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Physicochemical studies of closed loop insulin delivery system based on intelligent carboxymethyl cellulose hydrogel

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Diabetes mellitus type 1 (T1DM) is a form of diabetes mellitus that results from the autoimmune destruction of the insulin producing β-cells in the pancreas. This study aims to establish the safety and efficacy of a hybrid closed loop. Physicochemical studies, we investigated for a closed loop insulin delivery system based on intelligent carboxymethyl cellulose (CMC) hydrogel. The hydrogel was synthesized by free radical polymerization of acrylamide (AAm) in the presence of CMC in atmospheric condition. Immobilization and loading of glucose oxidase (GOx) and insulin was done by the swelling diffusion method. GOx plays an important role to make the synthesized hydrogel responsive to blood glucose level as a model of an artificial pancreas. The loaded hydrogels were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), high- performance liquid chromatography (HPLC) and thermo gravimetric analysis (TGA). Thermodynamic parameters such as $\Delta\mu$, ΔH, ΔS, E_a, and coefficient diffusion were also calculated. The results indicate that hydrophobic forces play a major role in drug-hydrogel interactions.

Keywords: Carboxymethyl cellulose (CMC), Diabetes, Hydrogel, Insulin, Thermodynamic

The Diabetes mellitus represents a group of diseases of heterogeneous etiology, characterized by chronic hyperglycemia and other metabolic abnormalities. Approximately 5-10% of the world population is affected by type 1 diabetes, which results from the autoimmune destruction of the insulin producing β-cells in the pancreas[1-3]. The traditional insulin administration which measuring the glucose concentration and insulin delivery are two separated processes, is called open-loop insulin delivery system. A new system for insulin delivery is called closed loop insulin delivery system, which these two processes are coupled in it that leads to decrease in the risk of hyperglycemia [4-7]. Hydrogels consisting of natural or synthetic polymers have been synthesizing and applied over the past two decades. Hydrogels were used in the biomedical field, tissue engineering, diagnostics, regenerative medicine, drug and protein delivery systems *etc*. Polymeric hydrogels have been used several times as a drug carrier in drug delivery systems, because they have the potential to improve protein stability and increase the duration of the therapeutic effect[7-10].

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Carboxymethyl cellulose (CMC) is a linear polymer of β-D-glucose, in which some of the hydroxyl groups of cellulose was substituted with carboxymethyl groups. Low toxicity, biocompatibility, biodegradability and economical advantages of CMC make it a good choice to be utilized in drug delivery systems[4-12].

Although polymers have been studied extensively as a drug carrier in the insulin delivery system, designing intelligent drug delivery systems based on glucose oxidase as a specific glucose sensor, demands further investigation. In this investigation CMC-PAAm hydrogels as intelligent insulin delivery system was synthesized by free radical polymerization. Immobilization and loading of glucose oxidase (GOx) as a biosensor and insulin was performed by swelling diffusion method. Glucose oxidase (GOx) is a glucose-specific enzyme that catalyzes glucose to gluconic acid and hydrogen peroxide. By increasing the glucose concentration in phosphate buffer solution (PBS), more hydrogen peroxide will be produced which cause degradation of hydrogel structure and insulin release. The rate of hydrogel degradation and release of insulin increases with increasing the glucose concentration. This can provide a controlled release of insulin from the

hydrogel-like an artificial pancreas. The optimization of the drug loading capacities (DLC) of the hydrogel was performed by changing the amount of methylenebisacrylamide (MBA) cross linker in the hydrogel structure[11-16]. Thermodynamic parameters such as Δμ, ΔH, ΔS, E_a and coefficient diffusion were also calculated. These studies indicate that insulin loading process is spontaneous and entropy driven. Hydrophobic forces are also a dominant factor in insulin-hydrogel interactions.

Materials and Methods

Carboxymethyl cellulose sodium salt (Viscosity of 2% solution 3000 mpa.s) was purchased from Chem Food, Iran. N,N'-Methylenebisacrylamide (MBA) was purchased from SD fine, India. Acrylamide (AAm) for synthesis was obtained from Merck Millipore. Glucose oxidase (GOx) was a gift from PishtazTebZaman research and development Co. Human insulin 100 IU/mL was purchased from Exir pharmaceutical Co., Iran. Ammonium persulfate (APS) was obtained from Novinshimiar, Iran.

Synthesis of CMC-g-PAAm hydrogel

Synthesis of the CMC-g-PAAm hydrogel was performed using CMC as a biopolymer, AAm as a monomer, APS as an initiator and MBA as a cross linker in an aqueous medium. Briefly, CMC/AAm mixtures consisting of the 1.00 g of CMC polymer and 2.00 g of AAm monomer were dissolved in 60 mL of distilled water at room temperature under magnetic stirring. The definite concentration of initiator (APS) and cross linker (MBA) was added to the reactor under an atmospheric condition at 80°C for 30 min. Then, ethanol was used to remove the water and soluble fractions. Finally, the products were dried in an oven at 50°C overnight.

Insulin Loading and Enzyme Immobilization

Immobilization and loading of GOx and insulin were performed by the swelling- diffusion method. For this purpose, GOx and insulin were dissolved in PBS 1X solution and the hydrogel samples kept in this loading medium. When swelling equilibrium completed, the swollen hydrogels were taken out and rinsed thoroughly with PBS. Then, the hydrogels were weighed to calculate the swelling ratio and freeze dried at −40°C for 24 h[17,18].

Calibration Curves

To construct the calibration curve, the absorbance of the insulin in PBS was measured by the UV-visible spectrophotometer (Camspec M 350).

Optimization of the Loading and Swelling Capacity Characteristics

HPLC and UV-visible spectroscopy was used to determine the amount of unloaded insulin in the remaining loading medium. Drug loading capacities (DLC) was calculated as follow:

$$DLC(\%) = \frac{W_{IL}}{W_{HG} + W_{IL}} \times 100 \quad \ldots (1)$$

where, W_{IL} is the weight of insulin-loaded in the hydrogel and W_{HG} is the weight of the dry gel sample[17].

In order to estimate of swelling capacity, the hydrogels were deride to a constant weight and were kept in distilled water at room temperature and then the hydrogels were periodically weighted after soaking the surface water by tissue paper.

The swelling capacity of the hydrogels was calculated according to the following Equation:

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

where, W_t and W_0 are the weights of the swollen gels at the time and dry samples, respectively[19].

Techniques and Methods

SEM, model AIS 2100 from Soren technology, was used to obtain morphological information of the freeze-dried hydrogels. TGA was performed under a nitrogen atmosphere at a heating rate of 10°C/min by Perkin Elmer Pyris Diamond thermal analyzer. FTIR spectra were recorded on a Bruker Tensor 27. The chromatographic separation was done using a Knauer 1100 HPLC analyzer with a column C18 25 × 4.0 mm, methanol as a mobile phase and eluent flow rate of 1.0 mL min^{-1} at room temperature. The volume of the sample injected was 20 μL. The system was equipped with a UV detector. Detection of insulin in PBS solution was carried out by Camspec M 350, UV-VIS spectrophotometer at 280 nm.

Thermodynamic Studies

Diffusion Coefficient and Activation Energy

The diffusion coefficient of insulin through the hydrogel was calculated by the Equation (3) and was obtained from a linear regression of C_t/C_∞ *vs.* $t^{0.5}$ [20-24].

$$\frac{C_t}{C_\infty} = 4\left(\frac{D_t}{\pi r^2}\right)^{0.5} \quad \ldots (3)$$

where, C_t and C_∞ are insulin concentration in the hydrogel at loading and infinite time, respectively. D is the diffusion coefficient, t is the loading time and r

is the mean radius of the loaded hydrogel particles that was calculated through the application of tools software.

The maximum coefficient and the activation energy for diffusion were calculated by the Equation (4).

$$D_T = D_0 e^{\frac{-E_a}{RT}} \quad \ldots (4)$$

where, D_T is the diffusion coefficient of insulin through the hydrogel at temperature T, E_a is the activation energy for diffusion, D_0 is the maximum diffusion coefficient, T is the loading temperature and R is the ideal gas constant.

Affinity, sorption enthalpy and entropy

The apparent sorption affinity was calculated using Equation (5)

$$\Delta\mu = -RT \ln \frac{a_f}{a_s} \quad \ldots (5)$$

where, $\Delta\mu$ is the apparent sorption affinity, T is the loading temperature, R is ideal gas constant, a_f is the activity of insulin in the hydrogel and a_s is the activity of insulin in the loading medium[21,22].

Based on the Langmuir sorption isotherm, the activity of insulin was calculated by Equation (6).

$$a_f = \frac{D_f}{S_f - D_f} \quad \ldots (6)$$

where, $[D_f]$ and $[S_f]$ are insulin activity within the hydrogel and the saturated insulin concentration of the medium, respectively.

Saturated drug concentration was obtained from the reciprocal of intercept of the linear regression of $1/[D_f]$ *vs.* $1/[D_s]$, where D_s is insulin concentration in the loading medium.

ΔH was calculated using the Equation (7) and was obtained by the slope of the linear regression of $\Delta\mu/T$ *vs.* $1/T$.

$$\Delta H / T = \Delta\mu / T + C \quad \ldots (7)$$

where, ΔH is the apparent sorption enthalpy and C is a constant.

The apparent sorption entropy was calculated according to Equation (8)[25-30].

$$\Delta\mu = \Delta H - T\Delta S$$

$$\Delta S = \frac{\Delta H - \Delta\mu}{T} \times 1000 \quad \ldots (8)$$

Results and Discussion

CMC-g-AAm hydrogel was synthesized by free radical polymerization in the presence of an MBA as a cross linker agent and APS as an initiator. To develop a closed loop insulin delivery system, immobilization and loading of glucose oxidase and insulin were performed by the swelling diffusion method. The designed intelligent hydrogel has good sensitivity and responsibility to the glucose concentration, the system acts as an artifical pancreas which the releases of insulin reversibly change with the level of glucose centration.

Table 1 also indicates that hydrogels with higher MBA content have lower DLC and Swelling capacity percent. Figure 1 shows the Swelling Capacity percent of hydrogels in distilled water. It can be seen that the lower amount of MBA has a higher Swelling Capacity and DLC. The reverse relationship between the swelling capacity and the concentration of cross linker is a well-known behaviour[18,19].

The effect of glucose concentration on the kinetic release of insulin was studied (Fig. 2). The insulin can be released to some extent by diffusion, even if no polymer degradation occurs. But, the release of insulin can be affected significantly in the presence of glucose.

Thermo gravimetric analysis (TGA) is given in (Fig. 3). The initial weight loss up to 120°C is due to water evaporation of the hydrogel. The main degradation of CMC-g-AAm hydrogel starts

Table 1 — Effect of MBA on DLC and Swelling Capacity percent of the synthesized hydrogels

Code	CMC (g/dL)	MBA (g/dL)	SR%	DLC (%)
a	1.67	0.33	190	74
b	1.67	0.40	170	67
c	1.67	0.47	140	61
d	1.67	0.53	106	57
e	1.67	0.60	90	49
f	1.67	0.67	60	30
g	1.67	0.73	40	23

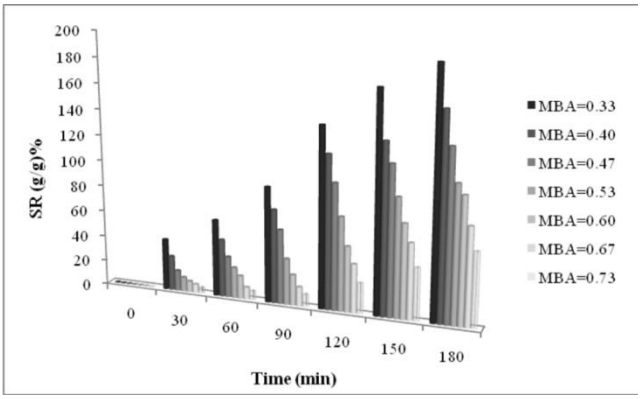

Fig. 1 — Effect of MBA on SR % (swelling capacity) of the synthesized hydrogels at room temperature

temperatures above 300°C. As can be seen from the (Fig. 3A), about 37% weight loss takes place in the temperature range of 300-500°C. The values T_{50} are 466°C and 352°C for the CMC-g-AAm hydrogel and insulin-loaded CMC-g-AAm hydrogel, respectively. In thermo grams of insulin-loaded hydrogel, the weight loss at 258°C is associated with the temperature of insulin decomposition (Fig. 3B) similar behavior was also reported by Shyong *et al.*[28].

Results of HPLC for the optimized hydrogel shows that the amount of unloaded insulin in loading medium is 15% of the initial value, which confirmed 85% of insulin was loaded into the hydrogels (Fig. 4).

The scanning electron microscope was used to investigate the surface morphology of the hydrogels. SEM micrograph demonstrates that the synthesized hydrogel have good porosity, whereas in insulin-loaded hydrogel, the pores were fully occupied (Fig. 5A & B).

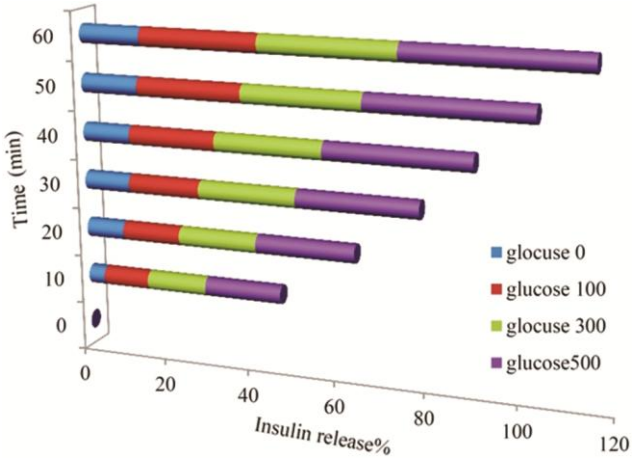

Fig. 2 — Effect of glucose concentration on insulin release

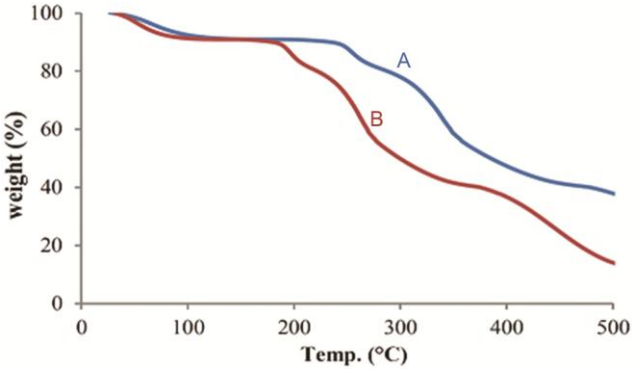

Fig. 3 — TGA thermograms of (A) unloaded hydrogel; & (B) insulin-loaded hydrogel

FTIR spectrum of the hydrogels is shown in (Fig. 6). A new absorption band in the spectrum of the hydrogel at 1670 cm^{-1} is attributed to the C=O stretching mode of amide groups and verifying the formation of CMC-g-AAm (Fig. 6A). In the spectrum of insulin-loaded hydrogel (Fig. 6B) a strong absorption band at 1127 cm^{-1} for C-N stretching mode, which is confirmed by a new peak at 780 cm^{-1} for bending N-H mode clearly indicate that insulin is loaded on the hydrogel. The stretching band for –NH of insulin overlaps with the –OH stretching band of CMC.

The thermodynamic parameters such as the diffusion coefficient (D_T), activation energy (E_a), affinity ($\Delta\mu$), sorption enthalpy (ΔH) and entropy (ΔS) were studied.

The diffusion coefficient is an important parameter of chemical species entrapped into polymeric systems.

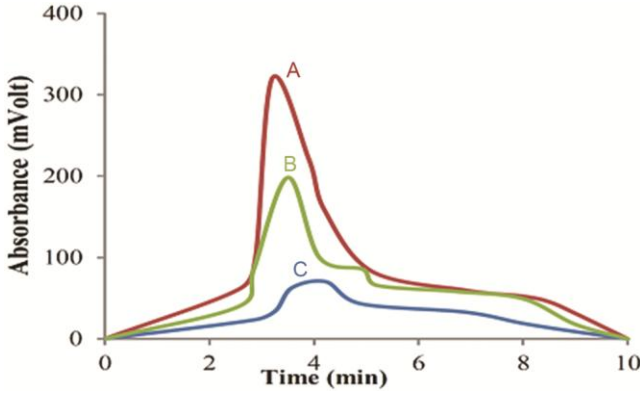

Fig. 4 — Chromatograms of (A) insulin in loading medium before load; (B) insulin control 4.4 (v/v%); and (C) insulin in loading medium after load

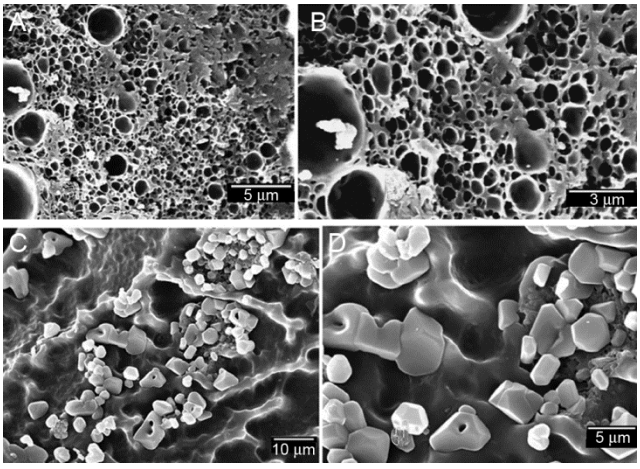

Fig. 5 — SEM image of dried CMC hydrogel (A) 7000×; (B) 15000×; and insulin- loaded CMC hydrogel (C) 3000×; and (D) 7000×

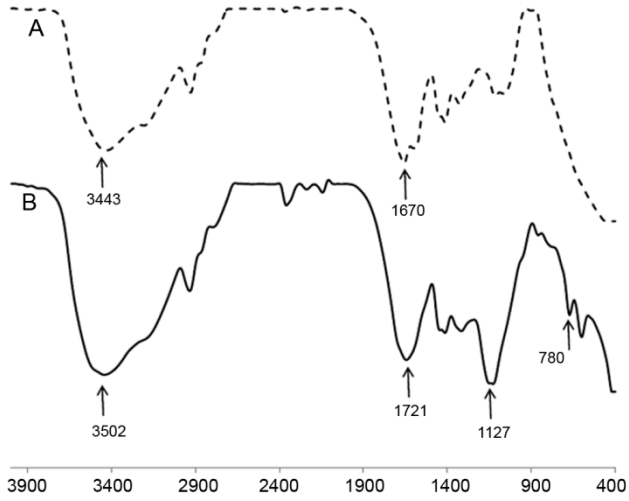

Fig. 6 — FTIR spectra (A) unloaded hydrogel; & (B) insulin loaded hydrogel

Using Pepas model the diffusional exponent (n), the rate constant (c) and the diffusion coefficient (D) of the solvent in the matrix could be calculated. The insulin release data can be fitted by Pepas model.

In order to calculate the diffusion coefficient, C_t/C_∞ was plotted *vs.* $t^{0.5}$ and then regressed into a linear line with intercept set to zero as is shown in (Fig. 7A). Diffusion coefficient was obtained by substituting the slop value into the Equation (3).

$$D = (0.1787\ \text{min}^{-0.5} \times (9.5mm/4)^2 \times 3.14 = 0.565\mu m^2/\text{min}$$

$$D = 5.65 \times 10^{-13} m^2/\text{min}$$

Figure 7B demonstrate the plot of *ln* D_T *vs. 1/T*. The maximum diffusion coefficient was obtained by keeping intercept equal to zero. The activation energy was calculated as follow:

$$Ea = -(-1190) \times 8.314 = 9263.5 Jmol^{-1} = 9.893 kJmol^{-1}$$

Insulin activity in the hydrogel was calculated by Equation 6.

The saturated drug concentration of the hydrogel $[S]_f$ was obtained from the reciprocal intercept of the linear regression of *1/*$[D_f]$ *vs. 1/[Ds]* (Fig. 7C). As follow:

S= 1/58.29 mol/kg S=1.715×10^{-2} mol/kg

$$a_f = \frac{5.91\times10^{-3}}{(1.715\times10^{-2} - 5.91\times10^{-3})} = 5.258\times10^{-1} molkg^{-1}$$

V (L/kg) was calculated from the linear regression of *ln*(a_f) *vs. ln*(a_s) (Fig. 7D), as follow:

$$V = e^{0..3886} Lkg = 1.475 Lkg$$

$$a_f(mol/L) = 5.258\times10^{-1} mol/kg/1.475 L/kg$$

$$a_f(mol/L) = 3.56\times10^{-1} mol/L$$

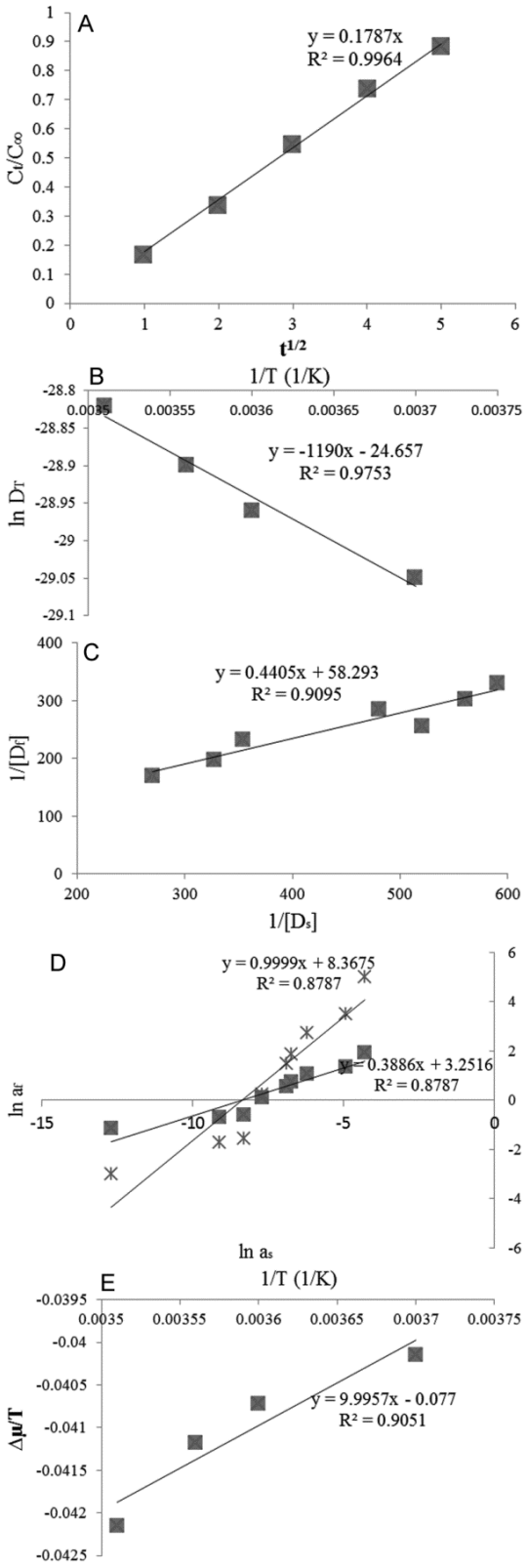

Fig. 7 — (A) C_t/C_∞ as a function of $t^{0.5}$; (B) Ln (D_T) as a function of 1/T; (C) $1/D_f$ as a function of $1/D_s$; (D) Ln (a_f) as a function of ln (a_s); and (E) $\Delta\mu/T$ as a function of 1/T

The apparent sorption affinity was calculated using Equation (5) as follow:

$$-\Delta\mu = 8.314\,J/Kmol \times 274K \times 5.198$$

$$-\Delta\mu = 11845.8\,J/mol$$

$$-\Delta\mu = 11.841\,kJ/mol$$

The negative value of Δμ (Δμ=-1190 kJ/mol) indicates that insulin loading is spontaneous and the process is exothermic.

The apparent sorption enthalpy was obtained from the slope of the linear regression of *Δμ/T vs. 1/T* (Fig. 7E): ΔH=10.358 kJ/mol.

By substituting apparent sorption enthalpy and affinity in Equation 8, apparent sorption entropy was obtained as follow:

$$\Delta S = \frac{\Delta H - \Delta\mu}{T} \times 1000$$

$$\Delta S = 79.695(J/molK)$$

Positive values of the thermodynamic parameters; ΔH=10.358 KJ/mol and ΔS=79.695 J/mol K indicate that hydrophobic interactions are playing the major role in the binding process of insulin-hydrogel and also prove the insulin-loading process is entropy driven.

Conclusion

The CMC-g-AAm hydrogel was synthesized in the presence of an MBA as a cross linking agent by a free radical polymerization method. Immobilization and loading of glucose oxidase (GOx) and insulin was done by the swelling diffusion method. The MBA content was optimized to improve the insulin loading characteristics of the hydrogel. Characterization of the intelligent hydrogel was investigated by various techniques such as FT-IR, TGA, HPLC and SEM. various thermodynamic parameters including diffusion coefficient, activation energy, affinity, sorption enthalpy and entropy, also were investigated. The negative value of the apparent sorption affinity (Δμ= −1190 kJ/mol) indicates that insulin loading is spontaneous and the process is exothermic. Positive values of the thermodynamic parameters such as the apparent sorption enthalpy (ΔH=10.358 kJ/mol) and apparent sorption entropy (ΔS=79.695 J/mol.K) indicate that hydrophobic interactions are playing the major role in the binding process of insulin-hydrogel and prove insulin loading process is entropy driven and also the hydrophobic forces play a major role in drug-hydrogel interactions.

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