IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 2 Issue 6, June 2015.
www.ijiset.com
ISSN 2348 – 7968

Novel Biodegradable Methyl Cellulose Hydrogels For Controlled Releases Of Protein

Prof. Dr.Mohammed Ali Mutar

Chemistry Department , University of Al-Qadisiya, Iraq

E-mial : mohammeddw73@gmail.com

Abstract

This work describes the synthesis of new biodegradable hydrogels based on (methyl cellulose-co-methyl methacrylate), by free radical polymerization in the presence of benzoyl peroxide as initiator and ethylene glycol di methacrylate as across linking agent. And protein was loaded in polymeric matrix during polymerization. The concentration of protein releases was measured by UV-Vis spectrophotometer and the release of two model proteins with different molecular weights albumin (M=44. 3 kDa), BSA (M=66 kDa) from (MC-co-MMA) gels with a varying initial water content was studied. FTIR was performed to find out the total conversion of methyl cellulose and methyl methacrylate into biodegradable hydrogels.

The effect of PH on the release of protein was also studied, and the results showed that the maximum releases of protein were arranged as follows: PH=7 > PH =4 > PH=2

The water content (WC) was measured for all percentages of hydrogels at 37°C and it was observed that the percentage 90% of methylcellulose swelling more than the percentages (70%, 50%, 10%) methyl cellulose hydrogels.

Keywords*:* Hydrogels, releases of protein, biodegradable, methyl cellulose, water content

1.Introduction

There was, during the past decades, rapid development of protein –based pharmaceuticals and it has tremendously increased the need for suitable delivery systems, leading to guarantee a safe and controlled delivery of protein accuse drugs. Hydrogels, because of their an inherent biocompatibility, show good opportunities as protein delivery system or engineering scaffold. The hydrogels have hydrophilic, soft and rubbery nature ensures minimal tissue irritation and a lower tendency of cells and protein to adhere to the hydrogel surface. In order to design hydrogels in which network formation is established by chemical or physical crosslinking, a variety of both natural and synthetic polymers have been used [1].

In a recent way, biotechnology made it possible the production of large quantities of pharmaceutically active protein. Such protein can be used for prophylactic (e.g. Vaccines) and for therapeutic purposes as well (e.g. Treatment of cancer or vascular diseases).Yet, there is a number of problems related to the use of these protein – based charges. The first problem is the stability of the drug ought to be ensured during manufacturing and storage of the pharmaceutical formulation [2-4].The second problem is that oral administration is not possible because of the

ISSN 2348 – 7968

low pH and proteolytic activity in the gastro intestinal tract .In general ,protein have a short half-life after parenteral administration (e.g. .intravenous injection), the thing that made repeated injection or continuous of the protein necessary to obtain a therapeutic and this is the third problem. The way to overcome are one or more of these problems, is that a large number of delivery system has been designed and evaluated in order to release pharmaceutically active proteins during the last years [5]. The systems based on polymers are among these systems and they are the almost successful so far[6].

The Hydrogels are based on hydrophilic and hydrophobic polymers ,and the latter are cross linked to prevent dissolution in water .An important factor is the crosslink density that determines the water content of the gels at equilibrium swelling. The hydrogels are interesting devices for the delivery of proteins, because they can contain a large amount of water [7,8,9].

In this present study, polymerization of biodegradable methyl cellulose hydrogels have been designed and evaluated systems for the controlled release of proteins. Also a study effect of the concentration of crosslinking, swelling measurement have been made as well as an examination of the effect of PH on the release of protein.

2-Experimental

2.1 The materials

Methyl Metha acrylate (MERCK), Methyl cellulose (BDH), Benzoyl peroxide (HIMEDA), Ethylene glycol dimethacrylate (MERCK), Bovine serum albumin (HIMEDA), albumin (HIMEDA), Acetic acid (BDH) and N, N dimethyl formulated DMF (HIMEDA).

2.2Apparatus

(Oven) Trivp International Crop. Italy, (FTIR 8400S) Fourier Transform infrared spectrophotometer, Shimadzu, Japan, (UV-1650 PC) Ultraviolet-visible spectrophotometer, Shimadzu Japan, was used to measure the protein adsorption.(Hot plate stirs) BibbyStrlintd. UK (PH meter) Hanna, Romania.

2.3 Polymerization of Methyl cellulose-Based Hydrogels

(2) gm. of Methyl cellulose with different concentrations of EGDMA (0.0025, 0.005, 0.0075, 0.01) moles as a crosslinking agent was polymerized. The polymerization begins when using 0.0012 moles from benzoyl peroxide dissolved in 5 ml DMF as initiator. In order to avoid oxygen inhibition ,nitrogen was bubbled in all the solutions for 3 hrs. The reaction was refluxing for 3 hrs. at 60°C.After polymerization was complete, the hydrogel was carefully removed from the mold and dried in oven at (37°C) for overnight. The dry weight of each hydrogel was weighted also [10].

ISSN 2348 – 7968

2.4 Methacrylate- derivatives Methyl cellulose

Polymerization methyl cellulose by reaction methyl cellulose with methyl methacrylate by free radical polymerization in different ratios(10%,70%,50%,90%). Hydrogels were formed after the addition of 0.0012 mol from benzoyl peroxide as initiator,0.0025 moles (EGDMA) as crosslinking agent and 1ml of N,N,N´,N´-tetramethlene ethylene diamine (TEMED) was added as accelerator[11].

BSA and albumin was dissolved in a small amount of water and then added to reaction mixture, nitrogen was bubbled in all the solutions for 3 hrs. The reaction was refluxing for 3 hrs. at 60°C.After polymerization was complete, the hydrogels were removed carefully, and then dried in vacuum oven at (37°C) for overnight [12].Table (2-1) show the Hydrogel MC/MMA were Synthesized Using Four Different Mass Ratios.

2.5 Swelling Measurement

The water content was determine by immersing the hydrogel (0.1gm) in 100 ml of distill water for extended period time .Excess water was removed from surface by blotting with lens-cleaning tissue just before measurements. The equilibrium water content in distill water was determined by the ratio of the weight of water in the hydrogel to the total weight of the hydrogel at hydration equilibrium.EWC was calculated by using the following equation:

$$EWC = \frac{Ws\text{-}Wd}{Ws} \times 100$$

Where Ws and Wd correspond to the weight of the swollen sample and dried sample, respectively [13].

2.6 Protein Release

A loaded hydrogel sample is used in order to determine the amount of protein (albumin,BSA) release from the hydrogel network. The sample is dried and weighted (0.1 gm), and then immersed in 100 ml from different pH (2, 4 and 7) and temperature (37)°C.The amount of protein release was evaluated spectrophotometer at λmax 279.0 ,278.0 nm respectively each 2 hrs.[14].

3. Results & Discussion

3.1 Synthesis and Characterization

3.1.1 Synthesis and Characterization of Hydrogel (MC-co-MMA)

The (MC-co-MMA) was synthesized from the reaction of MMA with MC in the presence of EGDMA as crosslinking agent and BPO as initiator by refluxing it with DMF as solvent for 3 hrs. The mixture was gently stirred while nitrogen purged through the mixture to remove any dissolved oxygen. This reaction was shown in Scheme 1

ISSN 2348 – 7968

FTIR Spectrum

The FTIR Spectrum of (MC-co-MMA), is shown in Figure (3-1): which indicates absorption band at; 3425 cm-1to (-OH str), 2908cm-1 ,2839cm-1 to (C-H str of polymer backbone), 1712cm-1 to (C=O str ester group), 1373cm-1 ,1319cm-1 to (C-O-C str) and 1064 cm-1 to (-C-O of C-OH str) [15,16].

3.2 Calibration Curve for Albumin

A standerd curve was constructed by varying the amount of albumin in the range of (0.01-0.09) g.L-1.The solution was prepared from stock solution using de ionized water as solvent. The absorbance of the solution was measured at λmax(279) against solvent. The regression analysis shows the linear relationship between the concentration of the albumin and the absorbance, the plot is shown in figure (3- 2).The results indicate that the method is quite suitable for the analysis of the protein in this concentration range. Table 2: show the Absorbance of (Albumin) in Various Concentrations. The concentrations calculate by plotting of absorbance as a function of concentration. If a linear straight line is obtained, the slope of the straight line can be used to calculate the molar absorptive.

3.3 UV-visible Spectrophotometric Analysis

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

3.4 Calibration Curve for BSA

A standerd curve was constructed by varying the amount of BSA in the range of (0.01-0.09) g.L-1.The solutions was prepared from stock solution using de ionized water as solvent .The absorbance of the solution was measured at λmax(278) against solvent. The regression analysis shows the linear relationship between the concentration of the BSA and the absorbance, the plot is shown in figure (34) .The results indicate that the method is quite suitable for the analysis of the protein in this concentration range. Table(3-1) show the Absorbance of (BSA) in Various Concentrations

3.5 UV-Visible Spectrophotometric Analysis

Generally, molecules that absorb in the UV region at a certain wavelength will contain suitable chromophore. The spectrum consisting, of a plot absorbance, percent transmittance as a function of wavelength is automatically obtained using a scanning spectrophotometer. The absorptive or molar absorptive of many substances at a specified wavelength is listed in various tables in literature. Figure(3-5) shows the UV spectra of (BSA).

ISSN 2348 – 7968

3.6 Effect of PH on the Release of Protein

The protein release rates from the biodegradable hydrogel (MC-co-MMA) which have been measured at PH 2,4 and 7 as shown in figure 7 and 8. Particularly, the protein release rate at PH=7, exhibit the highest release rate, which may be related to the higher swelling ratio of the hydrogels, because of the hydrogen bonding of the hydrogels and the protein causing the higher swelling ratio.

While in PH 2 and 4 the decrease in the amount of protein release may be related to the lower swelling ratio of the hydrogels. On other hand the release rate decreased with an increasing protein size for example the BSA release less than albumin release. For a given protein, the initial water content of the methyl cellulose hydrogel has a large effect on the release rate .Further, a decreasing water content of the gel resulted in a decreasing release rate. For gels with a high equilibrium water content (Fig.3-7 and 3- 8) the diffusion of proteins in these highly hydrated gels could be effectively described by the free volume theory. On the other hand, the release of the proteins from hydrogels with a low hydration level was marginal and did not follow the free volume theory, indicating that in these gels screening occurred [17].

Effect of Swelling on the Release of Protein

Figure(3- 9) represents the water content to time for different percentages of methyl cellulose, the water content of the hydrogel increases with higher percentage of methyl cellulose because of the contain of (–OH) groups which can make hydrogen bonding with water molecule but decreases when adding methyl methacrylate because of the contain of many methyl groups that make swelling behavior and the expansion water of the polymer network very poorer and kept its chain rigid in resulting hydrogel; clearly that the hydrophilic monomer releases protein more than hydrophobic monomers due to hydrophobicity [18].

3.7 Effect of cross linker Concentrations on The Release of Protein

Hydrogels are based on hydrophilic polymers, which are cross linked to prevent dissolution in water. The crosslink density is an important factor that determines the water content of the gels at equilibrium swelling. Because hydrogels can contain a large amount of water, they are interesting devices for the delivery of proteins[19,20]. First, the hydrated matrix results in good compatibility with proteins [21,22] as well as living cells and body fluids [23]. Second, many parameters, such as the water content, the amount of crosslinks and possible protein-matrix interactions, can be used to control the release of proteins from hydrogels. These parameters can be made time dependent by swelling and/or degradation of the gel. Since crosslinking is performed with highly reactive crosslinking agent and at a high pH, the gels have to be loaded after preparation. This leads to a low entrapment of the protein and a rapid release(Figure 3-10).

Conclusion

The main factors that determine the swelling ratio and release of protein for the hydrogel system or the network density, molecular structure and composition concentration. It was found the maximum swelling ratio and the

ISSN 2348 – 7968

releases rate with the higher percentage of methyl cellulose. The swelling ratio and releases of protein are decreased by varying the crosslinking agent concentration the results show the lower swelling ratio and slow release rate when used higher concentration from cross linker. PH effect on swelling ratio and releases was found at the higher release rate at PH=7 than other PH, due to the higher swelling ratio of the hydrpgels and hydrogen bonding interaction between protein and polymer network.

References

[1]CrommelinD.J.A., SindelarR.D. (Eds.), Pharmaceutical Biotechnology, **1997**.Harwood ,Academic, Amsterdam.

[2] Arakawa ,T., PrestrelskiS.J., 'et al.'**1993**Carpenter, Adv. Drug Delivery Rev.,10: 1-28.

[3] Manning ,M.C., Patel ,K. 'et al.'**1989**, Pharm. Res.,6, 903-915.

[4] Chen,T., **1992**.Drug Devlivery. Indus. Pharmacy**,** 18, 1311.

[5] Park,K., Park,H., 'et al.'**1993**.Technomic, Basel.

[6] SandersL.M. and Hendren,R.W. (Eds.), **1997**.Protein Delivery, Pharmaceutical Biotechnology,Plenum, New York vol. 10, 1-43.

[7] Park,K., and Park,H., **1993.**Biodegradable Hydrogels for Drug Delivery, Technomic, Basel.

[8] Cheng J., Park, K. **1995**,Carbohydrate Polymers, 28, 69-76.

[9] Andrade J.D., MED. **1973.**Instrum, 7, 110-121.

[10]Renjha, M.N and Khan, **2013.**S, JFAM. 2 (1):30-40.

[11] Ferreira, p., Calvinho, p., 'et al.'. **2006**.H. M Brazilian JPS,.42(3):239-227

[12] VanDijk-WolthuisKettenes-van den Bosch W.N.E., J.J., **1997**.Macromolecules. , 30: 3411-341

[13] Bengani,C.L., And Leclerc,J., 'et al.'. **2012**.Journal of Colloid and Interface Science,386:441-450

[14] Ball, B. A., and Wilker, C., 'et al.'**1992**. J.,Am. J. Vet. Res., 53: 1294-1297.

[15] Pavia,L.D.,Lampman,L.G.,Kris,S.G.,andVyvyan,R.J . **2009** .Introduction to Spectrophotometer 4 th.bookcole general learning.

[16] Rao, C.M.R. **1963.**Chemical application of infrared spectroscopy. Academic press. New York.

[17] Putney, S.D and Burke PA. **1998.**Improving protein therapeutics with sustained-release formulations. NatBiotechnol,16:153–157.

[18] Sannino, A., Esposito, A., 'et al.', **2004**.M. J. Mater. SCI - Mater. MED.11 (4):247-253.

[19] AndradeJ.D.,**2000**. MED. Instrum. 7: 110-121.

[20] HubbellJ.A., **1996**.J. Controlled Release. 39: 305-313.

[21] PeppasN.A. and ScottJ.E., **1992**J. Controlled Release. 18: 95-100.

[22] EndeM.T. and PeppasN.A., **1997.** J. Controlled Release., 48, 47-56.

[23] SidebothamR.L., **1974** .Adv. Carbohydr. Chem. 30: 371-444.

ISSN 2348 – 7968

Acknowledgments

The authors gratefully acknowledge partial support of this work by Chemistry department of education college of University Qadisyia

Scheme 1: Copolymerization and crosslinking of (MMA-co-MC)

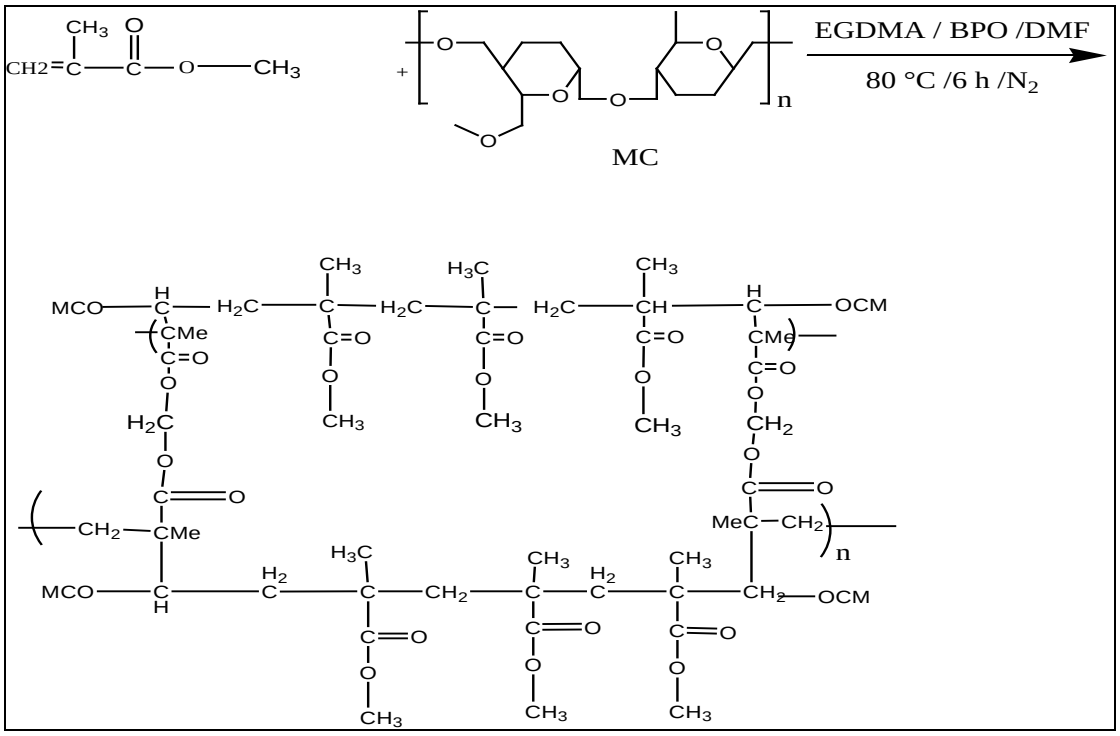
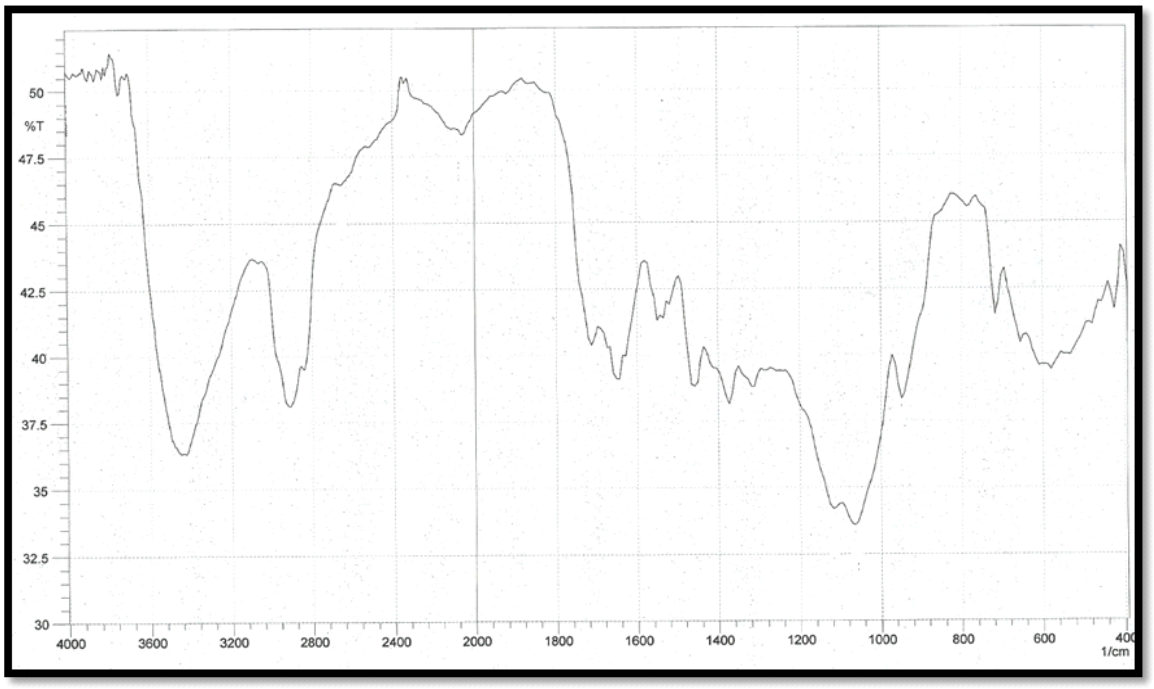


Table (2-1) MC/MMA Hydrogel were Synthesized Using Four Different Mass Ratios.

	MC%	Wt gm	MMA%	Wt gm
1	90	1.8	10	0.2
2	70	1.6	30	0.4
3	50	1	50	1
4	10	0.2	90	1.8

ISSN 2348 – 7968



Figure(3-1)FTIR Spectra of (MC-co-MMA)

Table (3.1) The Absorbance of (Albumin) in Various Concentration.

Conc. $g.L^{-1}$	Abs.
0.01	0.12
0.02	0.21
0.03	0.3
0.04	0.41
0.05	0.5
0.06	0.57
0.07	0.69
0.08	0.76
0.09	0.83

ISSN 2348 – 7968

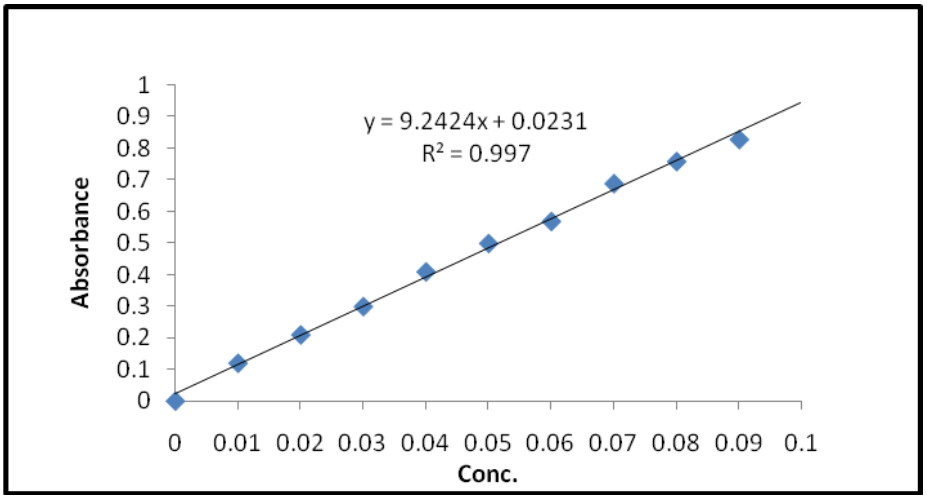


Figure (3.2) The working Calibration Curve for The Data of (Albumin)(the Absorbance in 1cm cell) at λmax 279.0 nm

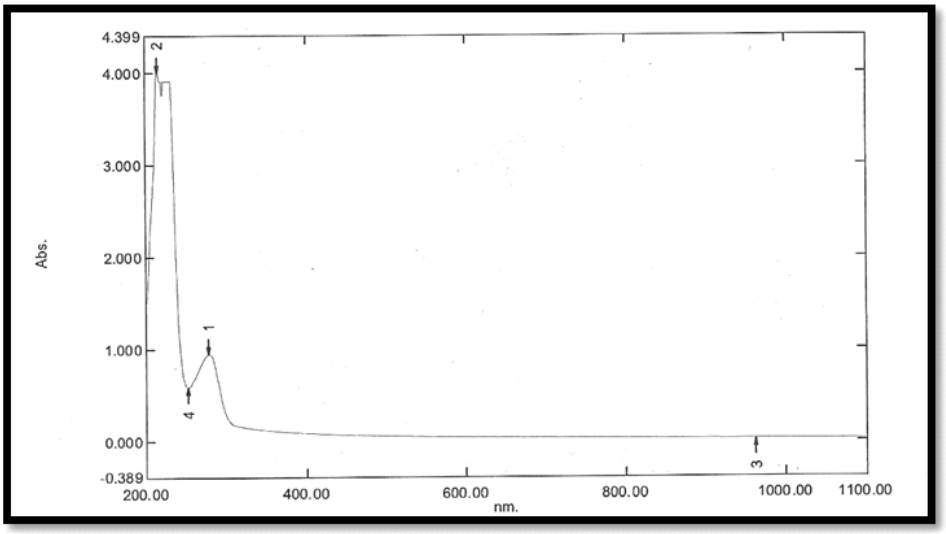


Figure (3.3) UV spectra of albumin

ISSN 2348 – 7968

Table (3.2) The Absorbance of (BSA) in Various Concentration

Conc. $g.L^{-1}$	Abs
0.099	0.01
0.197	0.02
0.3	0.03
0.37	0.04
0.48	0.05
0.57	0.06
0.65	0.07
0.71	0.08
0.83	0.09

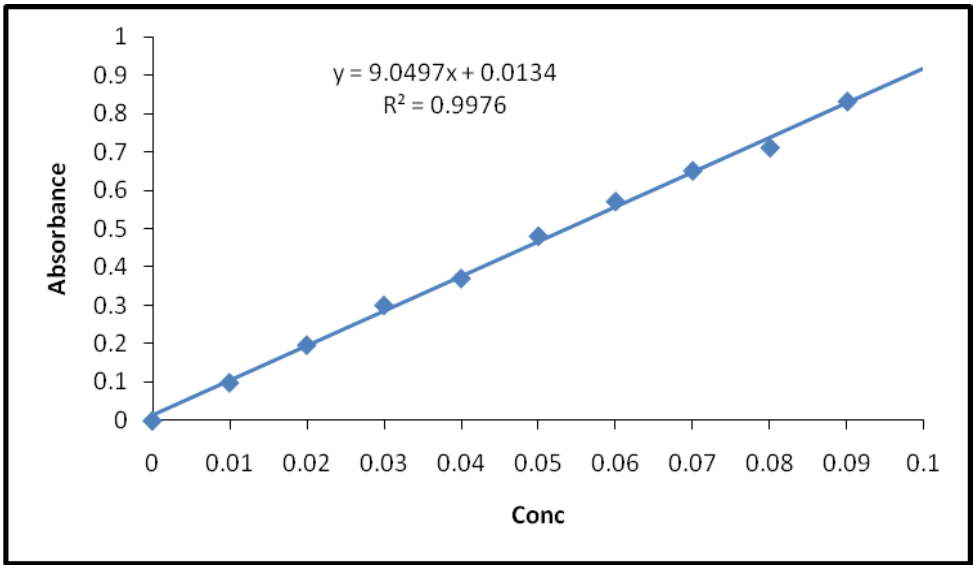


Figure (3.4) The working Calibration Curve for the Data of (BSA)(the Absorbance in 1 cm cell) at λmax 278.0 nm

ISSN 2348 – 7968

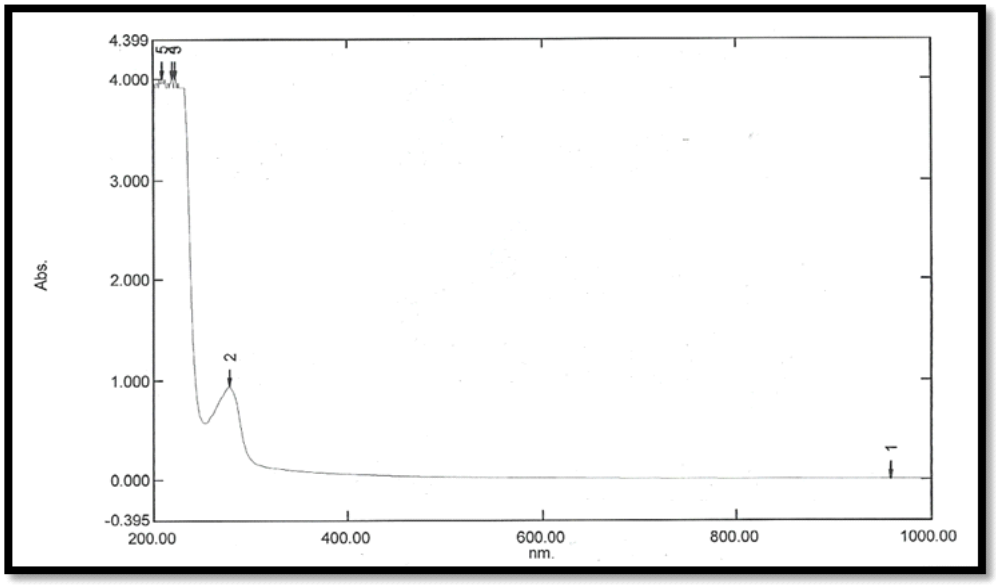
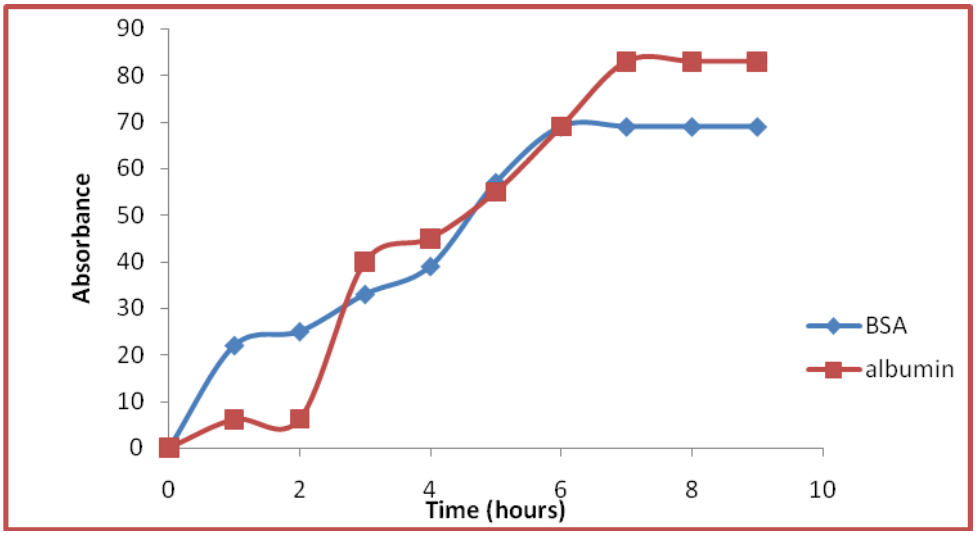
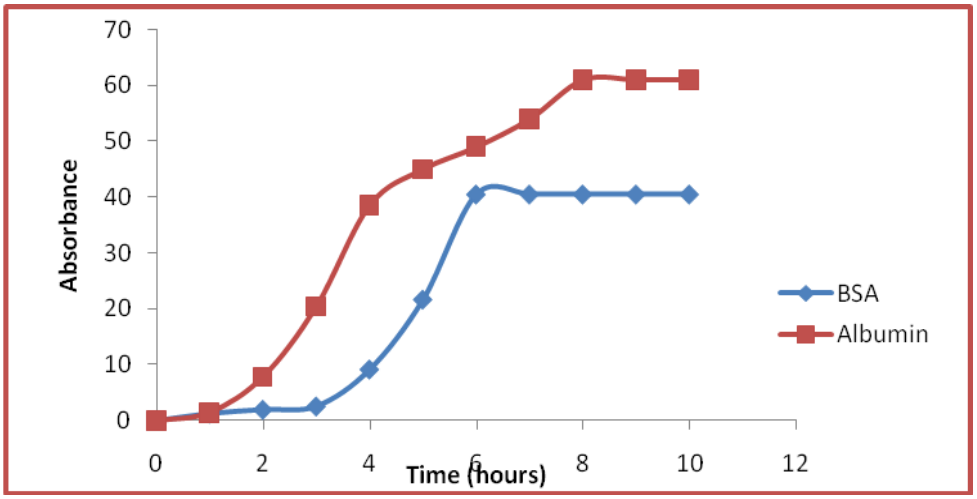


Figure (3.5) UV spectra of BSA

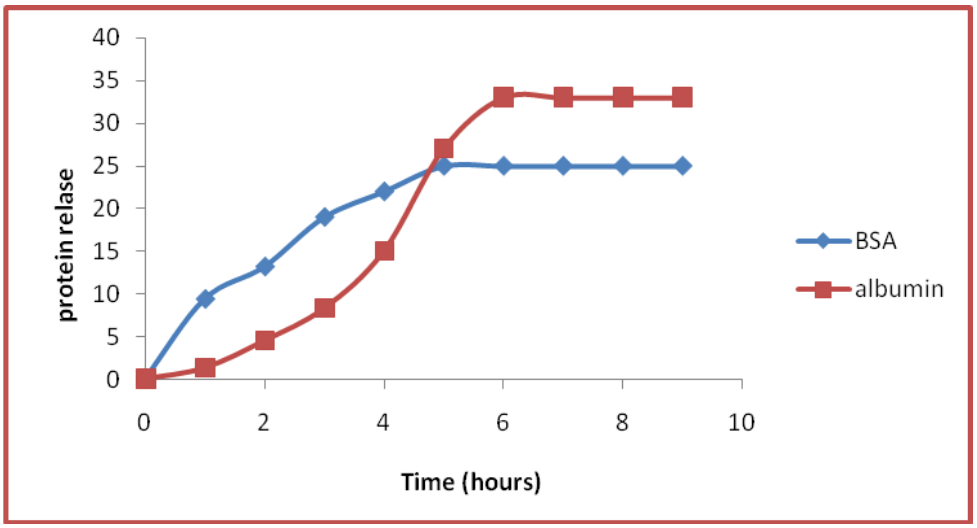


Figure(3-6) Protein Release at PH =7

ISSN 2348 – 7968



Figure(3-7) Protein Release at PH=4



Figure(3-8) Protein Release at PH =2

ISSN 2348 – 7968

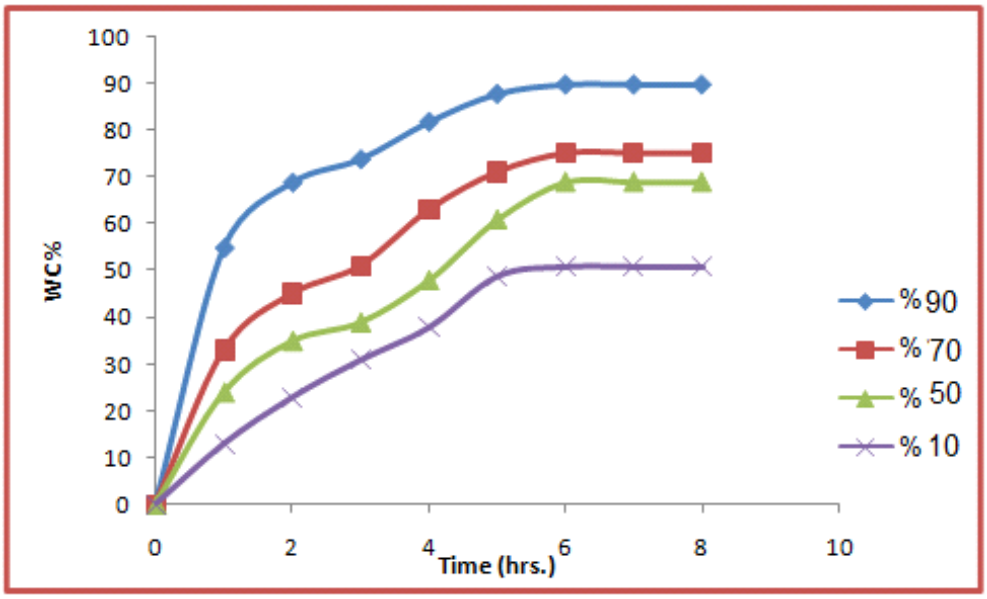
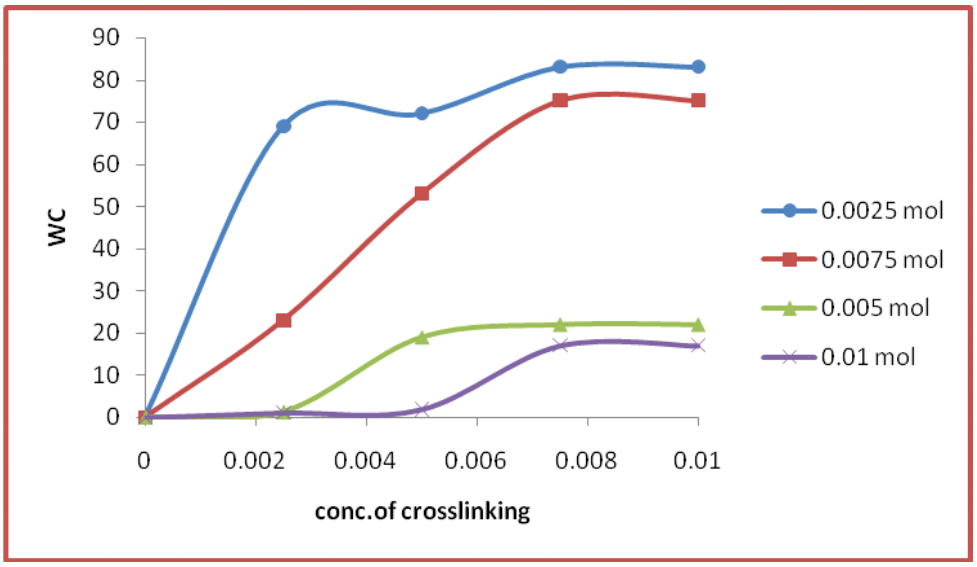


Figure (3-9) Equilibrium Water Content (EWC) for Different Ratios of MC(10%,30% ,50%,90%)



Figure(3-10)Effect of Cross linker EGDMA Concentration on the Swelling Behavior of Hydrogel (MC-co-MMA)