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Preparation of Metformin HCl-loaded Chitosan Microspheres and In vitro Characterization Studies

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Behzad Mokhtare*, Meltem Cetin*°, Rukiye Sevinc Ozakar*, Hatice Bayrakceken**

Introduction

Chitosan is a biodegradable, biocompatible and non-toxic natural polymer and shows good bio-adhesive characteristics due to the electrostatic interaction of its positive charge with mucus or negatively charged residues on the mucosal surface[1,2].

Metformin has antihyperglycemic effect from the biguanide class, and recently, it has been shown that it has also antineoplastic and chemopretentive potentials[3,4]. Metformin has a relatively low (50-60%) bioavailability due to its primary absorption from the small intestine and also short biological half-life ($t_{1/2}$) in the range of 0.9-2.6 hours. The absorption of metformin may be increased with a decreased intestinal motility. Due to repeated applications of high doses of metformin (in immediate-release formulations) for an effective treatment, the incidence of side effects (e.g. vomiting, anorexia, nausea, lose weight, diarrhoea and taste disturbance) increases, and hence patient compliance reduces[5,6]. The development of modified-release systems for metformin is carried out to improve its bioavailability, to reduce its dosing frequency and to decrease its gastrointestinal side effects[7].

The purpose of this study was to prepare and *in vitro* characterise (size, zeta potential, surface morphology, encapsulation efficiency and drug release from microspheres, FT-IR and DSC analysis) metformin HCl-loaded CS microspheres.

* Ataturk University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 25240, Erzurum, Turkey
** Ataturk University, Faculty of Engineering, Department of Chemical Engineering, 25240, Erzurum, Turkey
° Corresponding author: E-mail: melcetin@atauni.edu.tr; melcetin@hotmail.com

Materials and Methods

Materials

Chitosan (Protosan UP CL 113) was purchased from FMC Biopolymer-Novamatrix Co., Norway, metformin HCl was a generous gift from Sandoz Ilac Sanayi&Ticaret AS, Turkey, and glutaraldehyde solution (Grade II, 25% in H_2O) was obtained from Sigma-Aldrich Co. USA. All other chemicals and reagents used as they were received were of analytical grade.

Preparation of the metformin HCl-loaded CS microspheres

Microspheres were prepared by emulsion-crosslinking method. Briefly, chitosan aqueous solution (2%, w/v) containing metformin HCl (100 mg) and Tween 80 (2%, w/v) was added drop by drop into liquid paraffin containing a surfactant (Span 80; 2%, w/v), and stirred using an IKA® dual-speed mixer, RW20.n (Germany) for 30 min. Then, 2.5 mL of glutaraldehyde solution (Grade II, 25% in H_2O) was added dropwise by a syringe needle (26 G) and stirred for 1 h. After centrifugation (3000 rpm, 30 min., 15 °C), microspheres were washed several times with petroleum ether and re-suspended in pure water. Petroleum ether residue was evaporated in a rotary evaporator (45 °C, 20 min.) then, microspheres were lyophilized for 48 h.

Particle Size and Shape Evaluation

The shape of microspheres was characterized by SEM (Inspect S50, FEI, USA). Particle size of microspheres was determined using a particle size analyser (Mastersizer 2000; Malvern Instruments Ltd., UK).

Drug Content of Microspheres

Lyophilized microspheres (20 mg) suspended in 15 mL of PB pH 6.8 in amber vials were vortexed for 2 min., and then mixed at 750 rpm for a further 3 h in the dark for the complete extraction of metformin HCl. This dispersion was centrifuged at 5000 rpm for 20 min. at 15 °C. The drug content in supernatant of each sample was measured using a validated UV method at 232 nm. The experiment was performed in triplicate.

In vitro Release Studies

In vitro release studies were performed using dialysis sac method in the two different release medium (PB pH 6.8 and HCl pH 1.2). Microspheres (20 mg) were suspended in PB pH 6.8 or HCl pH 1.2 and sealed in a dialysis membrane (molecular weight cut off 14000 Da). The sealed dialysis membrane was placed in an amber vial containing 15 mL of medium, and maintained

at 37±0.5°C, 50 rpm. At pre-determined time intervals, samples (3 mL) were withdrawn from the release medium and replaced with fresh release medium. Prior to analysis, all samples were centrifuged at 12500 rpm for 10 min, and their drug content was measured by means of a validated UV method at 232 nm (for PB pH 6.8) and 209 nm (for HCl pH 1.2). The experiment was performed in triplicate.

FT-IR and DSC analysis

A Perkin-Elmer Spectrum One model FT-IR was used to record the IR spectra of CS, metformin HCl, blank and metformin HCl-loaded microspheres prepared in KBr disks in the region of 4000–400 cm^{-1}.

DSC curves were recorded using a Netzsch STA 409 PC Luxx® model DSC. The instrument was calibrated using several standards (Bi, In, Ni, Zn, Al, Ag, Sn, Au) and alumina pan was used as reference. The DCS runs were conducted under 60 mL/min of nitrogen flow and in the temperature range of 25-400°C

Statistical Analysis

Statistical evaluations were performed using Mann-Whitney U test (SPSS Statistics 20.0 program; SPSS Inc., IL, USA) ($p<0.05$ presents the statistical significance; mean±SD).

Results and Discussion

Polymers were used widely in the pharmaceutical field as flow controlling agents in liquid dosage forms, film coatings, binders in tablets and preparation of modified release systems. Especially, natural polymers (e.g. alginate, chitosan) are widely preferred because of being economical, easily available, biodegradable, biocompatible, non-toxic, and capable of modification chemically[8]. Chitosan has mucoadhesive and permeability-enhancing properties due to its positive charge/cationic nature. Therefore, it is an important polymer to prepare oral drug delivery systems because of extending the residence time of delivery systems and increasing drug–membrane interactions at absorption sites and improving oral bioavailability[9]. In this study, CS microspheres were prepared using emulsification-crosslinking method. SEM image shows that the microspheres have spherical shape (Figure 1). The particle sizes of blank and metformin HCl-loaded microspheres were 9.06±1.32 and 9.21±2.16 μm ($p>0.05$), respectively (Table 1).

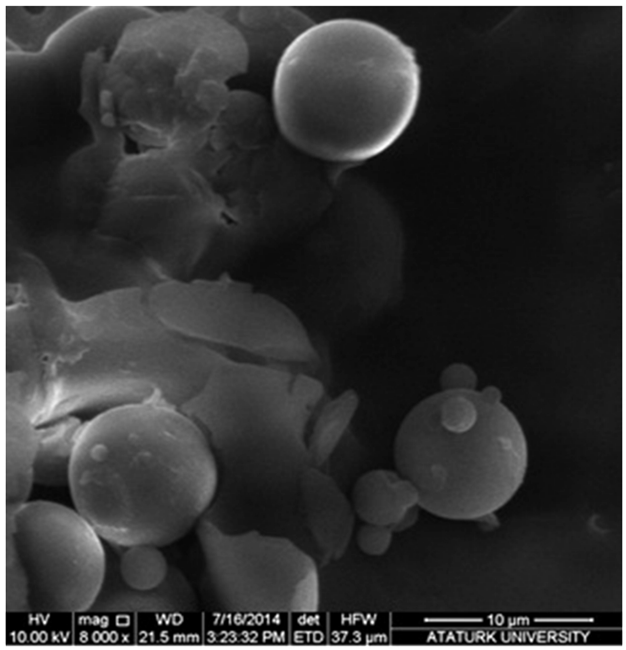

Figure 1
SEM image of metformin HCl loaded-CS microspheres

TABLE 1
The particle sizes, encapsulation efficiency and loading capacity of microspheres (mean±SD; n=3)

Formulation	Mean particle size (µm)	Encapsulation efficiency %	Drug loading %
Blank-CS MS	9.06±1.32	-	-
Metformin HCl-CS MS	9.21±2.16	20.05±6.92	1.44±0.47

The encapsulation efficiency and drug loading values for Metformin HCl-loaded microspheres are given in Table 1. Various factors (e.g. molecular weight and polymer type and the viscosity of phases used, drug-polymer ratio, drug solubility) influence drug loading into the microspheres[10-13]. In this study, the low drug loading value obtained is probably due to the high aqueous solubility of metformin HCl, and drug leakage to the aqueous medium during the preparation of microspheres.

Several studies are available in the literature in regard to metformin HCl-loaded CS microspheres. Sutar et al.[14] prepared metformin HCl-loaded CS microspheres using emulsification-crosslinking method, and also with

different concentrations of CS for sustaining the release of metformin HCl. The particle sizes of microspheres were in the range of about 534-700 μm. The particle size of microspheres increased with an increase in the polymer concentration. They reported that "drug loading and drug entrapment efficiency were found to be in an acceptable range" but did not give any numerical information. The *in vitro* drug release from microspheres was in the range of approximately 65-79% in 12 hours, and the best result was obtained for the formulation with a drug:polymer ratio of 1:4.

Wang et al.[15] prepared metformin HCl-loaded microspheres with a crude polysaccharide extracted from the Mactra veneriformis (MVPS) and also composite microspheres with MVPS and additive polymers (PVA-124, PEG-6000, and chitosan) by spray drying method. The production yield of microsphere formulations was in the range of ~46–70%. CS-MVPS microspheres have a smooth and spherical shape. The particle size of CS-MVPS microspheres was 4.2 μm. The encapsulation efficiency of the CS-MVPS microspheres was 89%. About 65% and 90% of metformin HCl were released from CS-MVPS microspheres within 25 min. and 90 min., respectively. In another study, Madsen et al.[16] developed metformin HCl-loaded bio-adhesive CS microparticles using spray drying method and investigated the bio-adhesion of CS microparticles to porcine buccal mucosa. The production yield and mean particle size of CS microparticles were 88% and about 6 μm, respectively. They reported that the retention of metformin depended on the characteristics of the irrigation medium (e.g. human saliva, and artificial irrigation medium such as phosphate buffer). Irrigation medium with high viscosity supplies a higher amount of water and thus increases the water uptake of microparticles, and consequently enhances the formation of electrostatic and hydrophobic interactions.

The in vitro release of microspheres was investigated in PB (pH 6.8) and also in HCl (pH 1.2). The cumulative drug release curves were given in Figure 2. Metformin HCl release from CS microspheres were about 36% and 98% in HCl pH 1.2 within 1 hour and 8 days, respectively. Furthermore, in PB pH 6.8 medium, ~22% and 89% of the loaded metformin HCl was released within 1 hour and 8 days, respectively. Slightly different drug release curves obtained for both release medium ($p=0.05$ for all time points). There are various critical factors that affect the drug release from CS microspheres such as swelling degree, pH of release medium, porosity, concentration of crosslinking agent and solubility of the drug. Drug release from CS microspheres is affected by changes in pH. The swelling degree and the drug release rate are higher in a medium with pH<6 than that of the release medium with pH>6 due to the protonation of CS free amine groups and thus the repulsion of the

polymeric chains, increasing in the degree of swelling and relaxation of the matrix structure in an acidic release medium[17].

Nayak et al.[18] evaluated the in vitro drug release from CS microspheres prepared using glutaraldehyde as the cross-linking agent (35% w/w) and 1:6 of drug:polymer ratio in PB pH 7.4, and reported that 70% of metformin HCl released from microspheres within 12 hours.

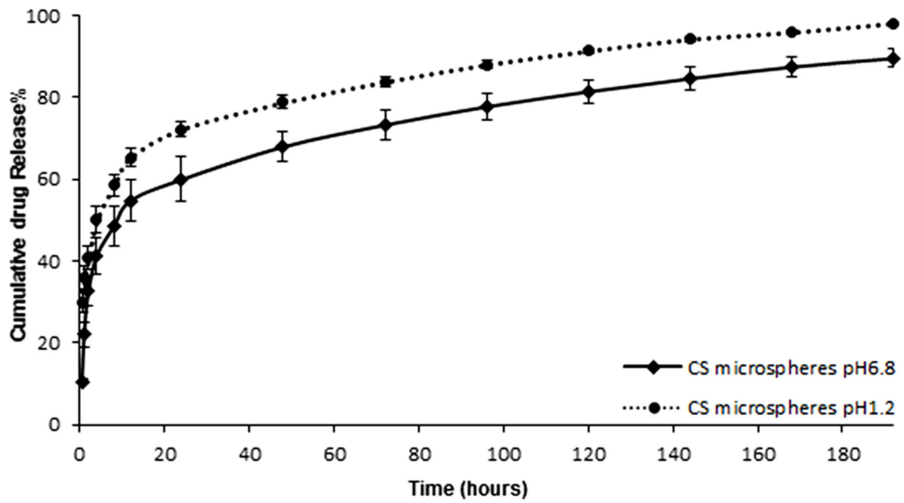

Figure 2
The release profiles of metformin HCl from the microspheres in HCl pH 1.2 and PB pH 6.8 (mean±SD, n=3).

Figure 3 shows DSC thermograms obtained for CS, metformin HCl and microsphere formulations. DSC thermogram of CS displayed an endothermic peak at around 80-100°C (Tpeak: 75.5°C) and an exothermic peak at around 280-300°C due to the evaporation of water, which forms the hydrogen bond with hydroxyl groups of CS and the degradation of CS, respectively[19-22]. The DSC curve of metformin HCl showed an endothermic characteristic peak at 236 °C, which is close to its melting point[5, 23-25] (Figure 3). The DSC scans of blank and metformin HCl microsphere formulations were shown in Figure 3. The endothermic peak of metformin HCl was not seen in the thermogram of metformin HCl-loaded microspheres due to the molecular dispersion of metformin HCl in the microspheres[25].

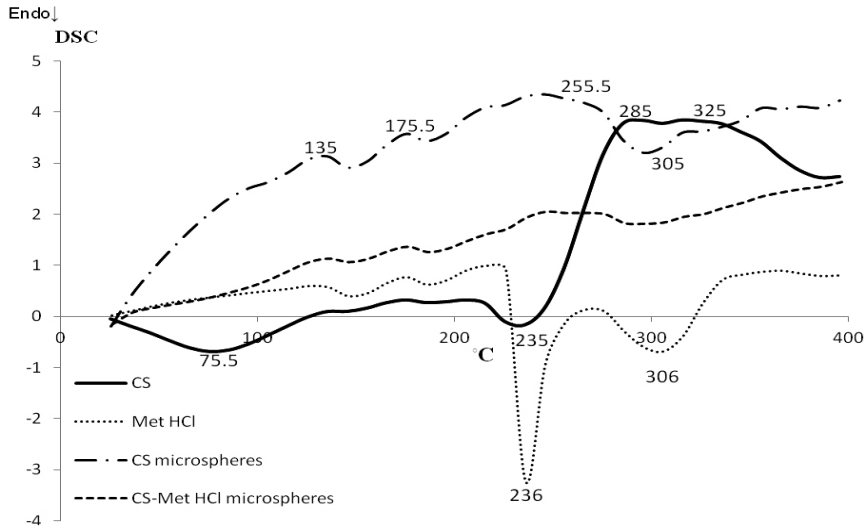

Figure 3
DSC thermograms of CS, pure drug, blank and metformin HCl-loaded microspheres.

The FT-IR spectra of metformin HCl, CS, blank and drug-loaded microspheres were obtained to determine the interactions between drug and polymer, and also the presence of drug in the microspheres (Figures 4-7).

The FT-IR spectrum of metformin HCl displayed peaks at 3367.79 cm^{-1} related to N-H asymmetric stretching, at 3293.10 cm^{-1} and 3149.27 cm^{-1} due to N-H symmetric stretching, 1621.90 cm^{-1} corresponded to C=N stretching, at 1557.70 cm^{-1} associated with N-H bending in plane, at 1472.57 cm^{-1}, 1447.01 cm^{-1} and 1417.93 cm^{-1} due to C-H asymmetric bending (-CH3), at 1166.68 cm^{-1} and 1061.30 cm^{-1} assigned to C-N stretching, at 936.41 cm^{-1} and 735.82 cm^{-1} corresponded to N-H wagging, at 632.29 cm^{-1} assigned to N-H rocking and also at 550.02, 530.34 cm^{-1} associated with C-N-C bending[26,27] (Figure 4).

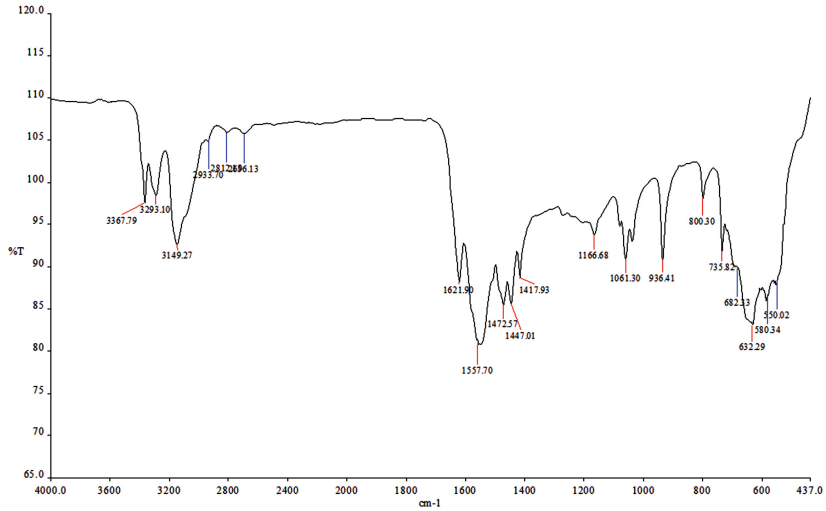

Figure 4
FT-IR spectrum of metformin HCl

The spectrum of CS presents peaks at 3232.49 cm^{-1} (NH_2 and O-H stretching), 2879.55 cm^{-1} (C-H stretching, CH_2), 1616.78 cm^{-1} (C=O stretching), 1146.85 cm^{-1} (bridge-O stretching), 1032.16 cm^{-1} and 1060.13 cm^{-1} (C-O stretching of amide group)[28-30] (Figure 5)

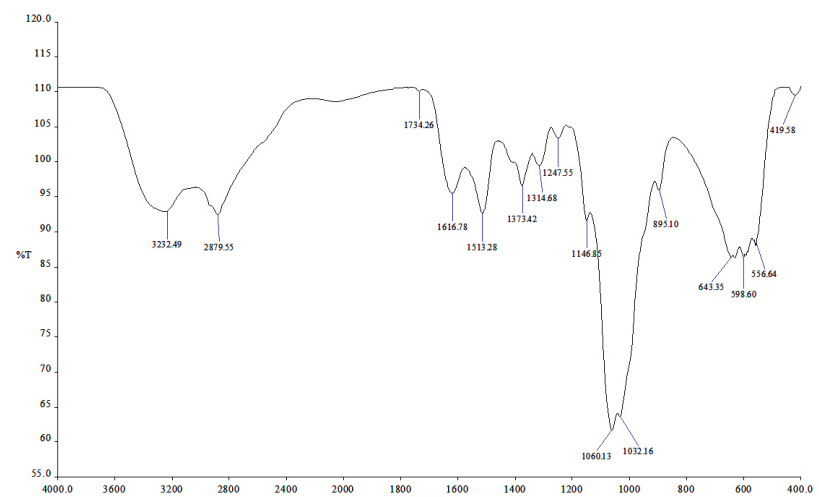

Figure 5
FT-IR spectrum of CS

The intensity of the peaks (at 2925.66 cm^{-1}, 1407.33 cm^{-1} and 1065.96 cm^{-1}) in the spectrum of metformin HCl-loaded microspheres increased with the presence of metformin HCl compared to the spectrum of blank microspheres (Figures 6 and 7), and this situation confirms the presence of metformin HCl in microspheres.

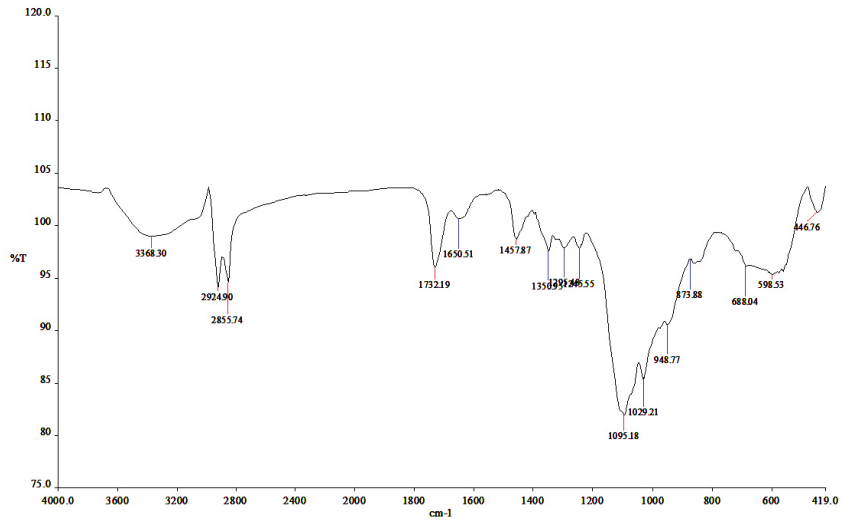

Figure 6
FT-IR spectrum of blank CS microspheres

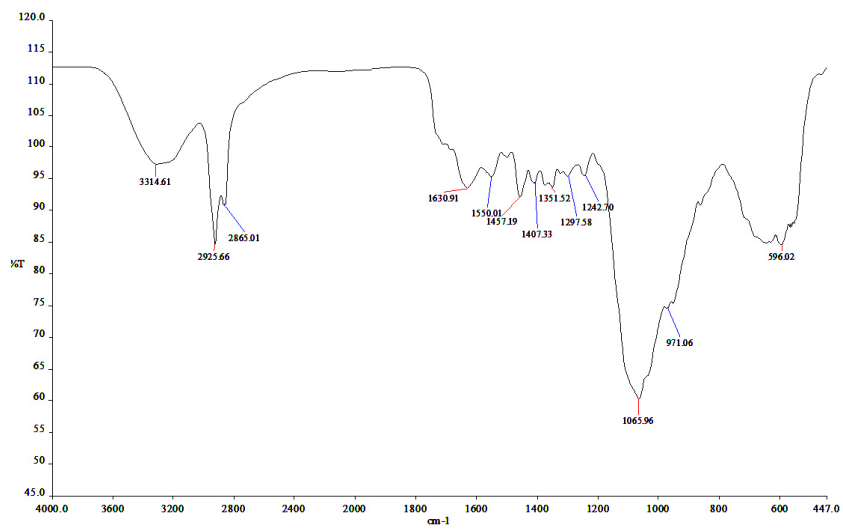

Figure 7
FT-IR spectrum of metfomin HCl-CS microspheres

Conclusion

The results of our study showed that non-aggregated and spherical metformin HCl-loaded CS microspheres with a mean diameter of 9.21±2.16 μm were prepared by an emulsification-crosslinking method. Positively-charged chitosan might be used to extend the release of metformin HCl from microspheres and increase the bioavailability of drug.

Acknowledgements

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Abstract

This study focuses on the preparation and *in vitro* characterisation of metformin HCl-loaded chitosan (CS) microspheres (MS). Scanning Electron Microscopy (SEM) image shows that the microspheres have spherical shape. The particle size and encapsulation efficiency (%) value of the metformin HCl-loaded microspheres were found as 9.21±2.16 μm and 20.05±6.92%, respectively. The obtained low drug loading value is due to the high aqueous solubility of metformin HCl, and drug leakage from microspheres to the aqueous medium. In HCl pH 1.2 and phosphate buffer(PB) pH 6.8 mediums, about 22-36% of metformin HCL was released within 1 hour and about 89-98% of the loaded metformin HCl was released in 8 days. Furthermore, microspheres were characterised using FT-IR and DSC. These data demonstrate a potential for sustained release of metformin HCl by using prepared CS microspheres.

Keywords: Chitosan, DSC, FT-IR, Metformin HCl, Microsphere

Özet

Metformin HCl-yüklü Kitosan Mikroküre lerinin Hazırlanması ve *In vitro* Karakterizasyon Çalışmaları

Bu çalışma, metformin HCl-yüklü kitosan (CS) mikroküre lerinin (MS) hazırlanması ve *in vitro* karakterizasyonu üzerine odaklanmıştır. Taramalı Elektron Mikroskobu (SEM) görüntüsü küresel mikroküre lerin hazırlandığını göstermiştir. Metformin HCl-yüklü mikroküre lerin partikül büyüklüğü ve enkapsülasyon etkinliği (%) değerleri sırasıyla 9.21±2.16 μm ve %20.05±6.92

olarak bulunmuştur. Metformin HCl'nin sudaki yüksek çözünürlüğü ve etkin maddenin sulu ortama kaçışı düşük yükleme etkinliği değeri elde edilmesine neden olmuştur. HCl pH 1.2 ve fosfat tamponu (PB) pH 6.8 ortamlarında yaklaşık %22-36 metformin HCl salımı 1 saat içerisinde ve yaklaşık %89-98 metformin HCl salımı ise 8 gün içerisinde gerçekleşmiştir. Ayrıca, FT-IR ve DSC mikrokürelerin karakterizasyonu için kullanılmıştır. Bu veriler, metformin HCl'nin sürekli salımı için CS mikrokürelerinin potansiyelini göstermektedir.

Anahtar Kelimeler: DSC, FT-IR, Kitosan, Metformin HCl, Mikroküre

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