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Research Article

Controlled swelling and *in vitro* release of insulin from Konkoli grafted polymethylacrylaminde hydrogel

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Abstract

The extended study of konkoli grafted polymethylacrylaminde (KG-g-poly (MAAm)) hydrogel synthesized in our earlier work from methacrylamide (MAAm) grafted, and N,N-methylenebisacrylamide (N,N-MBAAm) crosslinked Konkoli (*Maesopsis eminni*) galactomannan (KG), is necessary to establish its potential application as an oral insulin delivery system. In this study, the kg-g-poly (MAAm) Hydrogel swelling in typical pH media show an initial rapid swelling before stabilizing. The hydrogel attains its parabolic peak swelling in distill water (pH = 7). Swelling rises from a lower pH medium (pH = 2.2), and in a medium with a slight pH above this (pH = 7.4), swelling starts dropping. Comparing the media for the insulin release studies, the cumulative release drops to its parabolic trough in the distill water medium (pH = 7), a higher cumulative release was recorded at a lower (acidic) pH (pH = 2.2), and the highest cumulative release was recorded at a higher pH (pH = 7.4). The kinetics and mechanisms of these processes were presented while using the descriptive statistic of regression to fit the results from the processes to a power law. The release of insulin from kg-g-poly (MAAm) Hydrogel was also shown to increase with increase in glucose concentration in the media with an initial rapid release in all solutions, while in a typical intestinal pH media range, higher release at all concentrations was recorded at pH = 6.8.

Keywords: Hydrogels, swelling, insulin, kinetics and mechanisms.

1. Introduction

Hydrogels are polymeric materials that can swell quickly by imbibing a large amount of water without dissolving, hence exhibiting a thermodynamic compatibility with water. This phenomenon can be associated with the presence of hydrophilic functional groups in their structure, such as -OH, -COOH, -$CONH_2$, and -SO_3H [1,2]. Therefore, it can be accepted that the synthesis of these materials is primarily based upon the reaction of hydrophilic monomers, initiators and crosslinkers [3]. The water compactibility of hydrogels can be influenced by some changes in the system media which typically includes some stimulis (e.g. pH, temperature, light, electric or magnetic field, etc.) and some biological and chemical compositions [2-4]. Hydrogel-based drug delivery system is becoming a major area of research, and great attention has been paid to the stimuli sensitive in situ-forming hydrogels because of their potential applications in biomedical systems such as drug delivery [5].

In most cases drug release/delivery occurs when the gel is highly swollen or swelling in a manner typically controlled by the rate of swelling, the drug diffusion, or a coupling of swelling and diffusion. The different mechanisms for this drug release/delivery, for lots of many different types of release systems based on the materials has been formulated to propose the applications of these drug delivery devices within a typically narrow environmental condition [1]. For instance, the analysis of the mechanisms of water diffusion in swelling polymeric systems has gained considerable attention [6]. This explains that; when a hydrogel is brought into contact with water, it diffuses into the hydrogel and the network spreads out resulting in swelling of the hydrogel. The diffusion involves migration of water into pre-existing or dynamically formed spaces

among hydrogel chains. The swelling also involves the overall segmental motion resulting ultimately in amplified separation between hydrogel chains [6].

Diabetes mellitus is a typical health challenge and the prevailing therapeutic option for insulin administration to remedy it, other than the parenteral route (which requires frequent injections with needle), are other routes in which the controlled delivery of the drug from devices are designed to overcome the inherent barriers for insulin uptake across the gastrointestinal tract, mucosal membrane and skin [2]. These routes include; nasal route, pulmonary route, virginal route, transdermal route, ocular route and the oral route. These routes has come across a number of challenges, for instance, the oral route face probable degradation ascribed to the acidic environment in the stomach, metabolism in the liver and the proteolytic enzymes in the intestinal tract. The controlled release via these routes is regarded as a way to increase the efficacy while reducing side effects, and therefore improving the patient's quality of life [7].

In our earlier work [2], we synthesized a hydrogel from KG, characterize it and study the effect of the synthesis procedure on the hydrogel swelling with time, and at different media temperature. In furtherance to the study, we hereby study the swelling kinetics of the hydrogel in buffer solutions, the release of insulin from the hydrogel as a delivery device and its kinetics in different buffer media, buffered glucose solutions with different concentrations and different buffers of glucose solutions. This is to further establish the swelling characteristics and insulin release from the hydrogel, hence, more comprehensively presenting its potentials as an insulin delivery device in the oral insulin delivery system as an alternative therapy for diabetes mellitus.

2. Materials and Method

2.1 Materials

Konkoli (*Maesopsis eminii*) galactomannan was obtained from Baisa in Taraba State, Nigeria. Methacrylamide (MAAm), sodium hydroxide, hydrochloric acid, potassium chloride, dihydrogen potassium phosphate, sodium potassium tartrate and Folin's reagents are products of Merck-Schuchardt, Germany. Ammonium persulphate (APS), copper sulphate and N,N'-methylenebisacrylamide (N,N-MBAAm) obtained from S.D. Fine Ltd, sodium carbonate is Ranbaxy product, while insulin was obtained from Torrent Pharmaceuticals Ltd.

2.2 Synthesis of Hydrogels

The crosslinked KG-g-poly(MAAm) was synthesized using the procedure reported by Osemeahon et al. [8] as optimized in our earlier work [2]. According to this method, 1g of konkoli (KG) was introduced into 1.095 x 10^{-2} mol/l solution of APS. MAAm (7.03 x 10^{-1} mol/l) and N, N -MBAAm (6.45 mol/l) was again added, before the aqueous system was set at 65^{o}C for 2 h. The polymers thus formed were stirred for 2 h in distilled water and again 2 h in ethanol to remove the soluble fractions of the polymer and then dried in an aerated oven at 40^{o}C.

2.3 Swelling kinetics

Swelling kinetics of the polymeric network was carried out in distilled water and buffer solution with pH 2.2 and pH 7.4, in order to calculate diffusion exponent, gel characteristic constant and various diffusion coefficients for the swelling kinetics of the crosslinked polymer. The buffer solutions were prepared according the procedure outlined by Pharmacopoeia of India [9] as follows:

i. Buffer solution of pH 2.2 was prepared thus; 50 cm^3 of 0.2 M KCl and 7.8 cm^3 of 0.2 M HCl was introduced into a 200 cm^3 volumetric flask and made to the mark with distilled water.
ii. Buffer solution of pH 7.4 was prepared thus; 50 cm^3 of 0.2 M KH_2PO_4 and 39.1 cm^3 of 0.2 M NaOH was introduced into a 200 cm^3 volumetric flask and made to the mark.

For the swelling kinetics, the water absorbency of the hydrogel in the above solutions was determined using the teabag method as described by Pourjavadi and Mahdavinia [10]. The kg-g-poly(MAAm) sample (0.10 g) was placed in a pre-weighed teabag and immersed in 100 cm^3 of distilled water and allowed to soak for 2 h at 37^{o} C. The equilibrated swollen gel was removed from water and allowed to drain for about 20 mins, after which it was re-weighed. The absorbency (equilibrium swelling) was calculated using the following equation:

$$\text{Absorbency} = (W_s - W_d)/ W_d$$

W_s and W_d are the weights of the samples swollen in water and in dry state, respectively.

The diffusion exponent (n), gel characteristic constant (k) and various diffusion coefficients for the swelling kinetics of the hydrogels in these typical pH media were calculated using the generalized empirical equations which have been widely used to describe both the water uptake through the swelling glassy polymers and the drug release from these materials. In the case of water uptake, the weight gain, M_s, is described by the Eq (1)

$$M_s = kt^n \qquad [1]$$

k is a constant incorporating characteristic of macromolecular network system and the drug, while n also constant, is the diffusional exponent which is indicative of the transport mechanism are constants. Normal Fickian diffusion (Case I) is characterized by n =0.5, while Case II diffusion by n =1.0. When the value of n falls between 0.5 and 1.0, a mixture of Fickian and Case II diffusion is

characterized, and is referred to as non-Fickian or anomalous diffusion [11].

The evaluation of drug release from swelling systems was evaluated from the modification of Equation 1 as presented by Ritger and Peppas [12, 13] in which, M_t/M_∞ replaces M_s as follows;

$$F = M_t/M_\infty = kt^n \quad [2]$$

F is the fractional release, M_t is the amount of water absorbed at time t, M_∞ is the maximum amount absorbed. The initial diffusion coefficient (D_i), average diffusion coefficient (D_A) and late diffusion coefficient (D_L) were calculated from the Eqs (3) – (5), respectively [12, 13].

$$M_t/M_\infty = 4(D_i t/\ell^2)^{0.5} \quad [3]$$

$$D_A = 0.049\ \ell 2/t^{1/2} \quad [4]$$

$$M_t/M_\infty = 1 - (8/\pi^2)\exp[(-\pi^2 D_L t)/\ell^2] \quad [5]$$

M_t and M_∞ is drug released at time t and at equilibrium, respectively, D_i, D_A, and D_L are the initial, average and late diffusion coefficient, ℓ is the thickness of the sample, and $t^{1/2}$ is the time required for 50% release of drug.

2.4 *In-vitro* Release Studies of Insulin from Hydrogels

To study the in-vitro release of insulin from hydrogel, the following experimental steps/procedures were sequencially taken;

i. Insulin loading onto the polymer matrix

The loading of a drug onto hydrogels was carried out by swelling equilibrium method [14]. The hydrogels were allowed to swell in a drug solution of known concentration for 24 h at room temperature and then dried to obtain the release device.

ii. Preparation calibration curves

The procedure outlined by Singh *et al* [4] was followed. In this procedure, the absorbance of standard solutions of the reference substance at concentrations encompassing the sample concentrations were measured on a UV–Visible spectrophotometer and calibration graph constructed by plotting absorbance against concentration. The concentration of the drug in the sample solution was read from the graph as the concentration corresponding to the absorbance of the solution. Three calibration graphs of insulin were made to determine the amount of drug release from the drug-loaded polymeric matrixes, respectively.

iii. Drug release from polymer matrix

In vitro release studies of the drug was carried out by placing the dried-loaded (60 mg) sample in definite volume of releasing medium at 37˚C and the amount of insulin released will be assayed spectrophotometrically. This method reported by Lowry *et al* [15] was adopted thus; 1 cm^3 insulin sample solution (containing 10–100 mg of protein) was added to 4 cm^3 of reagent D and the contents mixed. After 10 min of incubation at room temperature, 0.4 cm^3 of Folin's reagent was added and the contents vortexed immediately and left for another 30 mins incubation for a blue coloration to be developed, before λ_{max} was taken. A blank with of distilled water, buffer solutions (with pH 2.2, 6.8 and 7.4) and buffered (with pH 6.8 and 7.7) glucose solutions (0.1 % w/v – 1% w/v) were further prepared using this procedure. The constructed calibration curves were then used to obtain the concentration of the insulin released from the sample taking into account the dilution factor [15]. The release percent of insulin was calculated from the following equation:

$$\text{Release (\%)} = \frac{Wt}{W\infty} \text{ x } 100$$

where W_t is the amount of the released insulin at time t and W_∞ is the total adsorbed insulin in the hydrogel structure.

Reagent D was prepared from a mixture of reagent A, B and C as follows;

Reagent A: sodium carbonate (2% w/v) in 0.1M sodium hydroxide solution was prepared by dissolving 20 g of sodium carbonate and 4 g of sodium hydroxide in 1L of distilled water.

Reagent B: copper sulphate solution (1% w/v) was prepared by dissolving 1g of copper sulphate in 100 cm^3 of distilled water.

Reagent C: sodium potassium tartrate solution (2% w/v) was prepared by dissolving 2 g of the salt in 100 cm^3 distilled water.

Reagent D: alkaline copper reagent: 1cm^3 each of reagents B and C will be mixed with 98 cm^3 of reagent A and vortexed.

2.5 Data Treatment

All measurements unless otherwise stated were run in triplicate, and mean values were reported as mean ± Standard Deviation (SD). Descriptive statistics of regression was performed to fit swelling and kinetics release using EXCEL 2007data analysis Add-in.

3. Result and Discussion

3.1 Swelling kinetics of Hydrogels

Figure 1 represents the dynamics swelling behavior of kg-g-poly(MAAm) hydrogel where the effect of pH on water uptake was studied in distilled water and buffers with pH 2.2 and pH 7.4 (simulated gastric and intestinal fluids respectively). The amount of water uptake per gram of the gel is highest distilled water (pH = 7.0), slightly higher than in pH 7.4, while the lowest was recorded in pH 2.2. The lower water uptake/swelling at pH 2.2 is expected of kg-g-poly(MAAm) hydrogel being an acidic hydrogel. However, swelling observed at this level shows that the pH 2.2 is higher than the pKa of the acidic

group in the kg-g-poly (MAAm) hydrogel [16]. The pH of the distilled water may mark the maximum ionization of the hydrogel, beyond which increase in pH will only increase the ionic strength, which will reduce osmotic pressure and result in deswelling or compression of the gel [16]. The observation may also be attributed to the fact that partial hydrolysis leads to the generation of new water interaction centers and especially new ion dipole interactions in the polymer chains, leading to the significant changes in the water uptake of these hydrogels [6]. Initially at all pH media, the rate of water uptake sharply increase and then begun to level off. A power law behavior is obvious from this, which prompts the evaluation of the diffusion exponent 'n' and gel characteristic constant 'k' for the swelling of polymers in different pH.

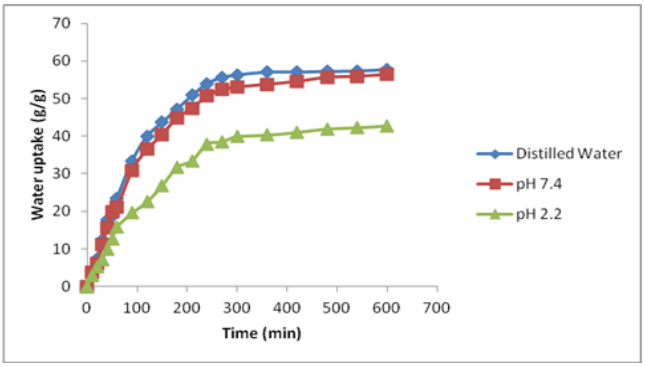

Figure 1: Swelling kinetics of kg-g-poly (MAAm) in different pH media

Descriptive statistics of regression was used to fit the swelling data to the power law expression given Eq. (2).The least squares estimates of the swelling data along with estimated correlation coefficient values (r) as presented in Table 6. This show that swelling kinetics followed Fickian diffusion mechanism for the swelling of the polymeric matrix of the hydrogel with high correlation coefficient for each swelling media. In the Fickian mechanism (Case I), the rate of diffusion of water molecules into polymer matrix is small as compared to the rate of polymer chain relaxation. The values of initial diffusion coefficients and late diffusion coefficient were observed to be less than the value of average diffusion coefficient. This indicates that in the initial and later stages of swelling, the rate of the penetration of water into the polymeric matrix was slow. This may be due to chain relaxation which could have started before the opening of the hydrogel structure leading to the slow rate of diffusion of water in the polymer networks at the initial stages of the polymer swelling. Also, there could be a formation of a thin layer of swollen gel on the polymer surface in which the polymer chains are slowly being hydrated and relaxed. But at the latter stages of swelling, all the polymer chains may have been completely relaxed and equilibrium swelling could have been established. Therefore in the start and latter stages the rate of diffusion of water molecules in the polymer networks will be slow.

Table 6: Results of Diffusion Exponent n, Gel Characteristic Constant k and Various Diffusion Coefficients for the Swelling Kinetics of Hydrogel Samples of kg-g-poly (MAAm)

Medium Diffusion	Gel characteristic Exponent n	constant K x 10^{-2}	Diffusion coefficient (10^4cm^2) Initial, D_i	Average, D_A	Last time, D_L
Distilled water	0.498	3.953	0.776	1.719	0.145
pH 2.2	0.495	3.529	0.675	1.643	0.128
pH 7.4	0.497	4.608	0.731	1.637	0.136

Correlation coefficient values, r: distilled water = 0.9584; pH 2.2 = 0.9689; pH 7.4 = 0.9598

3.2 *In Vitro* Release of Insulin from kg-g-poly (MAAm) Hydrogel

The release of water-soluble drugs, entrapped in a hydrogels, occur only after water penetrates the polymeric networks to swell and dissolve the drug, followed by diffusion along the aqueous pathways to the surface of the device. The release of drug is closely related to the swelling characteristics of the hydrogels, which in turn, is a, key function of chemical architecture of the hydrogels [4]. It has also been shown in this report that the swelling of this hydrogel increase when the pH of the medium changed from acidic to basic.

The scanning of 2.5 µg/mL of the volumetric solution of insulin in the UV range was determined at maximum lambda of absorbance at 758 nm to construct standard curves for release profiles in different solutions. The constructed graghs in Figure 2 showed good linearity with R^2 value of 0.999 and 0.998 in water and buffer solutions respectively.

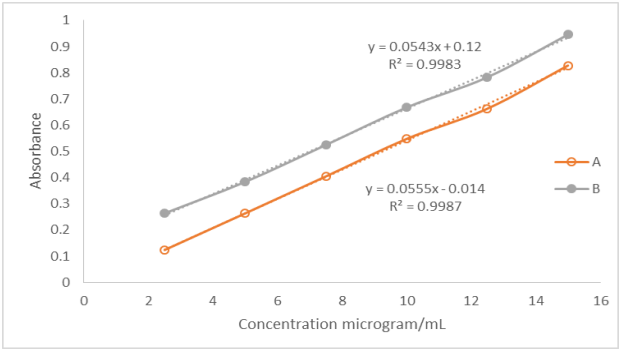

Figure 2: Standard Calibration Curves for Release of Insulin from Polymer Matrix (A = Deionised Water, B = Buffer Solutions)

Figure 3 shows the cumulative release of insulin from kg-g-poly (MAAm) at various pH at 37^{o}C. In all pH media the release pattern was biphasic, comprising an initial burst effect followed by an almost sustained continuous phase. This could be attributed to immediate dissolution and release of the accumulated drug on the surface or in the tunnels of the hydorgel. This part of the drug could diffuse rapidly when the gel came into contact with the release medium. After an initial burst release, follows the slower and steadier release of the insulin entrapped into the hydrogel at about the 300th minute in all the pH conditions. This behavior is similar to that reported by Khodaverdi et al., [5] and Zhou et al., [17]. The amount of drug release in pH 7.4 was highest and fastest compared the pH 2.2 and distilled water. Hydrogen bonding both between hydrogel molecules and the insulin molecules are more prevailing at lower pH, and this may result in hindered release of insulin [5].

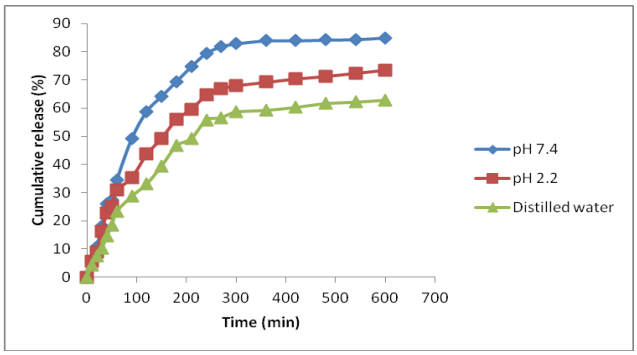

Figure 3: Cumulative Release (%) of Insulin from kg-g-poly (MAAm) in different pH Media

3.3 Kinetics and Mechanism of Insulin Release

Table 7 presents correlation values and release parameters determined from the results of power law model fitting of insulin profile (still from Equation 1-5). The correlation values and release data fitted the power model in the entire release medium. The values of n given in Table 4.2 are between 0.5 and 1.0 for the release of drug in pH 2.2 and pH 7.4 buffers. This indicates a non-Fickian or anomalous (Case II) diffusion mechanism for the release of insulin from the polymer matrix in these medium. It equally implies that the rate of polymer chain relaxation and the rate of drug diffusion from these hydrogels are comparable. Hence, insulin was released based on two simultaneous rate processes; water migrates into the hydrogel device and the insulin diffuses out continuously during the swelling of the hydrogel. The release of insulin in distilled water followed Case II diffusion mechanism where diffusion is very rapid compared to the relaxation process. In each release medium, the values of D_i for the released of insulin were observed to be higher than the values of D_A and D_L indicating faster initial rate of diffusion of drug from the polymeric matrix than the latter stages of diffusion. The implication of this is that more of the drug has been concentrated or retained near the surface of the polymer after drug loading onto the polymer, which will initiate rapid surface diffusion (or burst effect) of the drug from the release. After attaining certain concentration, the release of drug gets steady and controlled.

Table 7: Results of Diffusion Exponent n, Gel Characteristic Constant k and Various Diffusion Coefficients for the Release of Insulin from kg-g-poly(MAAm) Hydrogel

Medium Diffusion	Gel characteristic Exponent n	constant K x 10^{-2}	Diffusion coefficient (10^4cm^2) Initial, D_i	Average, D_A	Last time, D_L
Distilled water	1.123	0.072	16.340	12.159	1.492
pH 2.2	0.689	0.940	10.965	9.971	1.585
pH 7.4	0.623	2.236	9.002	9.895	1.329

Correlation coefficient values, r: distilled water= 0.9874; pH 2.2 = 0.9831; pH 7.4 = 0.9897

3.4 Glucose Sensittivity Response of Insulin Loaded kg-g-poly (MAAm) Hydrogel Systems

Figure 4 presents the release of insulin using kg-g-poly (MAAm) hydrogel in response to physiologically relevant trigger glucose levels (at 37^{o}C and 2 h). The device showed an increasing sharp response to insulin release in glucose solutions with increase in the glucose dose. This behavior is similar to that reported by Yin et al. [18] for a concanavalin A (Con A)-based hydrogels. The presence of glucose reduces crosslinking density in hydrogels and increases their hydrophilicity resulting in swelling [18]. The rapid release of insulin at high glucose concentration may also be due to the preferential absorption of the sugar molecules in the hydrogel, which will result in a parallel process evicting the insulin molecules.

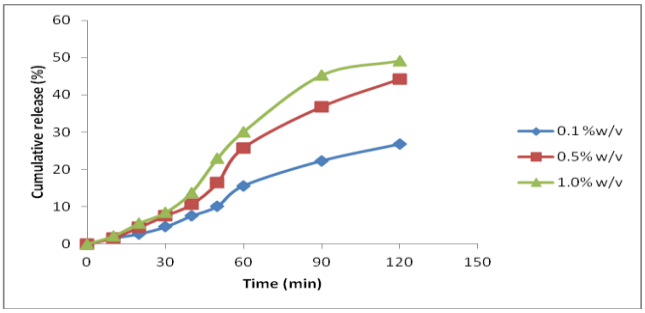

Figure 4: Cumulative Release (%) of Insulin from kg-g-poly (MAAm) in different concentrations of glucose solution with time

Figure 5 presents the release of insulin in glucose solutions with pH mimicking the intestinal conditions. The rate of insulin release in the two pH conditions increases with increase in the concentration of glucose. However, the release at the pH of 6.8 was higher. Decrease in pH causes ionization of the amino groups and this may lead to significant swelling, hence higher insulin release [18]. The phenomenon may also be attributed to the formation of hydrophilic glucose complexes in the hydrogel, in a reaction that is pH sensitive [19]. The presence of this complexes will influence the hydrophilicity of the hydrogel and hence its release of insulin in the glucose media as the pH changes. At 0.1% w/v (~5.5 mM) glucose concentration, the release of insulin was rapidly triggered from the hydrogel, and this is a promising phenomenon for the release of insulin in the system as the typical glucose concentration in the system ranges between 0.1 % w/v (5.5mM) and 1 % w/v (55 mM) [20].

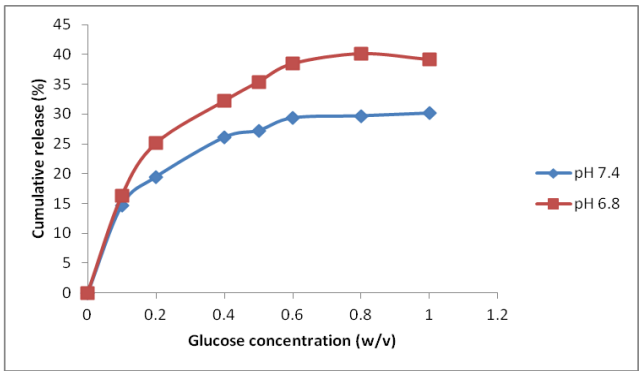

Figure 5: Cumulative Release (%) of Insulin from kg-g-poly(MAAm) in different concentrations of Glucose in buffered solutions

4. Conclusion

The result obtained from the pH-controlled swelling of konkoli grafted polymethylacrylaminde hydrogel and the pH-controlled cumulative release of insulin from the hydrogel has shown some of its intrinsic properties favorable for its application as an oral insulin delivery device. The kinetics and mechanisms of these processes as studied present the different behavior and interaction among the water molecules, insulin molecules, the hydrogel molecules and the polymeric network leading to the results obtained. Other cumulative insulin release result obtained from various media with different glucose concentrations range within which the physiological values will fall, and those obtained from typical pH-controlled glucose concentrations, shows that the hydrogel will release insulin trapped into its polymeric matrix in these media and also in a physiologically controllable manner, which is a promising attribute of a potential oral insulin delivery device.

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