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Protein Controlled Release Based On Copolymerization Of HEMA-Cs and HEMA-PVA

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Abstract

This work describes the synthesis of new biodegradable hydrogels based on 2-hydroxyethyl methacrylate, by free radical polymerization in the presence of benzoyl peroxide as initiator and ethylene glygol di methacrylate as across linking agent. And protein was loaded in polymeric matrix during polymerization. The concentration of protein release was measured by UV-Vis spectrophotometer and release. FTIR was performed to find out the total conversion of Chitosan and Poly Vinyl Alcohol into biodegradable hydrogels.

Keywords: Hydrogels, release of protein, biodegradable, Chitosan, Ploy Vinyl Alcohol.

Introduction

The poor stability and membrane impermeability of most native proteins make efficient delivery difficult. Different strategies that aim to protect protein integrity and activity as well as to aid intracellular delivery have been explored. Covalent approaches include genetic fusion of protein transduction domains]1,2 [and conjugation of polymers to free amine groups on the surface of proteins.]3-5[However, these approaches often suffer from alteration of protein activity due to modification of protein structure. Noncovalentbased polymer carriers that encapsulate protein cargo via electrostatic assembly]6,7[or hydrophilic and hydrophobic interactions have also been explored These methods employ various materials to effectively help the protein travel into cells, albeit
often suffer from instability in serum]8,9[.

Biodegradable hydrogels are useful for a variety of medical applications. In general, hydrogels have a good biocompatibility and the soft rubbery consistence resemble natural tissues, allowing for a favorable controlled interaction with living systems. Hydrogels may be used in the field of tissue engineering, as scaffolds to support and promote tissue regeneration and also as attractive systems for the controlled release of pharmaceutically active molecules]10,11[. A drug delivery vehicle positioned in the proximity of the site of disease or injury can release the drug in the desired location, this way reducing the side effects that usually result from systemic administration]12-15[. Additionally, depending on the crosslinking density, the hydrogels structure can restrict the diffusion of macromolecules, being able to deliver the therapeutic agent over extended periods of time. Indeed, improving in the patient compliance and extension of product life are major advantages of the drug delivery systems. Ideal systems for drug delivery are degradable, permeable, porous and capable of maintaining a desired shape]16-18[.

Due to extremely low bioavailability of protein drugs by oral administration, which is the most convenient mode of drug delivery, protein drugs are usually administered by parenteral route. One way of minimizing discomfort and improving patient compliance is to produce sustained-release formulations that deliver protein drugs continuously over long periods of time. Another challenge in protein drug delivery is to maintain the tertiary protein structure, which is essential to bioactivity. Exposure of protein drugs to unfavorable conditions during formulation tends to reduce their bioactivities]19[.

Most widely used approach for long-term delivery of protein drugs has been parenteral administration of protein drugs in microspheres made of biodegradable polymers. Preparation of protein drug-containing 1microencapsulation methods. A number of microencapsulation methods have been developed through the years, but none of the methods has been ideal for loading protein drugs. Each method has its own advantages as well as limitations. For further improvement in microencapsulation technologies, it is important to understand the strengths and drawbacks of each method]20[.

2.Expermental

2.1 The materials

2-ethylhexyl methacrylate (EHMA), Acetic acid, Benzoyl peroxide (BPO), Bovine serum albumin, Chitosan(Cs), Poly vinyl alcohol (PVA), Distilled water, N,N dimethyl formamide (DMF), Ethylene glycol dimethacrylate (EGDMA).

2.2 Apparatus

Oven ,Trivp International Crop .Italy, FTIR 8400S, Forier transform infrared spectrophotometer ,Shimadzu, Jaban, UV-1800 PC ,Ultraviolet -visible spectrophotometer,Shimadaz , Italy , Hot plate stir , Bibby Strlintd .UK, pH meter, Hanna ;

2.3 Copolymerization of Chitosan-co-HEMA hydrogel

2-hydroxyethyl methacrylate with chitosan was copolymerization by using different concentrations (0.0083,0.0066,0.005,0.0033,0.0016) mol .Cs was dissolved in acetic acid solution at room temperature, then the solutions were mixed by mechanical stirring for1 hr.

Then the solutions was added to HEMA monomer. Then added 0.0025 mole(EGDMA) as acrosslinking agent 0.0012 mole of BPO dissolved in 5 ml DMF was used an initiator.The mixture was refluxing for 6 hrs at 80°C,nitrogen gas was bubbled throughout solutions for about 6 hrs. When polymerization has been complete, the hydrogels was removed carefully,and then the hydrogel were dried in vacuum oven at (37°C) for overnight. The dry hydrogel of each was weighed[21].

2.4 Copolymerization of PVA-co-HEMA hydrogel

The copolymer was synthesized from reaction of HEMA with PVA by using different concentrations (0.00008,0.00006,0.00005,0.0003,0.000016) mol. (10)gm of PVA was dissolved in 90 ml distill water. The solution was refluxing at 90°C for 6 hrs and kept at room temperature for 24 hrs before use. Then the mixture was added to HEMA monomer. Then added 0.0025 mol (EGDMA) as crosslinking agent and 0.0012 mol of BPO dissolved in 5 ml DMF was used an initiator. The mixture was refluxing for 6 hrs at 80°C,nitrogen gas was bubbled throughout solution for about 6 hrs. When polymerization was complete, the hydrogels was removed carefully, and then the hydrogels was dried in vacuum oven at (37°C) for overnight. The dry hydrogel of each was weighed[22,23].

2.5 Swelling Measurement

Dried hydrogel pieces were used to determine the swelling ratio(Rs). The swelling ratio (Rs) was determined by immersing the hydrogels (0.1gm) in100 ml of different pH (pH=4, pH=7 and pH=10) and was allowed to soak for 11 days at different temperatures (37, 45, and 40) ^{0}C. After every 24hrs, they were removed from the water, blotted with filter paper to remove surface water, weighted and the (Rs) was calculated using Equation

$$Rs = (Ws-Wd)100 / Wd$$

Where Ws and Wd are the weights of swollen and dried hydrogels, respectively[24].

2.6 Protein Release

A loaded hydrogel sample is used in order to determine the amount of protein released from the hydrogel network. The sample is dried and weighted (0.1gm), and then immersed in 100 ml from different pH(4,7and10) and temperatures(37 , 40) ^{0}C. The amount of protein released was evaluated using UV-spectrophotometer at λmax 272nm each 24hrs. for 11 days [25].

3. Results & Discussion

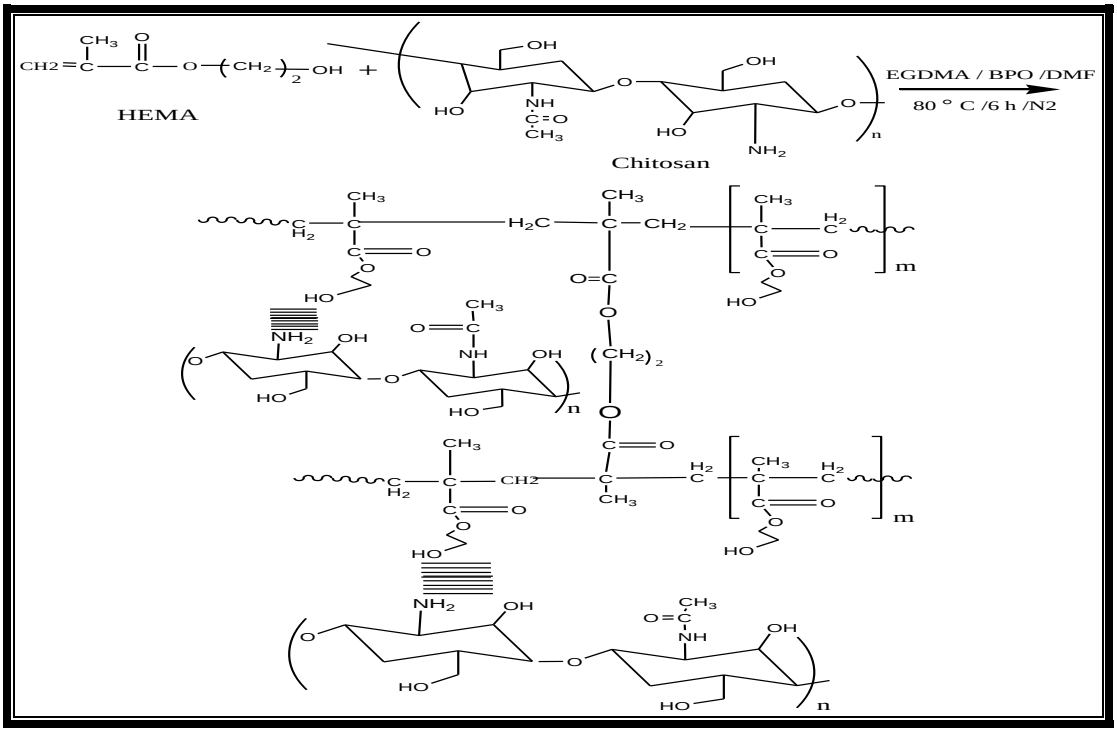
3.1 Synthesis and Characterization

3.1.1 Synthesis and Characterization of Hydrogel (Cs-co-HEMA)

The (HEMA-*co*-Cs) was synthesized from the copolymerization of (HEMA) with(Cs), using different (0.0083,0.0066,0.005,0.0033,0.0016) mol in the presence of EGDMA as crosslinking agent and BPO as initiator by refluxing it with DMF as a solvent for 6 hrs. The mixture was gently stirred while nitrogen purged through the mixture to remove any dissolved oxygen. This reaction was shown in Scheme(1-3).

FTIR Spectrum

The FTIR Spectrum of (HEMA-*co*-Cs),is shown in Figure (1-3); which indicates absorption band at 3422cm^{-1} due to (-OH str group in polymer), 3380 cm^{-1} to (N-H str of Cs), 2947cm^{-1} ,2885 cm^{-1} to (C-H str of polymer backbone),1720 cm^{-1} to (C=O str, ester group), 1650cm^{-1} to (N-H-C=O) 1164cm^{-1} ,1072 cm^{-1} to (C-O-C str) ,1026 cm^{-1}to (-C-O of C-OH str) and 1118cm^{-1} to (C-N str)**[**26-29]



Scheme (1-3) Copolymerization and Crosslinking With EGDMA of (HEMA–co-Cs)

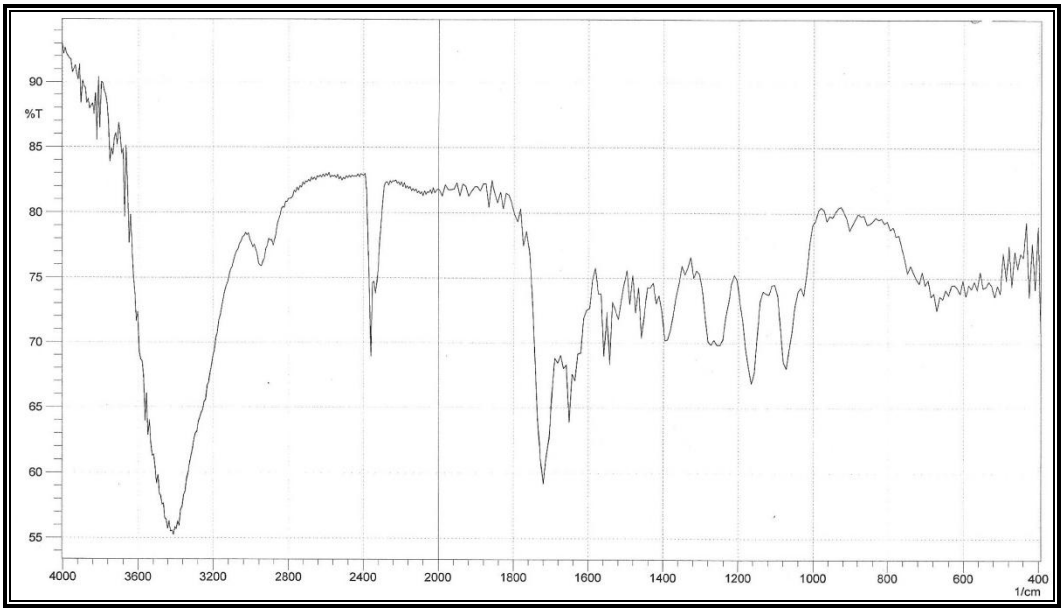
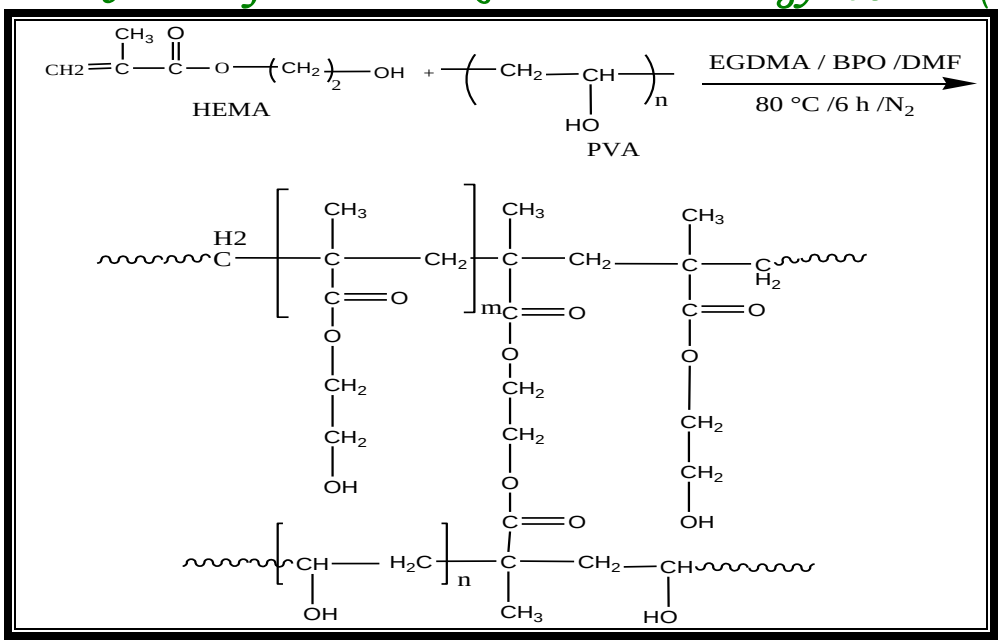


Figure (1-3):FTIR- Spectrum of Hydrogel (HEMA-co-Cs

3.1.2 Synthesis and Characterizationof Hydrogel (PVA-co-HEMA)

The (HEMA-*co*-PVA) was synthesized from the copolymerization of (HEMA) with (PVA),using different (0.00008,0.00006,0.00005,0.0003,0.000016) mol in the presence of EGDMA as crosslinking agent and BPO as initiator by refluxing it with DMF as a solvent for 6 hrs. The mixture was gently stirred while nitrogen purged through the mixture to remove any dissolved oxygen. This reaction was shown in Scheme (2-3)**.**



Scheme(2-3) Copolymerization and Crosslinking With EGDMA of (HEMA-co-PVA)

FTIR Spectrum

The FTIR Spectrum of (HEMA-*co*-PVA) ,is show in Figure (3-3); which indicates absorption band; The band in the range of 3425 cm^{-1} to hydroxyl group(-OH str in polymer),2947cm^{-1},2885cm^{-1} to (C-H str of polymer backbone),1728$cm^{-1}$to (C=O str,ester group),1164cm^{-1} ,1072 cm^{-1} to (C-O-C str) and 1026 cm^{-1} to (-C-O of C-OH str. [26-29]

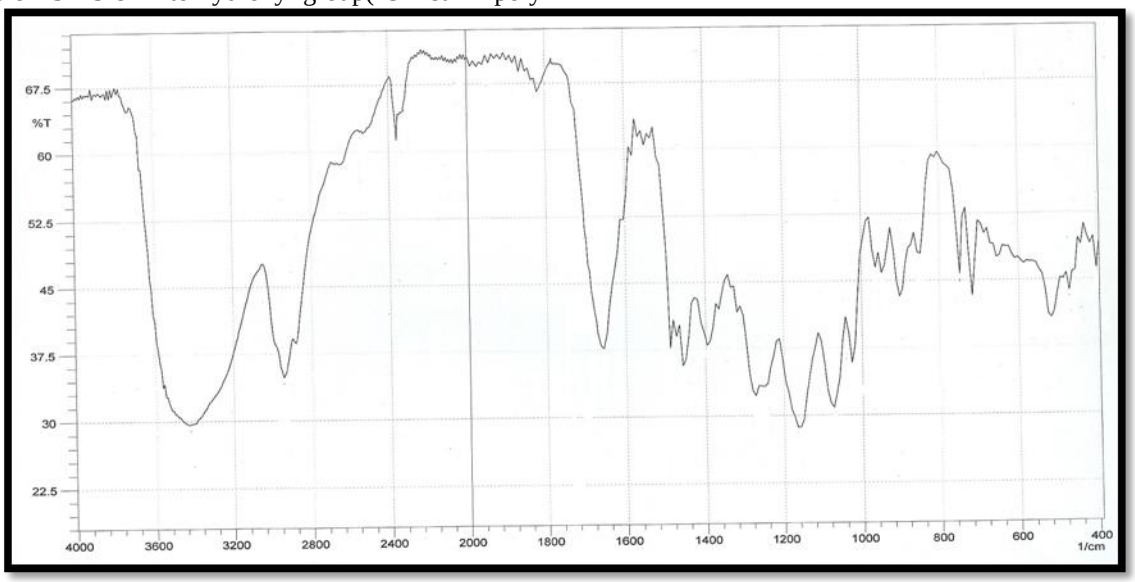
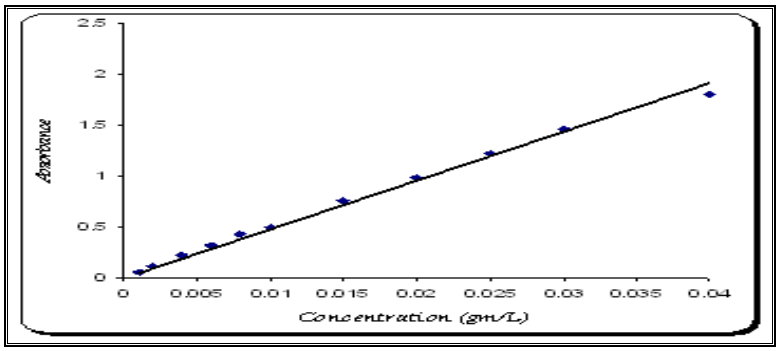


Figure (2-3):FTIR- Spectrum of Hydrogel (HEMA-co-PVA)

3.2 Calibration Curve for Albumin

A standard curve was constructed by varying the amount of protein in the range of 0.001 to 0.04 g.L-1. The Solutions was prepared from stock solution using deionized water as solvent. The absorbance of the solutions was measured at λmax 398.40 nm against solvent. The regression analy-

sis shows the linear relationship between the concentration of the albumin and the absorbance , the plot is shown in figure (5-3) The results indicate that the method is quite suitable for the analysis of the drug in this concentration range.



Figure(3-3) The working calibration curve for the data of albumin (the absorbance in 1 cm cell)at λmax 398.40 nm

3.3 UV- visible Spectrophotometric Analysis

Generally, molecules that absorb in the UV region at a certain wavelength will contain suitable chromosphere. The spectrum consisting, of a plot absorbance, percent transmittance as a function of wavelength is automatically obtained using a scanning spectrophotometer. The absorptivity or molar a absorptivity of many substance at specified wavelength is listed in various tables in literature. Figure (6-3) shows the UV spectra of albumin.

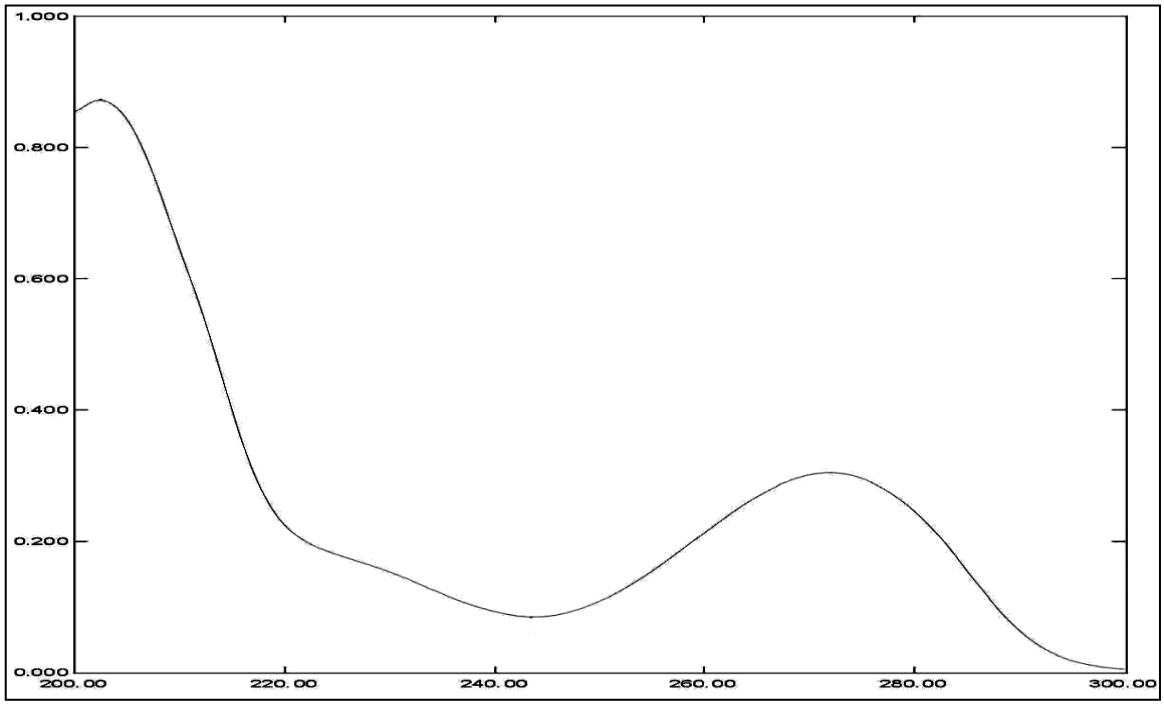


Figure (4-3)UV spectra of protein

3.4 The Effect of pH on the Release of Protein

The protein release rates from the HEMA hydrogels have been measured at pH 4,7 and 10 . Particularly, the albumin release rate at pH 10 which is the higher release rate may be related to the higher swelling ratio of the hydrogels, and the weak H-bonding interaction between protein and polymer network. And the high hydrophilicity results into the higher swelling ratio.

While in pH 7 and 7, the decrease in the amount of protein released may be related to the lower swelling ratio of the hydrogels, and The lower hydrophilicity cause decrease in swelling ratio. protein molecules were protonated and unable to form strong hydrogen bonds like at pH 7.2 with the gel matrix. [30]

Table (3-1) show the effect of pH on swelling in pH =4

Time	Abs.	Conc.	RS%
1	0.066	0.028	28
2	0.067	0.037	37
3	0.068	0.044	44
4	0.069	0.051	51
5	0.070	0.056	56
6	0.070	0.061	61
7	0.070	0.066	66
8	0.071	0.072	72
9	0.071	0.079	79
10	0.071	0.079	79

Table (3-2) show the effect of pH on swelling in pH =7

Time	Abs.	Conc.	RS%
1	0.066	0.024	24
2	0.067	0.038	38
3	0.068	0.051	51
4	0.069	0.060	60
5	0.070	0.068	68
6	0.071	0.075	75
7	0.071	0.079	79
8	0.071	0.081	81
9	0.072	0.086	86
10	0.072	0.086	86

3.5 effect of cross linker Concentration on the release of protein

The degree of flexibility of the polymer network chains is heavily influenced by the amount of crosslinking agent and type of crosslinking molecule within the polymer network. Highly cross linked imprinted polymer networks with short bifunctional crosslinking monomers have little to no flexibility within the network and have higher affinities, binding capacities, and selectivity compared to less cross linked polymers**.** Imprinted polymers of low crosslinking percentage demonstrate lower affinities, capacities, and selectivity which are a direct result of the flexibility in the polymer network. It is important to note that the degree of crosslinking agent is also important to the swelling behavior and the expansion of the polymer network. Highly cross linked materials do not exemplify swelling behavior because of the high crosslinking agent density.[31]

Table (3-3) show the effect of con. of EGDMA on swelling in con. 0.0025 mol

Time	Abs.	Conc.	RS%
1	0.065	0.059	59
2	0.065	0.064	64
3	0.066	0.068	68
4	0.066	0.073	73
5	0.067	0.076	76
6	0.067	0.084	84
7	0.068	0.089	89
8	0.068	0.093	93
9	0.069	0.97	97
10	0.069	0.097	97

Table (3-4) show the effect of con. of EGDMA on swelling in con. 0.0037 mol

Time	Abs.	Conc.	RS%
1	0.063	0.051	51
2	0.063	0.055	55
3	0.064	0.059	59
4	0.065	0.062	62
5	0.065	0.066	66
6	0.065	0.072	72
7	0.066	0.076	76
8	0.066	0.079	79
9	0.066	0.084	84
10	0.066	0.084	84

Table (3-5) show the effect of con. of EGDMA on swelling in con. 0.005 mol

Time	Abs.	Conc.	RS%
1	0.062	0.046	46
2	0.063	0.049	49
3	0.064	0.052	52
4	0.064	0.055	55
5	0.065	0.059	59

6	0.065	0.062	62
7	0.065	0.066	66
8	0.066	0.070	70
9	0.066	0.072	72
10	0.066	0.072	72

Table (3-6) show the effect of con. of EGDMA on swelling in con. 0.009 mol

Time	Abs.	Conc.	RS%
1	0.061	0.044	44
2	0.061	0.046	46
3	0.062	0.049	49
4	0.062	0.053	53
5	0.063	0.055	55
6	0.063	0.059	59
7	0.064	0.062	62
8	0.064	0.064	64
9	0.065	0.067	67
10	0.065	0.067	67

Table (3-7) show the effect of con. of EGDMA on swelling in con. 0.007 mol

Time	Abs.	Conc.	RS%
1	0.060	0.040	40
2	0.060	0.042	42
3	0.061	0.046	46
4	0.061	0.048	48
5	0.061	0.052	52
6	0.062	0.054	54
7	0.062	0.057	57
8	0.063	0.059	59
9	0.063	0.062	62
10	0.064	0.062	62

3.6 Effect of swelling on release of protein

The swelling ratio behavior of HEMA hydrogels were studied as a function of time and pH at 37 ^{0}C, The ability of a polymer network to absorb water was significantly hindered by the presence of crosslinks. Because of the hydrophilicity, the swelling behavior is highly dependent on the pH of the surrounding medium. In higher pH medium the hydrogels swelled more rapidly due to a large swelling force created by the electrostatic repulsion between the ionized acid groups and the osmotic pressure resulted from different concentrations of free ions within ionic network and the surrounding solution [101].

The pH was adjusted by preparation of buffers (4 ,7 and 10). Dried hydrogels were left to swell in a solution of desired pH. Swollen gels removed from the solution at regular intervals and weighed. The measurement was continued until a constant weight was repeated for each sample. This weight was used to calculate the swelling ratio:

$$Rs = (Ws-Wd)100 / Wd$$

Where Ws and Wd are the weights of swollen and dried hydrogels, respectively.

The observation that the time taken to achieve swelling ratio of the hydrogels decreased with increasing molar proportion of crosslinking agent (0.05-0.25) gm .

3.7 Protein (albumin) Release

A loaded hydrogel sample is used in order to determine the amount of protein released from the hydrogel network. The sample is dried and weighted (0.1gm), and then immersed in 100 ml from different pH(4,7 and 10) and temperatures(37 , 40) ^{0}C. The amount of protein released was evaluated using UV-spectrophotometer at λmax 398.40nm each 24hrs. for 11 days.

3.8 Drug Loaded

The synthesis of loaded hydrogels is similar to that of the unloaded ones. The difference is, after complete mixing of the monomer solution, Crosslinking agent and different amounts of drug are added and mixing operation continues for 4hrs.The drug content in the obtained polymer network was 0.1gm ,0.2gm and 0.3gm.

3.9 Differential Scanning Calorimetry (DSC)

The samples was measured 10 mg from sample in the system and heated in temperature 250 for 20 min. After that it was accrued on DSC curve, which it determine glass transfer (Ts), and crystal melting point (Tm), also it was determined the mixture states in preparation samples.

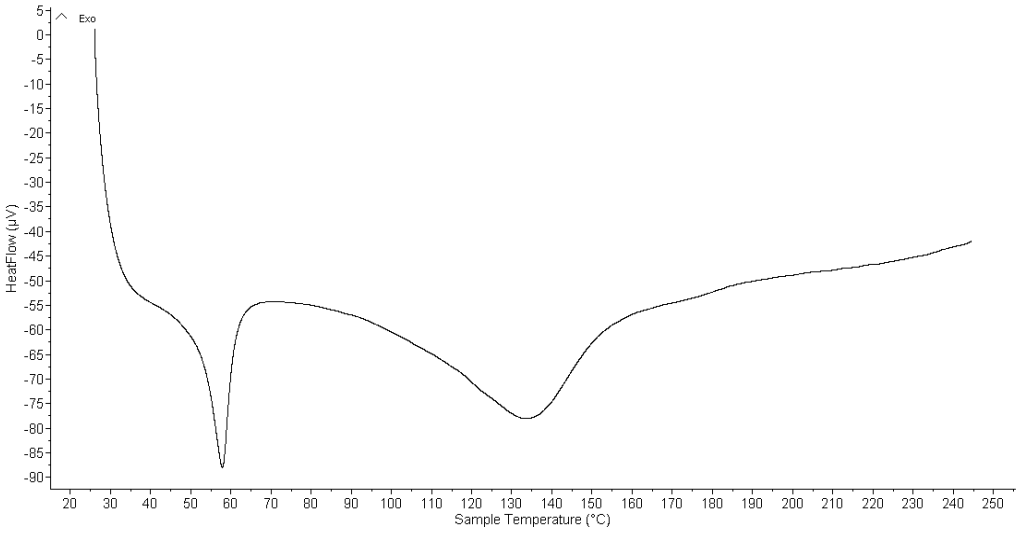


Figure (5-3) show the DSC curve for Hydrogel (HEMA-co-Cs)

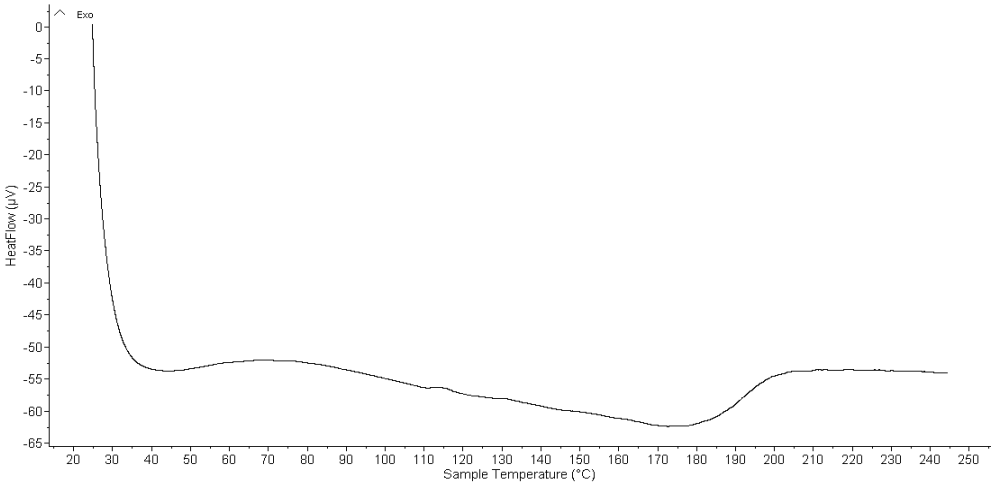


Figure (6-3) show the DSC curve for Hydrogel (HEMA-co-PVA)

Conclusion

Controlled-release polymer matrix system offers numerous advantages, not to avoid treating excess amounts of active substances, but also to offer the most suitable technical solution in special fields of application. The objective of controlled release systems is to protect the supply of the agent to allow the automatic release of the agent to the target at controlled rate and to maintain its concentration in the system within the optimum limits over a specified period of time.

The main factors that determine the swelling ratio and release of protein for the hydrogels systems are the network density, molecular structure and compositions concentrations. The superabsorbent hydrogel exhibited high sensitivity to pH and temperature. Concerning effect of pH on swelling ratio for all the hydrogel structures, it was found that the maximum swelling ratio can be arranged as follows :

$$pH=10 > pH=7 > pH=4$$

This arrangement due to the increase the hydrophilicity in the hydrogel, Concerning the effect of pH on release of protein for all the hydrogel structures, it was found that the higher release rate is at pH=10 than other pH, due to the higher swelling ratio of the hydrogels, and
increase the hydrophilicity in the polymer network. In relation to the effect of temperature on swelling ratio (Rs) for these four samples have high swelling ratio, it was found that the maximum swelling ratio is at 40 ^{0}Cthan at 37 ^{0}C, the reason is due to formulations that stored at higher temperature may be accounted for the more solution loss and dehydration of formulations than at low temperature. This greater loss or dehydration on storge at higher temperature had resulted into more solution uptake by the hydrogel during swelling and hence increase in the numerator value used in the formula for calculation of swelling ratio.

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