Sigma J Eng & Nat Sci 34 (1), 2016, 115-122

Sigma Journal Engineering and Natural Sciences
Sigma Mühendislik ve Fen Bilimleri Dergisi

Research Article / Araştırma Makalesi

PRODUCTION AND OPTIMIZATION METHOD OF SMART HYDROGELS BASED ON BIOCOMPATIBLE POLYMER FOR CONTROLLED INSULIN RELEASE

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Received/Geliş: 03.07.2015 Revised/Düzeltme: 29.10.2015 Accepted/Kabul: 17.11.2015

ABSTRACT

The aim of controlled insulin delivery study experiments is to produce the smart hydrogel to use insulin release for human body. Insulin entrapped p(HEMA-co-Eudragit L-100) hydrogels containing different ratios of 2-Hydroxyethyl methacrylate (HEMA) and Eudragit L-100 were synthesized by using ammonium persulfate (APS) as an initiator and ethylene glycol dimethacrylate (EGDMA) as a cross linker and new alternative ways were worked on to find and develop. Taguchi method was used and optimum synthesis conditions were determined. Insulin was entrapped into hydrogels as synthesis. After entrapped method, loading efficiency values were calculated. and the hydrogels produced were characterized by using Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning electron microscope (SEM) analysis.
Keywords: Hydrogel, insulin, Taguchi, Eudragit.

KONTROLLÜ İNSÜLİN SALIMI İÇİN BİYOUYUMLU POLİMER ESASLI AKILLI HİDROJELLERİN ÜRETİMİ VE OPTİMİZASYONU

ÖZ

Kontrollü insülin salım çalışmasının amacı, insan vücudu için kontrollü insülin salımını kullanarak akıllı hidrojel üretmektir. Farklı oranlarda 2-Hydroxyethyl methacrylate (HEMA) ve Eudragit L-100'ü içeren insülin tutuklanmış p(HEMA-co-Eudragit L-100) hidrojelleri, başlatıcı olarak amonyum persülfat (APS) ve çapraz bağlayıcı olarak etilen glikol dimetilakrilat kullanılarak yeni alternatif yollar bulunarak çalışılmıştır. Taguchi metodu kullanılmış ve optimum sentez koşulları belirlenmiştir. Sentezde hidrojeller içine insülin tutuklanmıştır. Tutuklama sonrasında, yükleme etkinlik değerleri hesaplanmıştır ve üretilen hidrojeller, Fourier Dönüşümlü Kızılötesi Spektroskopisi (FT-IR) ve Taramalı Elekton Mikroskobu kullanılarak karakterize edilmiştir.
Anahtar Sözcükler: Hidrojel, insülin, Taguchi, Eudragit.

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1. INTRODUCTION

For drug delivery systems, in present and future plans, biodegredable polymers are used and will be used more and more. Those polymers are affected by electricity, magnetic and pH of ambient. In these systems, while polymers are being destroyed, delivery occurs, and after that polymers will be threw away. The most important advantage of biodegredable polymers is not to need for surgery to remove them.

Hydrogels have been used essentially in the pharmaceutical field as carriers for delivery of different drugs, peptides etc. [1]. pH-responsive delivery systems are known in a number of different dosage forms, such as sustained-release tablets, micelles, microparticles and microspheres. Microsphere form is one of the prospect dosage forms because of its large specific surface area and high drug loading efficiency [2]. The researchers with an extensive research have been performed poly(hydroxyethyl methacrylate) as a synthetic hydrogel in the biomedical use of hydrogels due to its biocompatible and well-controlled rather than sustained-release delivery systems containing bioactive agents [3].

Zhang et al. (2012) [4] studied the preparation and characterization of nanoparticles based on thiolated Eudragit L-100 and unmodified polymer and evaluated their potential for the transportation of insulin in rats. The delivery system was a novel tool to improve the absorption of protein and peptide drugs.

Huynh et al. (2009) [5] investigated the profile of insulin release from mixtures of various pH/temperature-sensitive hydrogels and insulin in rats. The results showed that the diabetic rats were treated for more than 1 week with a single injection of the complex mixture containing insulin in a copolymer solution and the pH/temperature-sensitive insulin–hydrogel complex system had therapeutic potential.

Advances in polymer science and modifications of the backbone structures of biopolymers as copolymers, grafted copolymers, interpenetrating polymeric network hydrogels, polymeric micro-/nano-devices have contributed to the development of devices for oral insulin delivery. These systems resisted the variable pH medium before delivering insulin through various pathways in the intestine [6].

Hao et al. (2013) [7] studied a novel emulsion diffusion method to prepare enteric Eudragit L/100-55 nanoparticles by ultrasonic dispersion and diffusion solidification. In addition, the Eudragit L/100-55 nanoparticles showed a strong pH-sensitive release in vitro. The enteric Eudragit L/100-55 nanoparticle could be synthesized successfully via this method.

In previous study, Eudragit RL-PO was used as a polymer for preparation of acyclovir loaded nanoparticles due to its high permeability property and application for sustained release drug delivery systems by using nanoprecipitation method. Eudragit RL-PO is a copolymer of ethyl acrylate, methyl methacrylate and a low content of methacrylic acid ester with quaternary ammonium groups. The preliminary results showed that acyclovir loaded Eudragit RL-PO nanoparticles have an effect in sustaining drug release for a prolonged period [8].

Sadeghi (2010) [9] investigated acrylic acid and 2-hydroxyethyl methacrylate monomers were directly grafted onto chitosan using ammonium persulfate as an initiator and methylenebisacrylamide as a crosslinking agent under an inert atmosphere medium. Results indicated that the swelling capacity decreased with an increase in the ionic strength of the swelling medium. Moreover, the swelling of superabsorbing hydrogels was examined in solutions with pH values in range of 1-13.

Swelling behaviour of poly((2-dimethyl amino)ethyl methacrylate-co-BMA) was investigated by Emileh et al. [10]. The results indicated that the pH/temperature sensitive phase transition behaviour of the gels could be changed in various temperature/pH of the swelling enviroment at constant hydrogel composition.

The other researchers investigated in vitro and in vivo release data on pH-sensitive microspheres of Eudragit L100, Eudragit RS100 and their blend systems prepared by double

emulsion-solvent evaporation technique for oral delivery of insulin. The in vivo release studies on diabetic-induced rat models exhibited maximum inhibition of up to 86%, suggesting absorption of insulin in the intestine [11].

The goal of another study is to protect the sensitive drug from proteolytic degradation in the stomach and upper portion of the small intestine. In this work, we investigate the use of pH-responsive, poly(methacrylic-g-ethylene glycol) hydrogels as oral delivery vehicles for insulin. Insulin was loaded into polymeric microspheres. The insülin remained in the gel and was protected from proteolytic degradation [12].

In this study, insülin entrapped p(HEMA-co-Eudragit L-100) hydrogels containing HEMA and Eudragait L-100 were synthesized by using APS as an initiator and EGDMA as a cross linker. The optimum synthesis conditions were determined for hydrogels. Taguchi method was used as an optimization method for 16 experiments as 4 levels and 4 parameters. The analysis results (FT-IR, SEM) showed that the hydrogels were synthesized and entrapped method results were successful.

2. EXPERIMENTAL

2.1. Materials

APS used as an initiator was supplied from Sigma-Aldrich. Also, EGDMA, HEMA, Eudragit L-100 were supplied from Sigma-Aldrich (95%), Fluka (97%) and Evonik, respectively. Insulin was purchased from Balmumcu Chemistry.

2.2. Methods

Eudragit L-100 (0.3-0.6 g) polymer solution, insülin (7-28 mg) solution, HEMA (0.5-2 mL), EGDMA (0.005-0.02 ml) and APS (0.06 g) initiator was added to the reactor. Insulin based hydrogels were prepared by using 300 rpm stirrering with homogeneously mixing. After 24 hours waiting in tube, the hydrogels were cut with determined sizes (Figure 1). Taguchi method was used as an optimization method for 16 experiments as 4 levels and 4 parameters.

Figure 1. Insulin entrapped p(HEMA-co-Eudragit) hydrogels

2.3. Taguchi Method

For the Taguchi design and analysis of results, Minitab Release 13.20 Statistical Software was used after determined parameters. In classical methods one parameter was varied while

keeping all others constant. The Taguchi approach provides an opportunity to select a suitable orthogonal array depending on the number of control factors and their levels. The benefit of using a fractional factorial approach is the radical reduction in the number of experiments [13]. In this study, Taguchi's L-16 orthogonal array table was used to carry out experiments by choosing four parameters at four levels (Table 1).

Table 1. Taguchi Method

No	HEMA	Eudragit L-100	EGDMA	Insulin
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	1	4	4	4
5	2	1	2	3
6	2	2	1	4
7	2	3	4	1
8	2	4	3	2
9	3	1	3	4
10	3	2	4	3
11	3	3	1	2
12	3	4	2	1
13	4	1	4	2
14	4	2	3	1
15	4	3	2	4
16	4	4	1	3

2.4. Characterization

To analyze the hydrogels, Perkin Elmer Spectrum 100-FT-IR technique with Universal ATR sampling accessory was used to identify the chemical bonds of samples. The IR measurement range was selected as 4000–650 cm^{-1}. EVO LS10 Scanning Electron Microanalyzer (SEM) was used to take the micrograph of the sample. Sample was put on aluminum stubs by using conductive glue and was then coated with a thin layer of carbon.

2.5. Loading Efficiency

The loading efficiency of insulin in hydrogels were determined using UV spectroscopy (Shimadzu UV-1800) with 214 nm wavelength. The loading efficiency of insulin in hydrogels was determined after centrifugation of the dispersions and measurement of the insulin amount in the supernatant using UV. The drug loading efficiencies were calculated as described below Eq.(1) [4]:

$$\text{Loading Efficiency (\%)} = [(x-y)/x]*100 \quad (1)$$

where x, y were total amount of drug and amount of drug in supernatant, respectively.

3. RESULTS AND DISCUSSION

3.1. Taguchi Method Results

The experimental results were transformed into a signal-to-noise (S/N) ratio. This method used the S/N ratio as a measure of quality characteristics deviating from or nearing to the desired values. The optimum mixture was experiment 9 for 3-1-3-4 in Taguchi method by using S/N ratio (Figure 2).

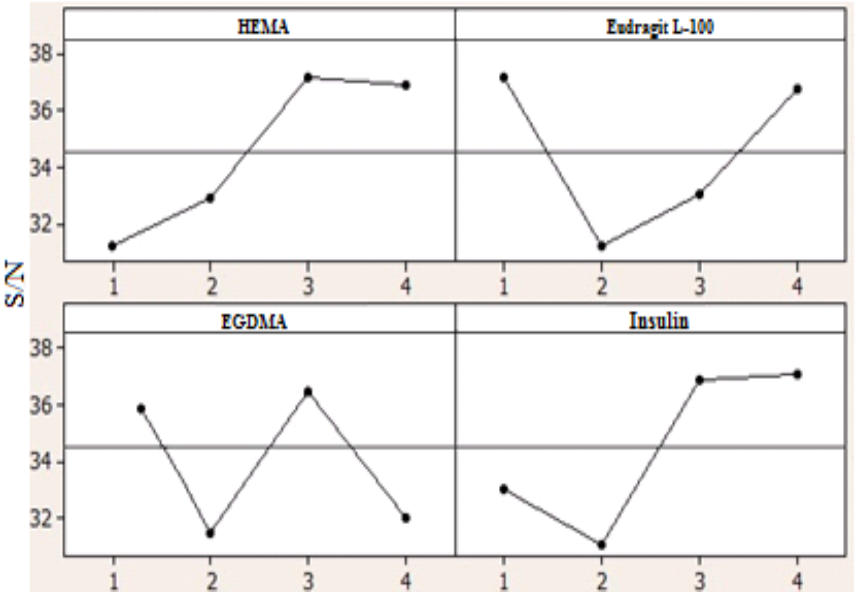

Figure 2. Taguchi Method Results

3.2. Characterization of Insulin Entrapped p(HEMA-co-Eudragit L-100) Hydrogels

The characteristic peak of Eudragit L-100 was at 3356 cm^{-1} observed due to the presence of O-H; 2919 cm^{-1} showed the CH-vibration. The peak observed in 1447 cm^{-1} was -CH_3 bending, and 1707 cm^{-1} and 1655 cm^{-1} showed C = O (ester) presence. The peak of HEMA was at 1717 cm^{-1} showed a carbonyl group C = O ester; the peak observed at 1386 cm^{-1} was = CH_2 band; 1448 cm^{-1} was asymmetrical methyl tendency. C-O stretching vibration of the ester carbonyl groups was seen 1074 and 1150 cm^{-1}. Insulin spectrum revealed two peaks; the Amide I (1655 cm^{-1}) and Amide II (1547 cm^{-1}). This band was due primarily to the C=O stretching, the out-of-phase C-N stretching and C-C-N bending vibrations. Amide II derived mainly from in-plane N-H bending, C-N and C-C stretching vibrations. These two peaks were present in the insulin-loaded spectrum. After insulin entrapment, particles spectrum presented a very similar aspect compared with insulin (Figure 3).

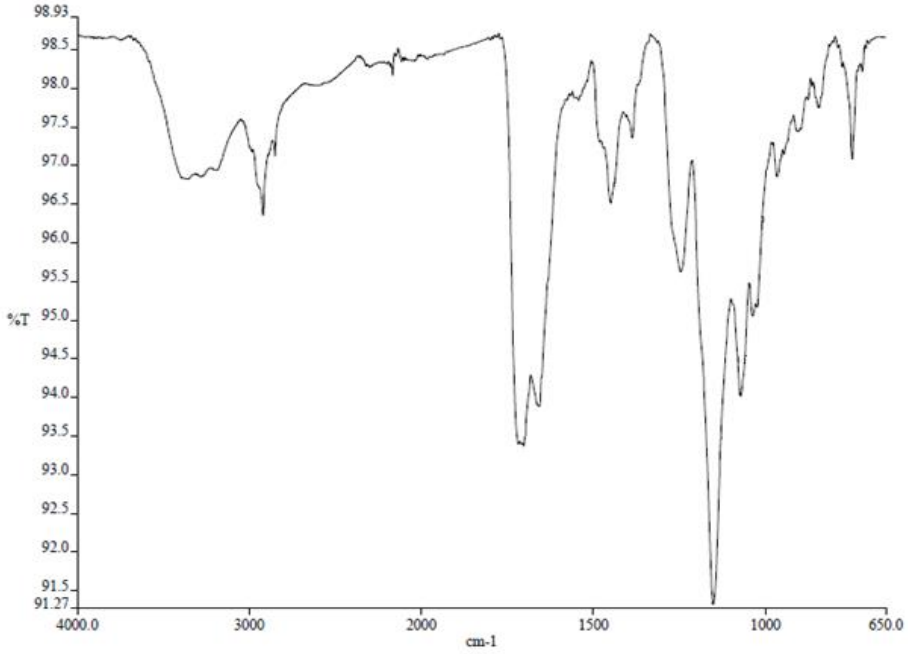

Figure 3. FT-IR spectrum of insulin entrapped p(HEMA-co-Eudragit) for experiment 9

SEM image of insulin entrapped p(HEMA-co-Eudragit) showed that desired pores were obtained for insulin release. Pore width changed between 1,790 µm and 10,26 µm (Figure 4).

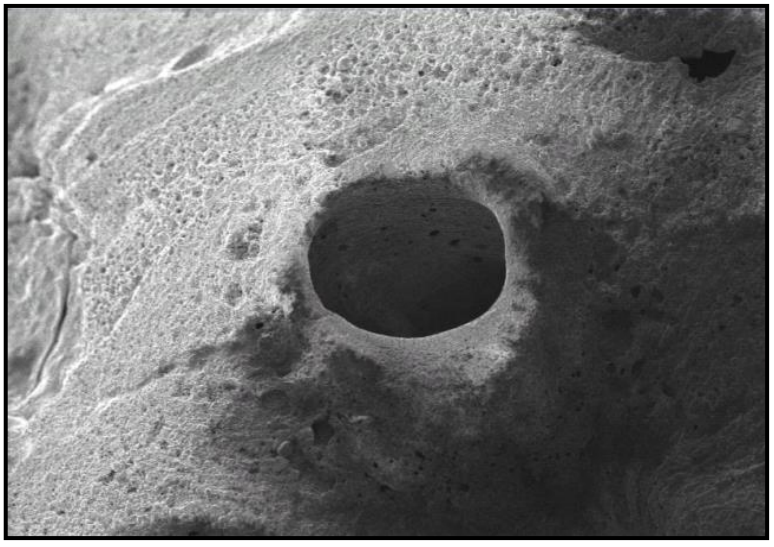

Figure 4. SEM analysis of insulin entrapped p(HEMA-co-Eudragit) for experiment 9

3.3. Loading Efficiency Results

The loading efficiency values were observed in the range from 62 (%) to 80 (%) (Table 2). It was seen that loading efficiencies of the insulin entrapped hydrogels were determined to vary inversely with the cross-link density (Table 1, Table 2).

Table 2. Loading Efficiency Results of Hydrogels

Experiment No	Insulin concentration (mg/mL)	Absorbans	Supernatant concentration (mg/mL)	Loading Efficiency (%)
1	7	3,515	1,35	80
2	14	-	-	-
3	21	3,609	6,7	68
4	28	3,672	10,3	63
5	21	3,587	5,46	74
6	28	3,575	4,76	83
7	7	-	-	-
8	14	3,585	5,32	62
9	28	3,639	8,4	70
10	21	3,631	7,98	62
11	14	3,533	2,38	83
12	7	3,522	1,75	75
13	14	3,580	5,04	64
14	7	3,532	2,31	67
15	28	3,629	7,84	72
16	21	3,580	5,04	76

4. CONCLUSION

In conclusion, a total of 16 experiments were performed owing to Taguchi method as an optimization method. Characterization results (FT-IR, SEM) showed that insulin entrapped p(HEMA-co-Eudragit) hydrogels were synthesized successfully. The optimum mixture was experiment 9 for 3-1-3-4 in Taguchi method by using S/N ratio. Loading efficiencies of the hydrogels were determined to vary inversely with the cross-link density. It was seen that this hydrogel could be evaluated in insulin release for diabetes treatment in future studies.

Acknowledgments / Teşekkür

This work was supported by YTU-2014-07-01-KAP03 and YTU-2014-07-01-GEP01 projects. There is no conflict of interests regarding the publication of this paper.

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