decomposition temperature T_{max} , crystalline temperature T_{cr} and heat of function ΔH_f which are important parameter give good information about the endo-and exo-thermic transition of the hydrogel as a function of temperature.

Thermoanalytical data recorded in (Table 5) give the thermal analysis characterizations of the four tested hydrogel samples cross-linked chemically. The CMC/P-b-CH hydrogel has shown T_g at 119.9°C means the hydrogel is thermaly stable, and its T_{max} and T_{cr} at 435°C and 400°C respectively (Table 5), Fig. (5). Means the hydrogel decomposed at 435°C and loss its structure and at 400°C start loss its crystalline structure and be ready for decomposition. The heat of function ΔH_f of the hydrogel gives two important transitions (Table 5), Fig.(5), one is endotherm of +45.8 J.g⁻¹, give indications that the hydrogel components (CMC and PVA) and the sodium ions present in the hydrogel would left the hydrogel has some crystalline portions in its morphology therefore it need

energy of 45.8 joule per gram for decomposition of those crystalline portions. Whereas the $-5.8 J.g^{-1}$ means the hydrogel has amorphous portions and on decomposition will liberate energy 5.8 Joule per gram of the hydrogel (El- Naggar *et al.*, 2017).

According to the Table 5, PE/P-b-CH, CS/P-b-CH and AA/P-b-CH hydrogel have shown thermal analysis data almost very close to CMC/P-b-CH hydrogel, where their T_g , T_{max} and T_{cr} Figs. (6-8) are recorded also at high temperature which means they have high thermal stability and even shows ΔH_f of two transitions one endo-and other exo-thermic transitions but with different magnitudes depends on crystalline and amorphous portions in the hydrogel structure (Fan *et al.*, 2016).

Table 5: DTA thermal data of chemically cross-linked hydrogels

Examined sample	$T_g(^{\circ}C)$	$T_{max}(^{\circ}C)$	$T_{cr}(^{\circ}C)$	$\Delta H_f(J.g^{-1})$
CMC/P-b-CH	119.9	435	400	+32.2; -5.8
PE/P-b-CH	103.7	420	408	+15.8; -6.0
CS/P-b-CH	111.6	431	399	+25.3; -25.9
AA/P-b-CH	114.2	420	416	+47.5; -7.2

The physical cross-linking hydrogels have shown thermal analysis Table 6 and Figs. (5-8) with completely different thermograms, The main differences recorded in physical cross-linked hydrogels in comparison with those cross-linked chemically are the little depression in their thermal parameters represent T_g , T_{max} and T_{cr} and especially in case of CMC/P-b-PH, CS/P-b-PH and AA/P-b-PH Fig. (5), Fig. (7) and Fig. (8) respectively due to their weak crystalline structure and this is clear from their ΔH_f , where their endo-thermic transitions are little depress in comparison with the chemical cross-linked hydrogels and their exo-thermic transitions are elevate which means the physical cross-linked hydrogels are less thermally stable due to the easier collapse of their systems (Akhtar and Ranjha, 2016), whereas PE/P-b-PH hydrogel (Table 3-6 and Fig. 6), where pectin with SHMP could increase its crystallinity and enhance the crystallographical structure of the hydrogel.

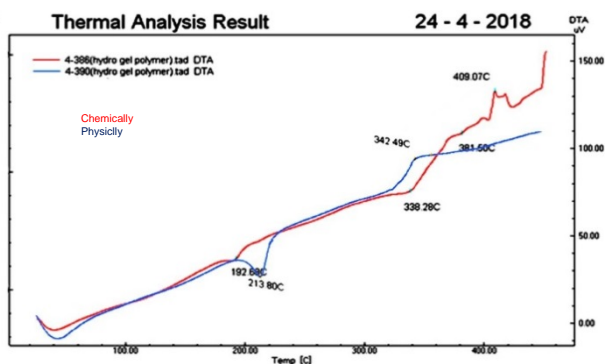


Fig. 5: DTA of CMC/P-b-CH & CMC/P-b-PH hydrogels

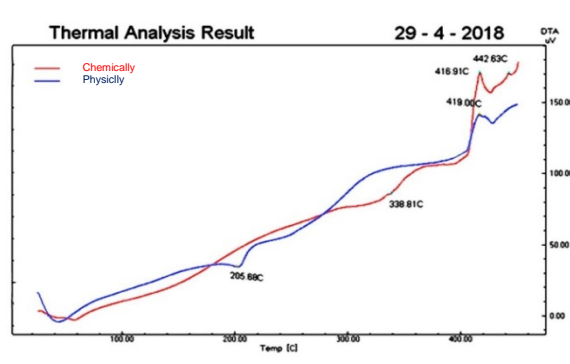


Fig. 6: DTA of PE/P-b-CH & PE/P-b-PH hydrogels

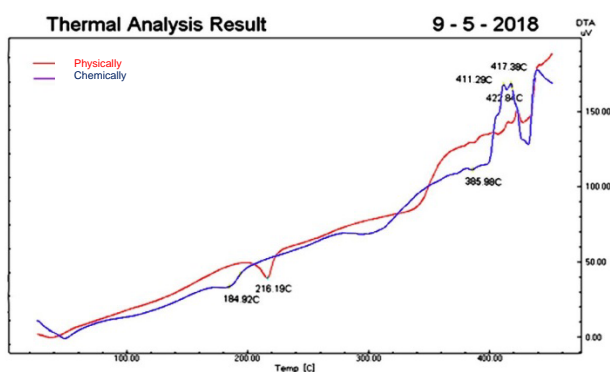


Fig. 7: DTA of CS/P-b-CH & CS/P-b-PH hydrogels

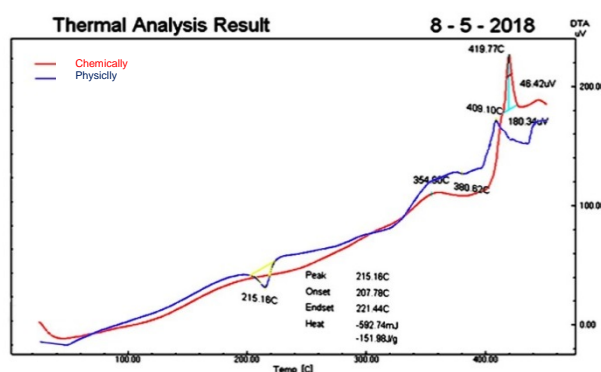


Fig. 8: DTA of AA/P-b-CH & AA/P-b-PH hydrogels

Table 6: DTA thermal data of physically cross-linked hydrogels

Examine sample	T _g (C°)	T _{max} (C°)	T _{cr} (C°)	ΔH _f (J.g ⁻¹)
CMC/P-b-PH	106.2	358	320	+16.3; -200.0
PE/P-b-PH	115.4	433	408	+11.4; -9.5
CS/P-b-PH	104.5	397	341	+15.1; -34.4
AA/P-b-PH	74.9	412	398	+13.8; -23.0

SEM images

SEM studies and composite morphologies of the tested hydrogels are illustrates in Figs. (9-12) where CMC/P-b-CH has shown in Fig. (9) folded surface and its blend components are homogenously distributed and its surface seems to be suitable for highly loading of liquids or solid materials. Whereas CMC/P-b-PH hydrogel Fig. (9) has shown smooth surface with a lot of pores and cavities and seems to be more compact (Pourjavadi *et al.*,2007).The PE/ P-b-CH hydrogel SEM image has shown irregular and folded surface with a lot of cavities Fig. (10), while PE/P-b-PH hydrogel Fig. (10) has SEM image shows smooth and porous surface and compact form. Same photograms have seen in case of CS/P-b-CH and CS/P-b-PH hydrogels Fig. (11), where the hydrogel's surface was rough with many folds and shown cavities between the folds with many pores distributed irregularly between folds (Fan *et al.*, 2016). Similarly the CS/P-b-PH hydrogels has shown less fold surface with cracks Fig.11 and white spots represent same unmixed well components of the hydrogel which effect of course on its homogeneity.

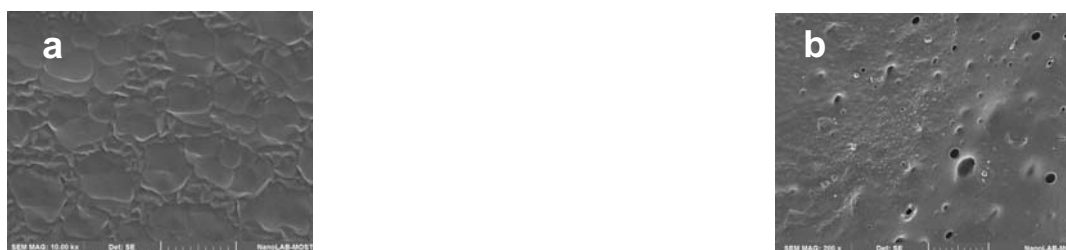


Fig. 9: SEM micrograph of a. CMC /P-b-CH and b. CMC /P-b-PH hydrogels

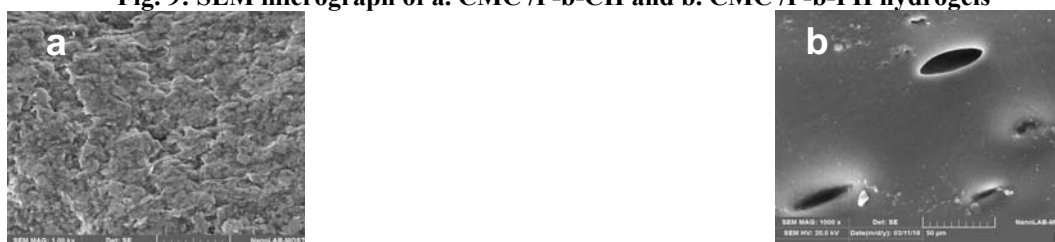


Fig. 10: SEM micrograph of a. PE /P-b-CH and b. PE /P-b-PH hydrogels

The AA/P-b-CH hydrogel has shown SEM image of transparent crystalline surface Fig. (12) contain cut away of compact crystals in its composite structure. In addition the cavities and pores are in between the crystals which will help for loading materials (Pourjavadi *et al.*,2007). The AA/P-b-PH hydrogel give highly crystalline structure in its SEM photograms Fig. (11), with un-homogeneous surface seems to be unable for loading materials in high quantities.

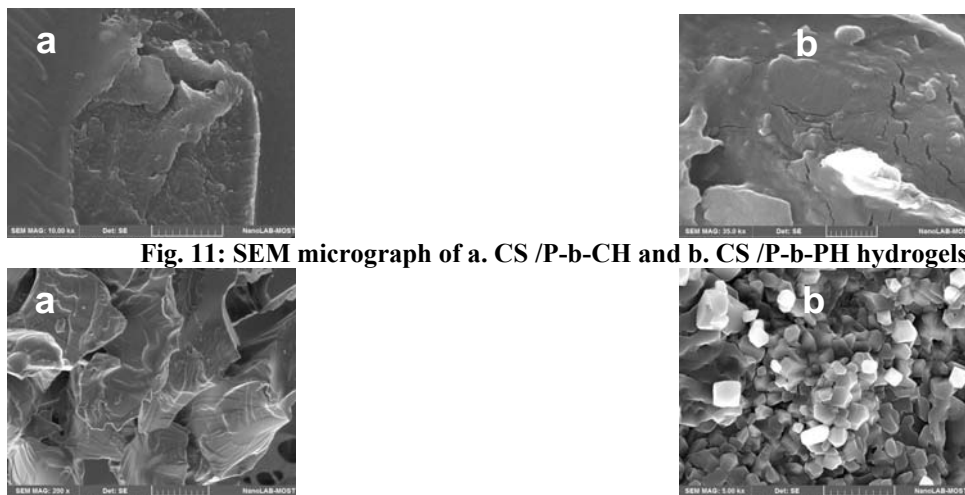


Fig. 11: SEM micrograph of a. CS /P-b-CH and b. CS /P-b-PH hydrogels



Fig. 12: SEM micrograph of a. AA/P-b-CH and b. AA/P-b-PH hydrogels

Studies on degree of swelling (S_w)

The degree of swelling of the prepared hydrogels in different pH swelling medium were investigated for best swelling conditions and in 0.1 N saline solution the degree of swelling also was examined. The degrees of swelling of CMC/P-b-CH and CMC/P-b-PH hydrogels were measured according to the Eq. 2, using swelling medium of pH4, pH7 and pH9 in addition to 0.1 N NaCl. Fig. (13) shows significant effects of pH or ionic solution of swelling medium on S_w (Salmawi, 2007). Where pH7 seems better pH swelling medium for CMC/P-b-CH hydrogel Fig. (13) while pH9 was better as pH swelling medium for CMC/P-b-PH hydrogel Fig. (13), because the basic medium would provide extra anions beside the anions of the physical cross-linker and as a result the repulsions between polymer chains will increase which elevate the hydrogel volume and finally increase its degree of swelling. Whereas, CMC/P-b-CH hydrogel has its maximum degree of swelling in pH7 Fig.(13), because the hydrogel is neutral and has no ionic structure and the swelling control factor is the penetration capability of the solvent molecules between polymer chains. In pH4, both hydrogels have shown low degree of swelling Fig.(13), because they shrink in acidic medium and especially CMC/P-b-PH due to neutralization of its anions with acid cations of the swelling medium Fig. (13). The swelling medium 0.1 N NaCl has a significant effect on the S_w of the hydrogels. The saline solution will effect on both CMC/P-b-CH and CMC/P-b-PH hydrogel Fig.13 because NaCl the strong electrolyte when dissociate will prevent hydrogen bonding in CMC/P-b-CH hydrogel and decrease S_w , beside it prevent the ionic interaction between polymer chains of CMC/P-b-PH hydrogel and decrease its S_w .

For PE/P-b-CH and PE/P-b-PH hydrogels are shown maximum S_w . Therefore, PE/P-b-CH at pH9 same like CMC/P-b-CH hydrogel Fig. (14), while PE/P-b-PH has shown more swelling in pH4 due to its carboxylic groups Fig. (14). However, Chitosan the cationic natural polymer its hydrogel CS/P-b-CH has high degree of swelling in comparison with physical cross-linked hydrogel Fig. (15), whereas AA/P-b-CH hydrogel has shown high swelling in pH9 due to repulsion between carboxylate anions of the acrylic acid and anions of the pH9 which elevate the swelling Fig. 16, while the AA/P-b-PH hydrogel shows higher degree of swelling in pH4 due to repulsion between both pH4 swelling medium and acrylic acid cations. In addition 0.1 N NaCl would help AA/P-b-PH hydrogel for more degree of swelling Fig. (16), due to anions repulsion between the hydrogel chains.

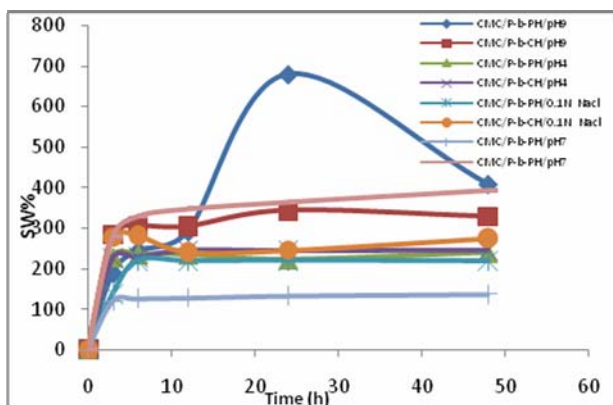


Fig. 13: Effects of pH or saline solution on S_w of CMC/P-b-CH and CMC/P-b-PH hydrogels

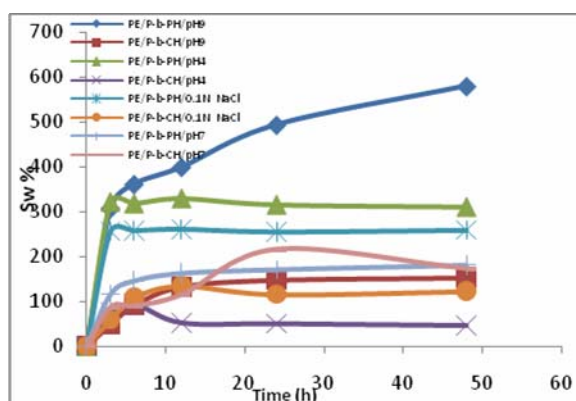


Fig. 14: Effects of pH or saline solution on S_w of PE/P-b-CH and PE/P-b-PH hydrogels

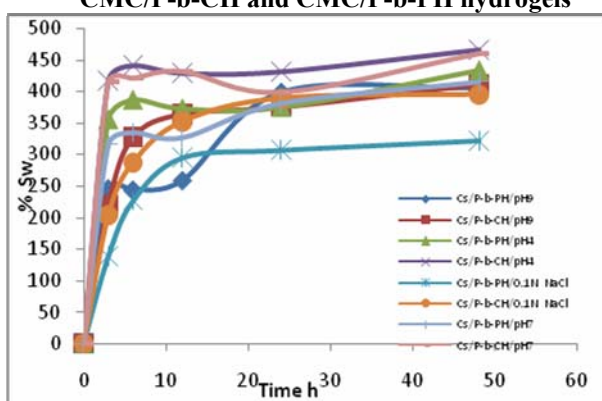


Fig. 15: Effects of pH or saline solution on S_w of CS/P-b-CH and CS/P-b-PH hydrogels

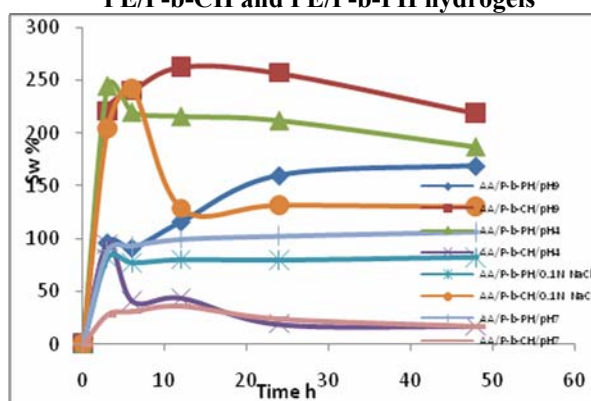


Fig. 16: Effects of pH or saline solution on S_w of AA/P-b-CH and AA/P-b-PH hydrogels

Loading of BSA on hydrogels

Different conditions of BSA loading hydrogels are considered due to their importance to reach maximum loading and to increase the efficiency of loading, therefore UV-Visible technique at λ_{max} 279 nm were depended for measuring the absorbance (A) of BSA loaded protein on prepared hydrogels. Loading pH and its time beside the concentration of BSA used for loading were investigated. The Eqs. (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 swelling degree and as the hydrogel swell more will be load more. Accordingly, because degree of swelling is affected significantly with pH, therefore pH control swelling and swelling play an important role in hydrogel loading. Fig. (17) has shown the efficiency of BSA loading (EL) versus pH solution where in which the hydrogel reach its efficient loading (Rizwan *et al.*, 2017). The time of loading also has significant effects on efficiency of loading, Fig.18 has shown the efficiency of BSA loading versus time in hours. Where the dependence of loading on time is in turn depend on degree of swelling of the hydrogel (Sriamornsak *et al.*, 2010). On account of the hydrogel is need sufficient time to reach its maximum degree of swelling and already when the hydrogel reach maximum swelling means the hydrogel will reach its maximum loading Fig. (18).

From other side, BSA concentration has shown an effect on the efficiency of loading (EL) percentage. Fig. (19) has shown the maximum loading of BSA which depends on the concentration of BSA in order to optimize the economical concentration of BSA in loading solution. The maximum loading of the hydrogels cross-linked chemically or physically are shown depended to their degree of swelling Figs. (17-19) and because physically cross-linked hydrogels have ionic interactions between their chains (Parhi, 2017), they show more effects from the loading conditions than chemically cross-linked hydrogels.

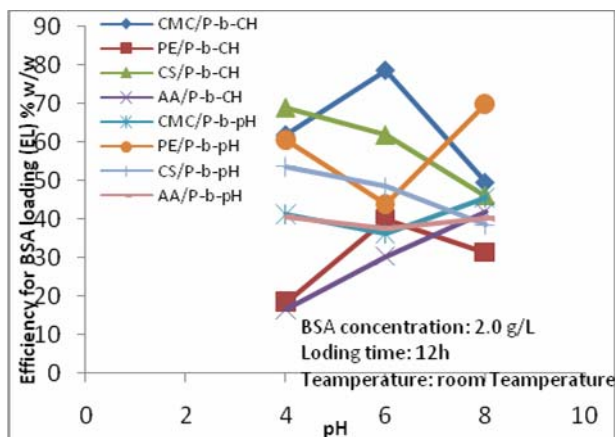


Fig. 17: Efficiency of BSA loading on chemically and physically cross-linked hydrogels for pH optimization

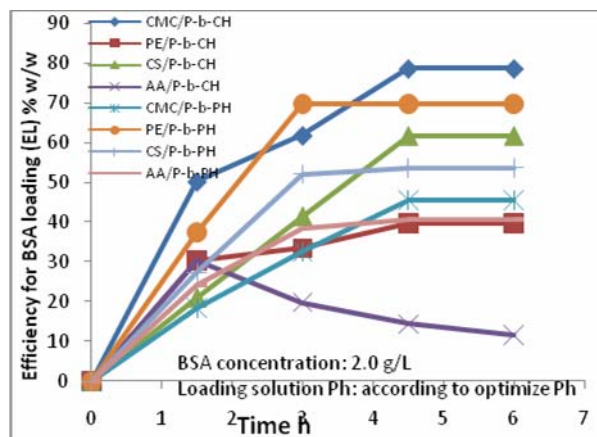


Fig. 18: Efficiency of BSA loading on chemically and physically cross-linked hydrogels for time optimization

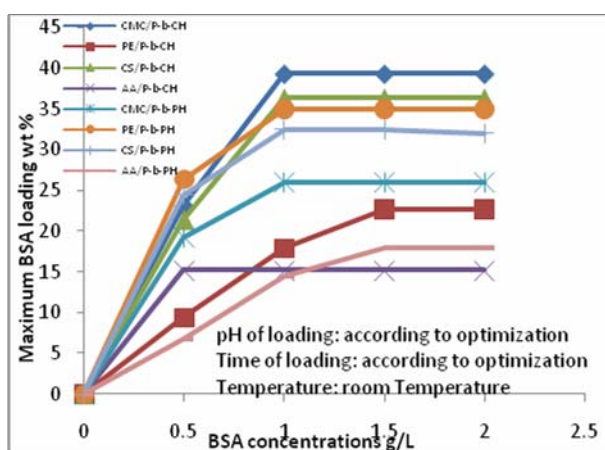


Fig. 19: Maximum BSA loading (L_{max}) chemical and physical cross-linked hydrogels versus concentrations of BSA

Studies on releasing of BSA from hydrogels

The BSA loaded hydrogels were examined in their cumulative release (R_{cum}) percentage for 12 hrs and each time intervals of 3hrs inside physiological saline (PS) solution which was prepared of 0.9% w/v. The release process was examined under two variable conditions are release medium temperature of 15, 25 and 40°C in addition to the PH of the release medium as pH4, pH6 and pH8 and Eq.5 has been depended in addition to the new calibration curve was prepared in PS solution for calculation the concentrations of BSA release depending on UV-Visible spectrophotometer for determination of the BSA release absorbance (A) at λ_{max} 279 nm.

The BSA protein was released in PS release solution because BSA hardly diffuse in D.W., Whereas the Saline ions prevent non-ionic interaction by ordering the structure of water (Rose, 1989). So that the BSA protein molecules absorbed on hydrophobic groups of the hydrogel could release easily in saline solution (Oztop *et al.*, 2003; Wang *et al.*, 2018; Asenjo and Andrews, 2011).

The degree of release of the hydrogel is in concert with its degree of swelling but this truth is in D.W. whereas it shows swelling loss in saline solution (Hosseinzadeh, 2013).

The CMC/P-b-CH hydrogel loaded with $\%L_{max} = 39.3 \text{ wt}\%$ BSA has shown depression in its $\%R_{sum}$ in comparison with its $\%L_{max}$ which means some of BSA protein was remained inside the hydrogel and not release completely and the hydrogel record its best release in pH6 and at 15°C Fig. (20), with $\%R_{sum} = 26.2 \text{ wt}\%$ and record release efficiency percentage of 66.7%. The CMC/P-b-CH hydrogel has shown minimum release in acidic and basic medium due to the ionic interaction with the ions of the release medium (El- Naggar *et al.*, 2017). Whereas the CMC/P-b-PH hydrogel

has shown its maximum release in basic medium and at 15°C Fig. 21. The $\%L_{max} = 26.0 \text{ wt}\%$ BSA of CMC/P-b-PH hydrogel would release $\%R_{sum} = 23.6 \text{ wt}\%$ with release efficiency percentage of 90.8% in pH8 release medium which increase the repulsions between the hydrogel chains and increase its swelling and finally its cumulative release.

The cumulative release of PE/P-b-CH hydrogel has reached to $\%R_{sum} = 18.4 \text{ wt}\%$ of $\%L_{max} = 22.7 \text{ wt}\%$ with release efficiency percentage of 81% in pH8 release medium Fig. (22), and at 15°C due to the hydrogel internal ions which are tended to anionic categories that enter in repulsions with the ions of the basic medium and therefore increase its degree of swelling and as result its cumulative release (Martínez *et al.*, 2014). Similarly, PE/P-b-PH hydrogel has shown its maximum release also in pH8 but at 25°C Fig. 23 for hydrogel has $\%L_{max} = 34.9 \text{ wt}\%$ BSA and its $\%R_{sum} = 29.5 \text{ wt}\%$ with release efficiency percentage of 84.5%.

The cationic natural occurring polymer chitosan was considered one of most famous pharmaceutical polymer (Gupta and Jabrial, 2007). Where the CS/P-b-CH hydrogel was loaded with BSA protein and its $\%L_{max} = 36.3 \text{ wt}\%$ and it was shown its maximum $\%R_{sum} = 31.6 \text{ wt}\%$ in pH4 release medium and at 15°C Fig. (24), and 87.0% efficiency release percentage.

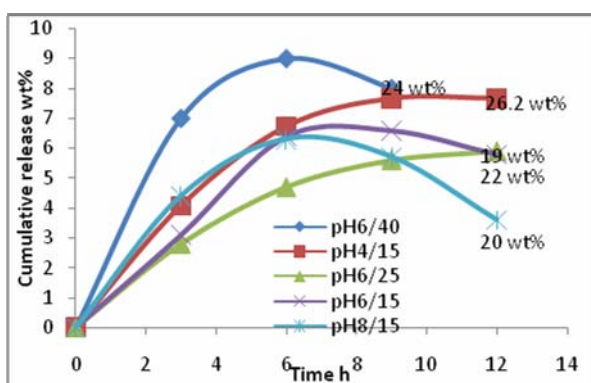


Fig. 20: % R_{cum} of CMC/P-b-CH hydrogel

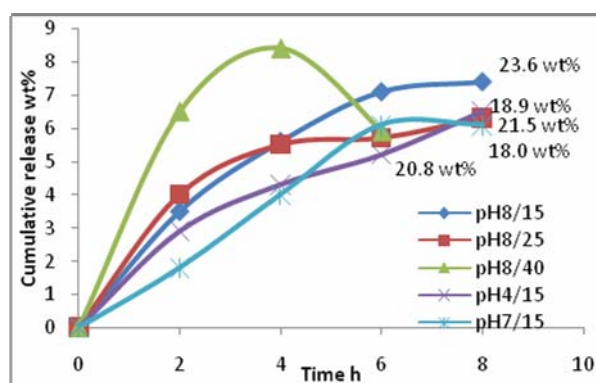


Fig. 21: % R_{cum} of CMC/P-b-PH hydrogel

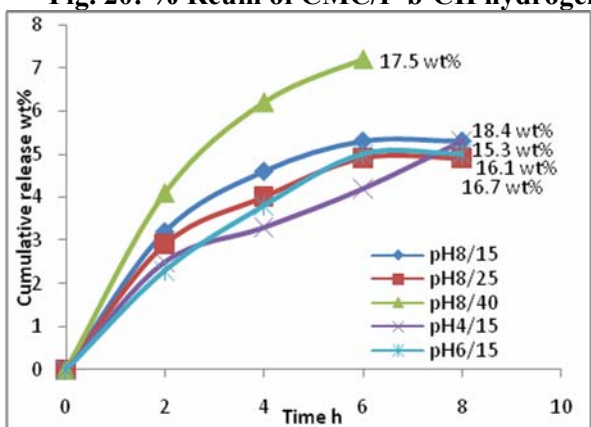


Fig. 22: % R_{cum} of PE/P-b-CH hydrogel

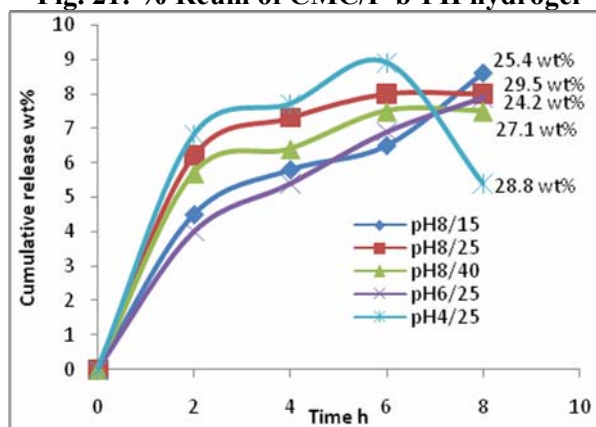


Fig. 23: % R_{cum} of PE/P-b-PH hydrogel

Similarly, the CS/P-b-CH hydrogel has shown its maximum release also in pH4 and at 25°C because of its cationic natural which do repulsions between the hydrogel cations and release medium cations and finally increase the degree of swelling and the maximum cumulative release. The Fig. (25), has shown CS/P-b-PH hydrogel with $\%L_{max} = 32.0 \text{ wt}\%$ BSA and it give $\%R_{sum} = 28.8 \text{ wt}\%$ with 90% efficiency release percentage.

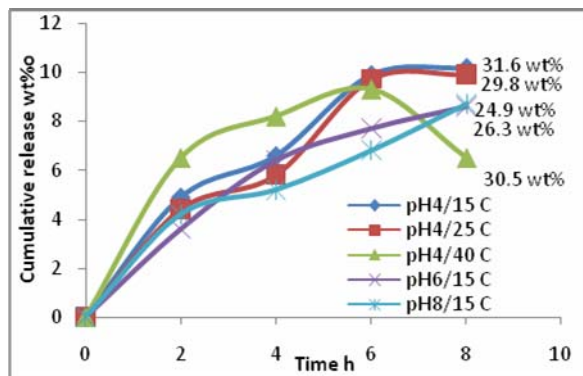


Fig. 24: % R_{cum} of CS/P-b-CH hydrogel

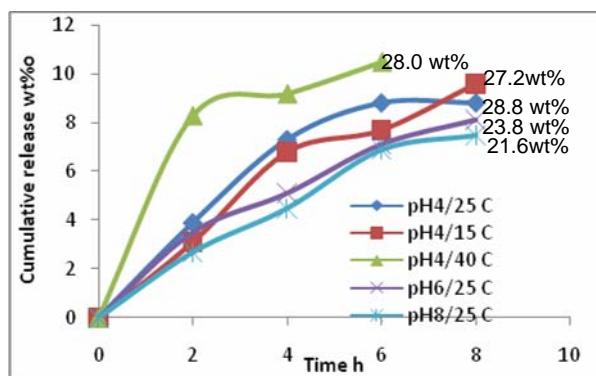


Fig. 25: % R_{cum} of CS/P-b-PH hydrogel

The crystalline AA/P-b-CH structure has shown low maximum loading between its crystals and in between its cavities therefore, its $\%L_{max} = 15.2 \text{ wt}\%$ and it give $\%R_{sum} = 12.6 \text{ wt}\%$ Fig. 26, and with almost high magnitude of efficiency release percentage 82.9% where BSA molecules like to leave the crystalline structure of the hydrogel in pH8 release medium due to the competition between BSA anions and the pH8 release medium anions. Also the AA/P-b-PH hydrogel release BSA weakly from hydrogel was loaded with $\%L_{max} = 18.0 \text{ wt}\%$ and its $\%R_{sum} = 15.9 \text{ wt}\%$ Fig. (27), with high efficiency of release percentage of 88.3%, but for this hydrogel the pH4 and 15°C temperature were suitable for best release due to the physical cross-linked hydrogel, where its cations help for more repulsions between the chains and its degree of swelling increase and finally it release efficiency percentage would increase.

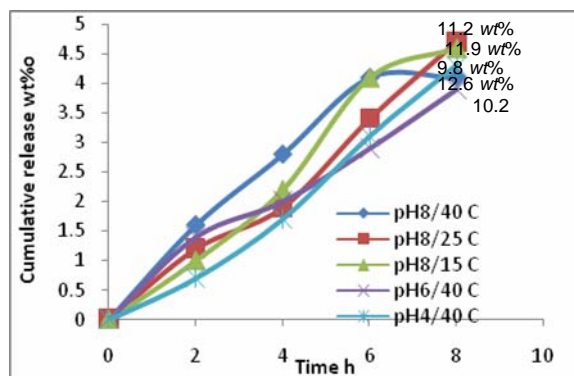


Fig. 26: % R_{cum} of AA/P-b-CH hydrogel

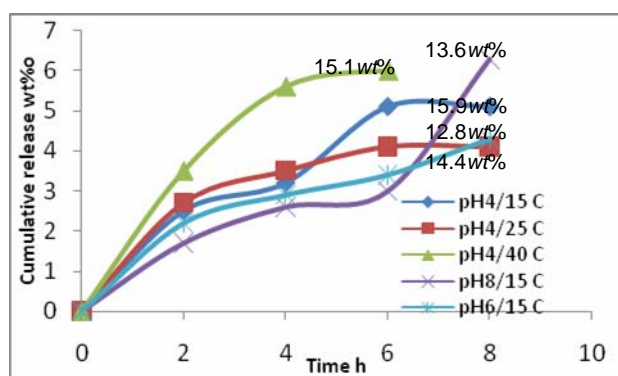


Fig. 27: % R_{cum} of AA/P-b-PH hydrogel

SEM characterization of some hydrogels after release

The SEM studies have shown the morphological surface of some studied hydrogels after release in order to be sure that the texture structure is still coherent and the hydrogel could be used next. The CMC/P-b-CH and CMC/P-b-PH hydrogels have shown SEM images with surface fill with many folded layers with clefts which may improve its next loading and releasing for different materials Fig.28a and huge of NaCl crystals are adhered on the surface of CMC/P-b-CH hydrogel. Whereas the CMC/P-b-PH hydrogel surface has SEM images with nice caves and holes most are open and empty and the hydrogel has geometric shape with compact tight form Fig.(28b), which means the hydrogel could be used many times.

The PE/P-b-CH hydrogel has shown SEM images Fig. (29a) with many clear pores on its surface beside white pots give indication of BSA molecules are remain in the hydrogel and the hydrogel is undamaged and could be used next. The texture surface of PE/P-b-PH hydrogel Fig. (29b) shows open holes and it looks like beehive with compact structure could be used next for loading and releasing.



Fig. 28: SEM micrograph of a. CMC/P-b-CH and b. CMC/P-b-PH hydrogels after release



Fig. 29: SEM micrograph of a. PE/P-b-CH and b. PE/P-b-PH hydrogels after release

CONCLUSION

The PVA blend hydrogels have shown homogeneous materials, the blending would improve the chemical and physical properties and stable them thermally and mechanically, and produce practical morphological surface contain qualifications, allow them for several uses.

The hydrogels were characterized using FTIR for their structure, XRD for their crystallographic structure, DTA and some thermal parameters for their thermal stability and SEM photograms for their morphological analysis. The hydrogels have shown their maximum swelling each in its suitable pH according to its functional groups. Loading of the hydrogels with BSA protein was success and reach its maximum at suitable time and pH of loading medium beside suitable BSA concentration. The loaded BSA hydrogels were allowed to release in PS solution and depending suitable pH and temperature of the release medium.

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