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تهیه و بررسی هیدروژل هوشمند زیستی به عنوان حامل سریع انسولین در دارورسانی خوراکی انسولین

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Preparation and Review of Biocompatible Smart Hydrogel as a Rapid-Acting Insulin Carrier in Oral Insulin Drug Delivery

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چکیده

در این مطالعه یک سیستم دارورسانی انسولین خوراکی بر پایه هیدروژل زیستی (PSH) هوشمند و حساس به pH بر روی یک پلیمر طبیعی با ساختار نانوسلولزی طراحی گردید. PSH از مخلوط آکریلیک اسید و ۲- هیدروکسیل اتیل متاآکریلات برروی یک پایه نانوسلولزی تهیه و توسط روش‌های FT-IR و SEM شناسایی شد. ساختارهای سنتز شده می‌توانند به تغییرات pH محیط وابسته بوده و منجر به ایجاد یک الگوی توزیع موزونی گردند. نتایج نشان داد که فاکتور اصلی مؤثر بر رفتار هیدروژل، توزیع دارو درpH محیط می‌باشد به طوری‌که سرعت آزادسازی دارو از هیدروژل در ظرف ۴۸ ساعت در یک محیط شبیه-سازی‌شده با بدن در SIF با pH=۹ و محیط پلاسمای طبیعی خون با pH=۷/۴ (۹۳٪) بیشتر از شرایط اسیدی (SGF با pH=۱/۵) بوده و نتایج برای تهیه انسولین خوراکی رضایت بخش می‌باشد.

واژه‌های کلیدی

آزادسازی دارو؛ هیدروژل‌های هوشمند؛ انسولین خوراکی؛ نانو سلولز؛ زیستی.

Abstract

In this study, an oral insulin delivery system, based on Biocompatible and smart pH sensitive Hydrogel (PSH) on to natural polymer nano-cellulose backbones has been designed. PSH is made of mixed acrylic acid and 2-hydroxyethyl methacrylate on the nano-cellulose backbones and has been identified by using FT-IR and SEM methods. Synthetic networks, can sense the pH environment changes and lead to the creation of a swinging distribution pattern. The results show that the main factor influencing the behavior of the hydrogels in drug distribution, is the pH environment, so that the rate of drug release from the hydrogel, within 48 hours in a simulated body environment in SIF with pH= 9 and blood plasma-neutral environment with pH= 7.4, (93%) has been in greater amount of it's release in acidic conditions (SGF with pH= 1.5) and the results have been satisfactory for the preparation of oral insulin.

Keywords

Drug Delivery; Smart Hydrogel; Oral Insulin; Nano-Cellulose; Biocompatible.

1. INTRODUCTION

Diabetes is a metabolic disorder in the body. The patient's ability to produce insulin in the body disappears or the body becomes resistant to insulin, so the produced insulin can't have the normal functioning.

Insulin is a hormone that converts the sugar and starches into energy in the body. It means that the sugar produced by eating food leads to the cells for metabolism. Without insulin, the glucose is not driven into the cells and increases the levels of blood sugar, which is called hypoglycemia so, the daily subcutaneous injection of one to several doses of insulin for diabetics to control blood sugar levels is essential. In addition to being difficult and painful, there are many physical and psychological complications. Including pain, redness and itching at the injection site, thickening of the skin where the injections usually take place in that area, the inability of injection by patient due to shaky hands and body, lack of awareness of patient to receive the proper dose of drug, reduction or excessive increase in drug level in the body and factors like it, have promoted

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researchers all over the world, to provide solutions in order to change the method of diabetes treatment [1].

Among all methods, receiving the oral insulin is the center of attention. But due to the destruction of insulin by the digestive enzymes and disappearance of drugs, this method requires extensive research. The method used in this research is based on oral insulin targeted drug delivery. Typical drug delivery systems in the body, including tablets, capsules, creams, ointments, lotions and aerosols (suspensions and emulsions). The therapeutic drug level in the patient should be much enough to meet patient needs until the next drug consumption. Unfortunately, it is observed that in an injection method reduction or the excessive increase of drugs in the body affects it's influence [2].

In delivery systems for insulin hormone, the most important issues concerning oral administration of peptides and proteins such as insulin is different Protease enzymes in the digestive system that analyze the subjected compounds quickly.

In this study, we attempt to retain the destructive effects of digestive enzymes and redirect to the proper place to influence, by smart Hydrogel synthesis of bio-pH, based on natural polymer nano-cellulose and putting the insulin on the cover. hydrogel particles of polymer networks have a three-dimensional configuration, which is able to absorb a lot of water with liquid bio that inflate on contact with water, but cannot be solved and doesn't lose adsorbed water under mechanical stress. The porous structure of hydrogel polymer is a basic concept for the controlled delivery of drugs.

natural hydrogel drug delivery systems have been widely studied. In the present study, by using the new formulation, a new form of oral insulin with synthesis of a smart, bio-anionic hydrogel were prepared on natural nano-cellulose polymer (2-hydroxyethyl methacrylate and acrylic acid), it has benefits, including easier system for synthesis, optimization of time, temperature, energy and costs, the use of nano-cellulose as a base polymer that is natural, cheap and available. synthesized hydrogels as a carrier of insulin act like a cover to prevent the destruction of insulin by stomach acid and digestive enzymes and pass the drug to release in the area with an appropriate pH.

In this study, by using a rapid-acting insulin, in addition to having the advantage of the rapid drug effect ,we can increase the duration of monitoring of drugs in the body with the smart hydrogel help. In addition to the rapid effect, immediately after taking the pill, patients need not take medicine for more than 48 hours, albeit in oral form and to improve the life quality of a diabetic patient like healthy people.

2. EXPERIMENTAL

Reagents: acrylic acid (AA), 2-hydroxyethyl methacrylate (HEAM), ammonium persulfate (APS), sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), hydrochloric acid (HC1), all were prepared by Merck company, nano-cellulose and methylene- bis-acrylamide (MBA) by Fluka Company. Distilled water was used as a solvent. The insulin aspart drug was purchased by (Novo Nordisk) company.

Apparatus**:** the spectrophotometer device, uv-visible t-80 model, spectrometer FT-IR necsus-670 model, scanning electron microscopy (SEM) LEO 1430VP model, pH meter PH/mV/Temp 86502 model, the scale GR200/Max210g /Min10mg/FA2104N model, the hothouse OF-02G and water bath equipped with temperature and time controller Lab Tech LCB-11 model was used.

2.1. Preparation of Hydrogel

The reaction of graft copolymer acrylic acid monomers (AA) and 2-hydroxyethyl methacrylate (HEMA) on nano-cellulose by ammonium persulfate (APS), as the radical initiator and methylene bis-acrylamide, as a network constructor factor was performed as follows;

35 ml of distilled water shed in a reactor equipped with a mechanical stirrer which has been in a constant-temperature bath, then a certain amount of nano-cellulose (2 grams) added to reactor and blend at a constant temperature (70 °C) uniformly with a mechanical stirrer, in two separate beakers, pour the certain amount of radical initiator ammonium persulfate (0.1 g) and the network constructor methylene bis-acrylamide (0.05 g) in 5 ml of the distilled water and mix until it completely gets dissolved. Then to start the reaction of a radical copolymer, ammonium persulfate solution added to reactor, and after 10 minutes a certain amounts of 2-hydroxyethyl methacrylate monomers (1.5 g) and acrylic acid (1.5 g) added simultaneously to the reactor. Then, we add the methylene bis-acrylamide to the reactor. At the same temperature (70 ° C) with stirring (stirrer speed 600 rpm) we let the copolymer reaction carried out and hydrogen is formed.

Then, the reactor is removed from the water bath and allow to cool to ambient temperature. Afterward, we dissolved certain amount of sodium hydroxide in 10 ml of distilled water and

pour into a reactor and with a mechanical stirrer, we mix it for 5 minutes to neutralize the acid groups (Acrylic acid).

We leave the hydrogel for 1 hour to complete the neutralization reaction. For dewatering, the hydrogel is cut into smaller pieces, we pour it in a beaker containing 200 ml of ethanol and let it dry by ethanol. By renewing the ethanol in every 3 hours, after 24 hours, the dried hydrogel is removed from ethanol and gets dried in a hothouse with a temperature of 40-50 °C for 12 hours. Then we grind the dried hydrogel to form a white powder.

The resulting powder is sifted with Mesh No.400 and kept in a container to use in next steps. We use a few hydrogel particles to provide FTIR spectrum with KBr to form KBr tablets, by covering part of powder hydrogel with a thin layer of gold using scanning electron microscope (SEM), the surface morphology of the gel was examined, too.

3. RESULT AND DISCUSSION

3.1. The reaction mechanism of hydrogel formation

In the first step, the initiator (APS), decomposes by heating and produces the sulfate anion radicals. After wards, the produced radical attacks to hydrogen OH group of nano-cellulose and nano-cellulose gets linked with the radical initial monomer. In addition, the crosslinking reaction, is performed in the presence of the crosslinker (MBA) so that it creates a three-dimensional network, the bonds formed between monomers were confirmed by FTIR spectrum.

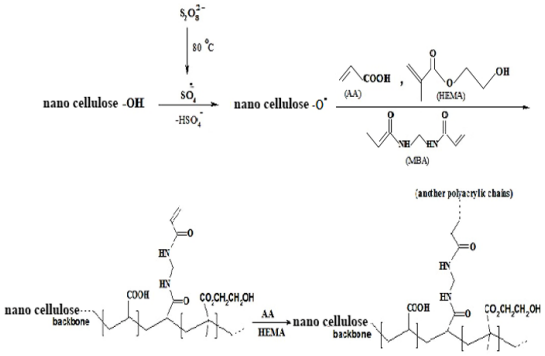

Fig. 1. The reaction mechanism of noncellulose-g-poly (acrylic acid-co-2-hydroxyethylmethacrylate) hydrogel formation**.**

3.2. Hydrogel FTIR spectroscopy

The FTIR spectrometry results, (the obtained acrylic monomers and gel) are shown in Fig. 2. Most of the changes in the structure of the hydrogel caused by the formation of covalent bonding grid (MBA) with acryl macromonomers. In the spectra of the hydrogel the characteristic band at 1581.11 cm^{-1} was attributed to C=O asymmetric stretching in the carboxylate anion. This was confirmed by another peak at 1397.46 cm^{-1} which is related to the symmetric stretching mode of the carboxylate groups. The main contribution to the absorption band in the 1732.05 cm^{-1} is due to the ester group from the poly (2-hydroxyethyl methacrylate). The formation of C-N bonds in the MBA will lead to loss of hydroxyl groups which has appeared as the reducing peak of hydroxyl groups (3425.02 cm^{-1}), compared to the reference peak.

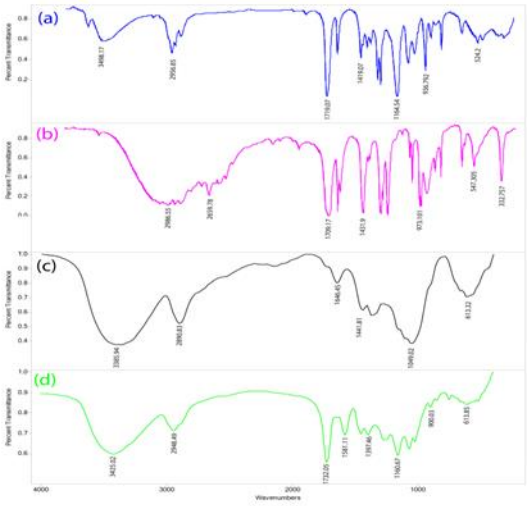

Fig. 2. FTIR spectrum of AA (a), nano-cellulose (b), HEMA (c) and hydrogel (d).

3.3. Hydrogel morphology and particle size

Fig. 3 shows the internal structure of dried hydrogel. Imaging has been made with a scanning electron microscope (SEM). Cross-microscopic images, confirms the formation of intelligent hydrogel particles biocompatible, particle porous for the imprisonment of drug. Since the pore size is associated with the water absorption rate, So, these samples must have quick absorption of water more than other samples with lower porosity. Cavities appearance is caused by water vapor loss during the polymerization process.

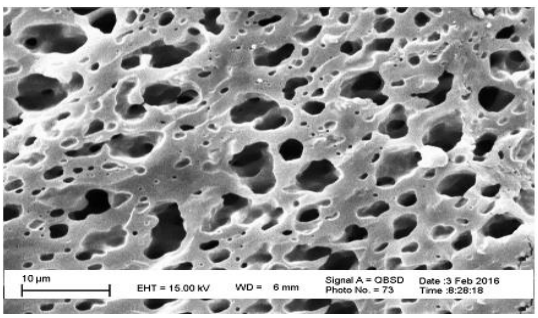

Fig. 3. SEM image of dried hydrogel.

3.4. Measuring the inflation rate of Hydrogel

Pure and dry hydrogen sample posed in 100 ml of distilled water (0.1 g) for 1 hour. After equilibrium, the swollen gel was obtained, which was dewatered by passing from a filter and hanging it for (15 minutes). The swollen gel mass was measured and inflation balance was calculated by using equation (1).

$$W.A = \frac{(Ws-WD)}{WD} \qquad (1)$$

Absorption (g / g) was calculated for weight of swollen hydrogels (Ws) per gram of dry hydrogel (W_D). Inflation samples were measured in various stages of research with the same conditions.

3.4. Study of smart hydrogel particles to changes of pH in the ambient.

At this stage, the solution of NaOH and HCI diluted with distilled water is used to get the desired basic (pH = 8) and acidic (pH = 3) conditions. In basic terms, ionization of the carboxyl group and increasing electrostatic repulsive force among groups COO^-, increases swelling capacity to 83 (g / g). While in acidic conditions, protonation of carboxylate groups, causes the loss repulsion between these groups and thereby reducing the amount of inflation. According to swelling- deswelling behavior of hydrogel among the pH changes and based on the graph shown in Fig. 4, synthetic networks can sense changes in pH ambient which leads to the creation of a swinging distribution pattern. Due to the reversible swelling behavior from hydrogel in different pH, taking advantage of this particle for oral delivery of rapid-acting insulin, has been studied [8].

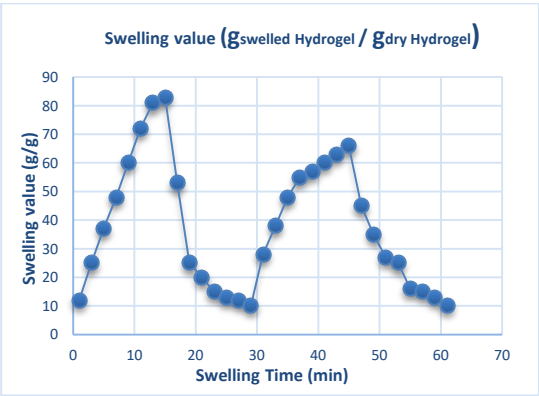

Fig. 4. The reversible swelling behavior of hydrogel in two pHs 8 and 3.

3.5. Loading of the drug in the hydrogel network

A completely exact weight (1 g) of the hydrogel optimized with mesh 400, with the average particle size (37 µm) immerse totally in a certain amount of the drug (60 units of insulin-aspart) and keep in a place away from light at room temperature for two days. After the completion of the swelling loaded hydrogel we filter the samples with drug and wash the particles with the amount of distilled water, till the attached drug to the surface be washed completely [6], [7]. In SEM images and FTIR spectrum of drug-loaded hydrogel, (Fig. 5) and (6), the trapped insulin in the pores of the hydrogel network, can be seen clearly.

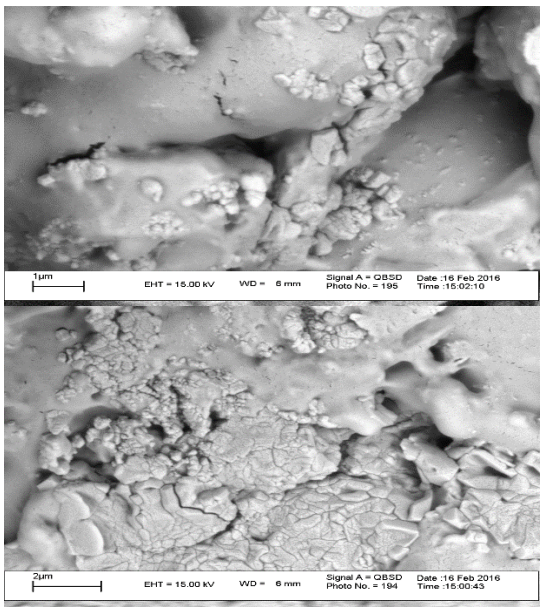

Fig. 5. SEM pictures of the drug- loaded hydrogel.

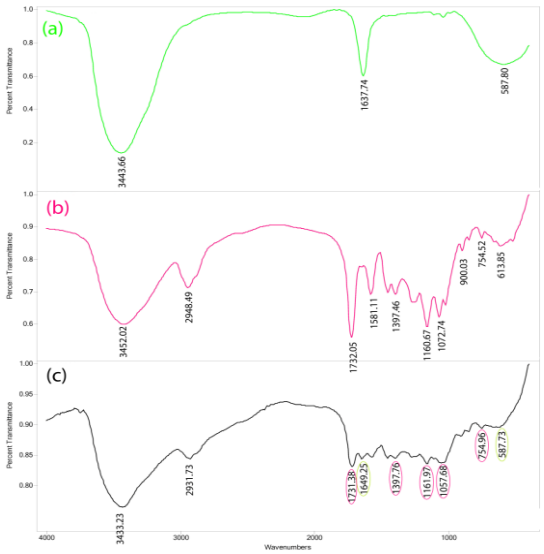

Fig. 6. FTIR spectrum of insulin-aspart drug (a), hydrogel (b) and drug-loaded hydrogel (c).

3.6. The testing of drug release

In this set of experiments, we put the specified amount (0.1 grams) of hydrogel, in beaker of 150 ml, containing 100 ml of buffer solution with certain pH in a water bath with a constant

temperature of 37 C. Gradually the influence of buffer solution into the hydrogel and swelling of loaded drug releases and over time, the drug concentration in the buffer solution is increased. To measure the drug concentration over its releasing time into the buffer solution, at certain intervals, once every two hours, the samples taken from the system (each 3.5 ml) measure the absorbance rate. To make this amount of sampling have less effect on system level, the same volume of sample buffer solution gets back into the system at the same circumstances and remains constant during the experiment.

3.7. Determination of the calibration curve

At this stage of the work, by providing seven different concentrations of the drug solution in terms of units of insulin (U) to milliliters (ml) of distilled water, using spectroscopy, UV, buffers's absorbance intensity in λ_{max} of insulin is taken, because the lack of buffers absorption in the λ_{max} of drug, providing one calibration curve for all three buffer pH of 1.5, 7.4 and 9 will suffice.

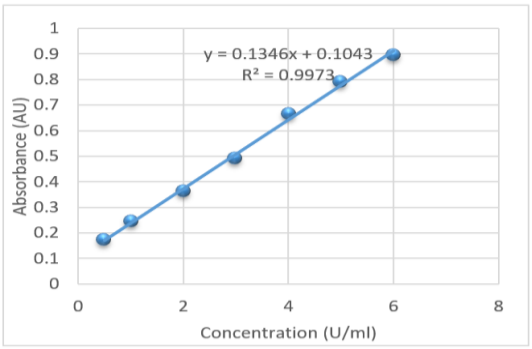

Fig. 7. Calibration curve of insulin, concentration is expressed in terms of insulin units per ml of distilled water (U/ml).

3.8. Evaluation of drug release

Chart related to the release of the drug into the hydrogel network, the pH of 1.5, 7.4 and 9, which respectively corresponds to the pH range in the acidic environment of the stomach, inert atmosphere blood plasma and the upper limit intestinal basic environment, is shown in Fig. 8. The hydrogel drug delivery is directly proportional to the amount of water absorption, when the water absorption rate is higher, the amount of drug release increases. As seen in Fig. 8, the drug release at pH 7.4, higher than its release in pH 1.5 and is pH 9, which is due to reduced water absorption of hydrogel in the lower and higher pH value [8] as mentioned above, in acidic conditions, Protonation of the carboxylate groups, causes loss of repulsion between these groups and thus reduces inflation, by increasing the basic properties of environment, ionization carboxylate groups and electrostatic repulsion force between COO- groups, causes swelling capacity increase, while in highly basic solutions, due to "charge screening effect" Na^+ ions to the environment, as a shield between carboxylate groups prevent anion-anion repulsion between these groups and reduces swelling and the capacity of the hydrogel inflation [9].

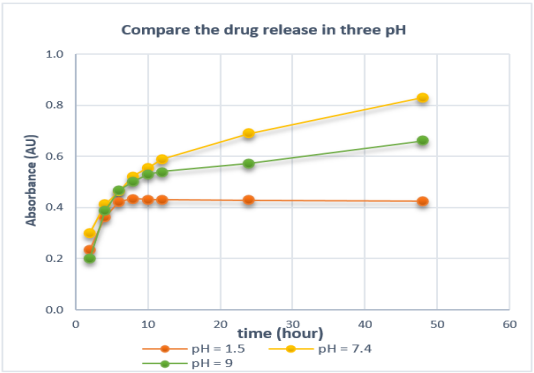

Fig. 8. The comparison chart of drug release from the drug into the hydrogel network of λ max over time and pH of 1.5, 7.4 and 9.

3.9. Study of percent drug release of insulin from the particles of hydrogel in different pH

In our evaluation, the percentage for the amount of drug release from the hydrogel network, Based on the total amount of drug loading in Fig. 8 and using the calibration curve graph reported in Fig. 7. has been calculated and presented in Fig. 9. drug release percent was calculated twice using the following equation:

$$\text{Released drug \%} = \frac{Rt}{L} \times 100 \quad (2)$$

where L and Rt represent the initial amount of drug loaded and the final amount of drug released at time t. Accordingly, in pH 1.5, 48% of the drug in 69% of the drug pH 9 and in pH 7.4 more than 93% of the total amount of loaded drug are released. As can be seen, the rate of drug release by hydrogels in pH 7.4 was more than the higher pH and lower than this amount.

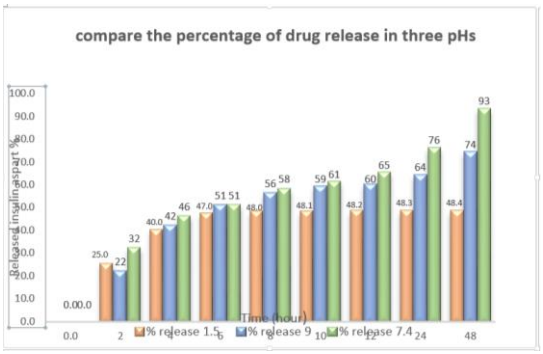

Fig. 9. The diagram of the percent of insulin release from the hydrogel, in the pH of 1.5, 7.4 and 9 within 48 hours.

3.10. Evaluation of drug release in simulated body environment, SIF and SGF

To achieve the desired results in the field of drug delivery, medication should distribute before treatment, in a system similar to the human body. Since body simulation, due to the special intricacies of the nervous, digestive and blood system is hard, so we tried the simulation to be done similar to blood plasma pH neutral medium (7.4). Thus, using the reference USP, with the volume delivery of appropriate amounts of Na_2HPO_4 and KH_2PO_4 with distilled water, the phosphate salt buffer solution with the desired pH (9, 7.4, 1.5) obtained. To investigate the drug release in simulated body the temperature was set on 37 °c.

The FTIR spectrum of drug release in pH 7.4, 9 and 1.5, is shown in Fig. 10 (a), 11 (a) and 12 (a), and the drug release chart in λ_{max} of drug (270 nm), with respect to time, is presented in Fig. 10 (b), 11 (b) and 12 (b). According to the results, in the 48 hours after dipping loaded Hydrogel with medicine, the amount of drug absorption has reached to 0.83 AU in the simulated body environment in pH 7.4. According to previous calculations, more than 93% of drug has been released uniformly in the blood plasma pH neutral medium and SIF (7.4) [10], [11].

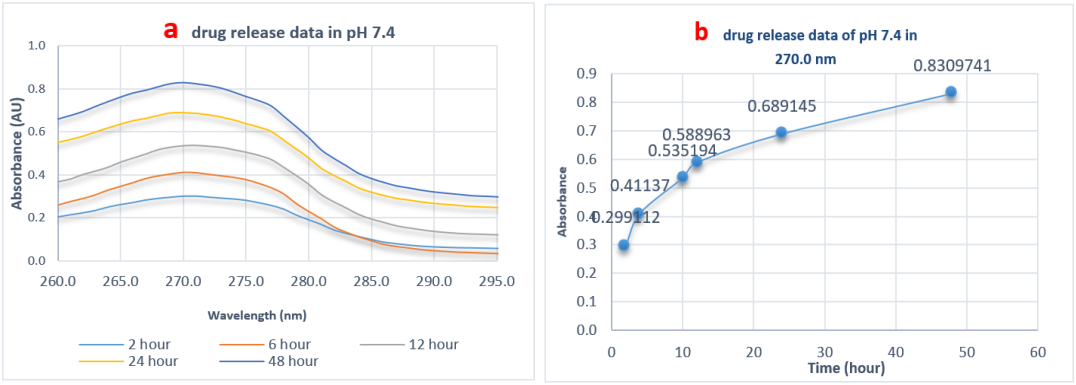

Fig. 10. UV-visible spectrum (a) and λ_{max} with respect to time graf (b), of insulin aspart release in pH = 7.4.

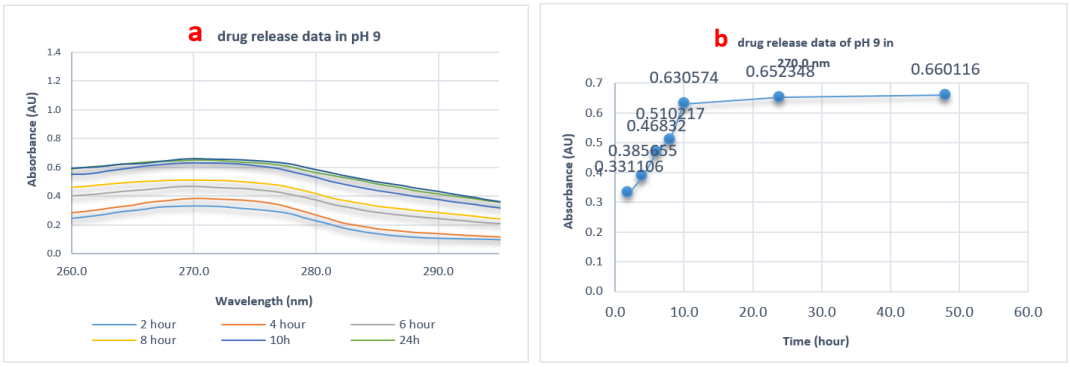

Fig. 11. UV-visible spectrum (a) and λ_{max} with respect to time graf (b), of insulin aspart release in pH = 9.

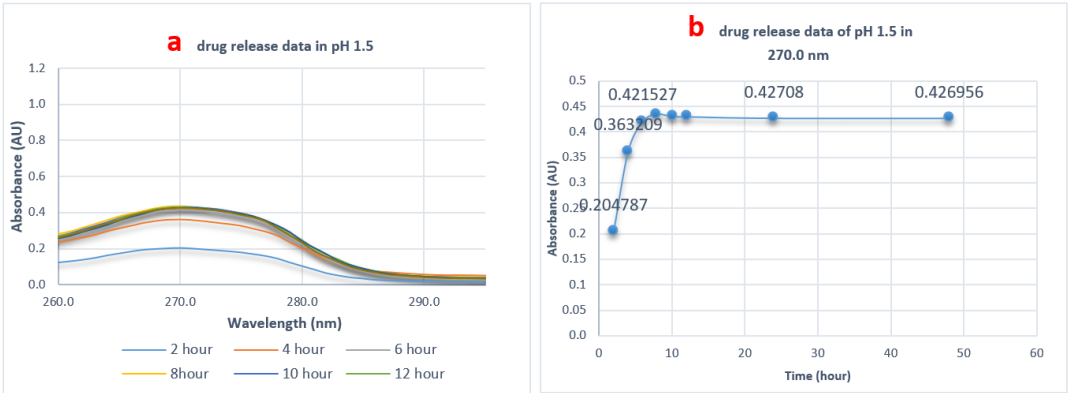

Fig. 12. UV-visible spectrum (a) and λ_{max} with respect to time graph (b), of insulin aspart release in pH = 1.5.

4. CONCLUSIONS

The smart biocompatible hydrogel with the combination of Acrylic acid monomers and 2-hydroxyethyl methacrylate on produced nano-cellulose was used for building and targeted delivery of oral insulin. According to studies, when the rate of hydrogel inflation is higher, by the loading of the drug, the water-soluble drug penetration into the hydro gel increases. Therefore, the concentration of the drug in the hydrogel network grows, the amount of hydrogel drug delivery is directly proportional to the amount of water absorption, when the water absorption rate is higher, the amount of drug release increases. Studies on the drug release PH of 1.5, 7.4 and 9 showed that the highest rate of drug release in PH=7.4 is in the 93% of the loaded-drug. Because of proton bearing in acidic PH of carboxylate groups and reduction of repulsion between them, the inflation rate decreases. In neutral pH and weak basic solutions, the electrostatic repulsive force between carboxylate groups, increases the capacity of inflation. While in highly basic solutions, due to "charge screening effect" Na^+ ions to the environment, as a barrier between carboxylate repulsive anion-anion groups has reduced between these groups, Reduces swelling and the capacity of the hydrogel inflation. Based on these results, the synthesized hydrogels for oral delivery of insulin has satisfactory conditions.

REFERENCES

[1] D.R. Owens, B. Zinman and G.B. Bolli, Insulins Today and Beyond, *Lancet* 358 (2001) 739–746.

[2] F.L. Buchholz and A.T. Graham, *Modern Superabsorbent Polymer Technology*, Wiley, NY, (1997).

[3] C.R. Kahn, Banting Lecture; “Insulin Action, Diabetogenes, and the Cause of Type II Diabetes”; *Diabetes* 43 (1994) 1066–1084.

[4] A.S. Hoffman, Hydrogel for biomedical applications, *Adv. Drug. Deliver. Rev.* 43 (2002) 3–12.

[5] A. Kikuchi and T. Okano, Pulsatible drug release control using hydrogels, *Adv. Drug Deliver Rev.* 54 (2002) 53-77.

[6] H.K. Ju, S.Y. Kim and Y.M. Lee, Ph/temperature-responsive behaviors of semi-IPN and comb-type graft hydrogels composed of alginate and poly(N-isopropylacrylamide), *Polymer*. 42 (2001) 6851–6857.

[7] Y.Y. Liu, X.D. Fan, T. Kang and L. Sun, A cyclodextrin microgel for controlled release driven by inclusion effects, *Macromol. Rapid Commun*. 25 (2004) 1912–1916.

[8] D. Saraydın, E. Karadag, H.N. Oztop and O. Guven, Adsorption of bovine serum albumin onto acrylamide-maleic acid hydrogels, *Biomaterials*. 15 (1994) 917-920.

[9] D.W. Jenkins and S.M. Hudson, Review of vinyl graft copolymerization featuring recent advances toward controlled radical-based reactions and illustrated with chitin/chitosan trunk polymers, *Chem. Rev*. 101 (2002) 3245-3273.

[10] M. Mahkam, L. Doostie and S.O.R. Siadat, Synthesis and characterization of acrylic type hydrogels containing azo derivatives of 5-amino salicylic acid for colon-specific drug delivery, *Inflammopharmacol*. 14 (2006) 72-75.

[11] M. Mahkam and M. Allahverdipoor, Controlled release of biomolecules from phsensitive network polymers prepared by radiation polymerization, *J. Drug Target*. 12 (2004) 151-156.