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Preparation and *In vitro* Evaluation of Cefadroxil Loaded Chitosan Microspheres

Sefadroksil İçeren Kitozan Mikroküre lerinin Hazırlanması ve *İn vitro* Değerlendirilmesi

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Abstract

The aim of this study was to prepare cefadroxil (CFD) loaded chitosan microspheres and to investigate the effect of different formulation parameters such as drug-polymer ratio, chitosan concentration, amount of glutaraldehyde and stirring rate on microsphere formation. CFD-loaded chitosan microspheres were prepared by simple coacervation method using glutaraldehyde as cross-linking agent. The particle size, morphological characteristics, thermal behaviour, encapsulation efficiency and *in vitro* release assessments of the formulations had been carried out. Chitosan microspheres between the size ranges of 4.7–9.8 μm were obtained. The stirring rate was found not effective to particle size of microspheres significantly. Production yield of all formulations was found to be higher than 90% and encapsulation efficiencies of 42–59% were obtained. The decrease of drug:polymer ratio caused an increase in encapsulation efficiency. DSC thermogram showed that the SF was in the crystalline state in the microspheres. *In vitro* drug release studies indicated that CFD-loaded microspheres showed sustained effect up to 6 weeks. Drug release rates were decreased upon increasing the chitosan concentration and the amount of glutaraldehyde. Drug release was evaluated kinetically and the data was fitted Higuchi kinetic model.

Keywords: Cefadroxil, microsphere, chitosan, sustained release, simple coacervation technique

Introduction

Controlled drug delivery systems offer some advantages compared to conventional dosage forms, which include reduced adverse reaction, toxicity and frequency of dosing, improved efficacy, patient compliance and convenience (Uchida and Goto, 1988; Ravi Kumar, 2000). Using novel microencapsulation techniques, varying the polymer ratio and molecular weight, microspherescan be developed as an optimal drug delivery system which will provide the desired release profile. The use of microsphere-based therapy allows drug release to be carefully observed to the specific treatment site through the choice and formulation of various

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drug-polymer combinations. The total dose of medication and the kinetics of release are the variables, which can be manipulated to achieve the desired results. Microsphere-based systems may increase the life span of active constituents and control the release of bioactive agents (Sinha *et al.*, 2004). In the cases where the therapeutic plasma level of a drug can only be maintained by a frequent daily dosing, sustained release would allow to reduce the number of daily applications. Consequently the compliance should be improved by sustained release systems for such drugs. The delivery system designed within this study could be considered as a guarantee for such a sustained release. Sustained-release preparations have frequently been prepared by dispersing the drug in the polymer matrices where the polymer is to act as a rate-controlling barrier (Kim *et al.*, 2000).

Chitosan, a natural linear biopolyaminosaccharide, is obtained by alkaline deacetylation of chitin (Muzarelli, 1977; Roberts, 1992). Properties such as biodegradability, low toxicity and good biocompatibility make the polymer suitable for use in biomedical and pharmaceutical formulations (Chandy and Sharma, 1990; Illum *et al.*, 2001).

Coacervation technique is one of the methods used for microsphere formation. In this technique, the polymer is solubilized to form a solution. This is followed by addition of a solute, which forms insoluble polymer derivative and precipitates the polymer. This process avoids the use of toxic organic solvents (Berthold *et al.*, 1996).

CFD, an antibiotic used in the treatment of bacterial infections, has a short biological half-life (1.5h) (Kim *et al.*, 2000; Kulkarni *et al.*, 2001). It is a semi-synthetic cephalosporin intended for oral administration. The antimicrobial spectrum of CFD includes important gram-positive and gram-negative pathogens usually associated with infections of the urinary and respiratory tracts (Buck and Price, 1977; Hartstein *et al.*, 1977; Tenrisaver and Santella, 1986). Due to its rapid elimination from the body, frequent dosing is essential. A sustained-release preparation of cefadroxil, therefore, thought to be advantageous (Uchida *et al.*, 1992).

In this study, cefadroxil (CFD) loaded chitosan microspheres were prepared by simple coacervation method and the effect of different formulation parameters such as drug-polymer ratio, chitosan concentrations, amount of glutaraldehyde and stirring rate on microsphere formation was investigated.

Experimental

Materials

CFD was kindly provided from Bristol Myers Squibb Inc., Turkey, chitosan (medium molecular weight, deacetylation degree >75%) purchased from Fluka, Germany. All other reagents and chemicals were of analytical grade.

Preparation of CFD-loaded chitosan microspheres

CFD-loaded chitosan microspheres were prepared according to simple coacervation technique (Berthold *et al.*, 1996). Sodium sulphate solution (20% m/v) containing CFD was dropped into chitosan solution in 1% v/v acetic acid and stirred (Ika Labortechnik, Germany) for 30 min. Then glutaraldehyde was added. After 1 h, the formed microspheres were separated by centrifugation (Heraeus, Germany) for 15 min at 6000 rpm, washed once with distilled water

and then freeze-dried (Lyovac GT 2, Leybold-Heraeus, Germany). The yield of microspheres thus formed was calculated (Eq. 1):

$$Y(\%) = \frac{\text{Practical mass (microspheres)}}{\text{Theoretical mass (polymer + drug)}} \times 100 \quad \text{(Eq. 1)}$$

Different formulation parameters (Table 1) were examined to study the effect of various factors on microsphere formation and properties as well. Each formulation was carried out in triplicate. Blank microspheres, omitting CFD, were prepared for comparison.

Table 1. Formulation of CFD-loaded chitosan microspheres

Formulation	Drug: Polymer ratio	Chitosan concentration % (m/v)	Glutaraldehyde amount (mL)	Stirring rate (rpm)
F1	1:1	0.50	1	500
F2	0.5:1	0.50	1	500
F3	1:1	0.25	1	500
F4	0.5:1	0.25	1	500
F5	1:1	0.50	2	500
F6	0.5:1	0.50	2	500
F7	1:1	0.25	2	500
F8	0.5:1	0.25	2	500
F9	1:1	0.50	1	1000
F10	0.5:1	0.50	1	1000
F11	1:1	0.25	1	1000
F12	0.5:1	0.25	1	1000
F13	1:1	0.50	2	1000
F14	0.5:1	0.50	2	1000
F15	1:1	0.25	2	1000
F16	0.5:1	0.25	2	1000

Scanning electron microscopy

For morphology and surface characteristics, prepared microspheres were coated with gold in an argon atmosphere. The surface morphology of the microspheres was then studied by scanning electron microscopy (SEM) (Jeol JSM-5600, Japan).

Fourier transform infrared analysis (FT-IR)

FT-IR spectrums of the CFD and CFD-loaded microspheres were measured in potassium bromide disks using a Perkin Elmer 1600 FT-IR spectrophotometer (USA).

Particle size analysis

Particle size of microspheres was determined using Sympatec Master Sizer (Germany). The d_{50} value was expressed as a mean particle size. The results were the average of three analyses.

Differential scanning calorimetric (DSC) analysis

Thermal analysis using DSC method was carried out on CFD and CFD-loaded chitosan microspheres. Samples were accurately weighed (~5mg) into aluminium pans and sealed. All of the samples were heated at 10°C.min^{-1} over a temperature 20-400°C under nitrogen.

Determination of drug content and encapsulation efficiency

The amount of CFD entrapped was determined by calculating the difference between the theoretical amount of drug in microspheres and the amount of non-entrapped drug. The drug content was determined spectrophotometrically (Schimadzu 1601, Japan) at 230 nm (n=3). Actual drug content (AC) and encapsulation efficiency (EE) were calculated (n=3) (Eqs. 2 and 3).

$$AC\,(\%) = \frac{M_{act}}{M_{ms}} \times 100 \qquad \text{(Eq. 2)}$$

$$EE\,(\%) = \frac{M_{act}}{M_{the}} \times 100 \qquad \text{(Eq. 3)}$$

where, M_{act} is the actual CFD content in weighed quantity of microspheres, M_{ms} is the weighed quantity of microspheres and M_{the} is the theoretical amount of CFD in microspheres calculated from the quantity added in the process.

In vitro release studies

In vitro release profiles of CFD from chitosan microspheres were examined in pH 7.4 phosphate buffer. 30 mg microspheres were suspended in falcon tubes containing 10 mL dissolution medium. The tubes were shaken (37±0.5°C, 175 rpm) with Forma orbital shaker (Thermo Electron Corporation, USA). At scheduled time intervals, samples were taken and supernatants separated by centrifugation. The released CFD was measured spectrophotometrically at 230 nm. After each sampling, the microspheres were resuspended in the fresh medium. *In vitro* release studies for all formulations were done in triplicate.

Kinetic Assesment

Drug release from the chitosan based CFD microsphere formulations (F1-F16) were kinetically evaluated to fit to zero order, first order and Higuchi kinetic models.

Results and Discussion

Characterisation of CFD microspheres

CFD was succesfuly encapsulated into chitosan microspheres. CFD-loaded chitosan microspheres were spherical in shape and smooth in surface (Figure 1).

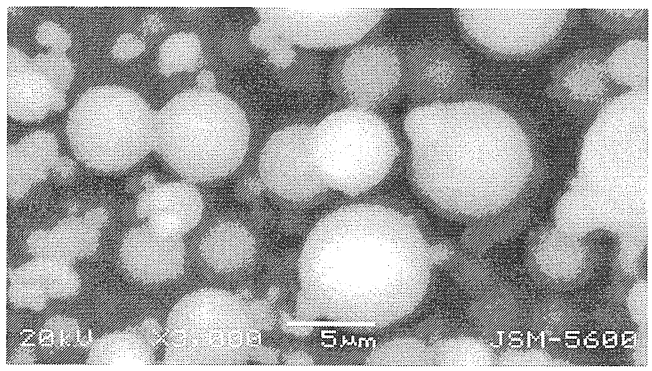

Figure 1. SEM photographs of CFD-loaded chitosan microspheres

The size of CFD encapsulated chitosan microspheres ranged between 4.7-9.8 μm is given in Table 2.

After encapsulation process the characteristic peaks of CFD were not altered in FT-IR spectra indicating no chemical interaction among drug, polymer and glutaraldehyde.

The physical state of the CFD loaded microspheres was assessed by thermal analysis. DSC provides information about physical properties of the sample, as crystalline or amorphous nature and demonstrates a possible interaction between different compounds of a mixture. According to the thermograms (Figure 2), CFD presented an endothermic peak centered at 207°C due to decomposition by melting. The results were in accordance with the literature (Kulkarni *et al.*, 2001). In the DSC curves of drug-loaded microspheres (F3, F9, F11),

characteristic peak of CFD was existed. The thermograms of CFD-loaded microsphere formulations showed that drug was in its crystalline form and no interaction between CFD and chitosan (Figure 2).

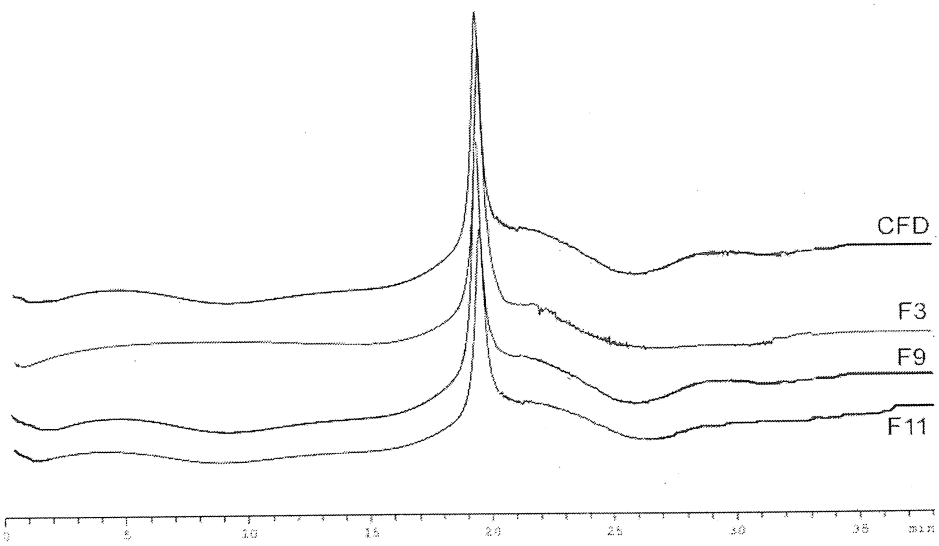

Figure 2. Differencial scanning calorimetry thermograms of CFD and CFD-loaded microspheres

Encapsulation efficiency and actual drug content

The production yield is relatively high percentage about 90-95% for all formulations and microsphere preparation parameters did not affect the yield. A low concentration of chitosan showed low encapsulation efficiency. Similar results were reported by Orienti *et al.* (1996). Encapsulation efficiency was found as 42-59% (Table 2). Decreasing of chitosan concentration led to decrease in encapsulation efficiency. Increasing of glutaraldehyde amount and decreasing of drug:polymer ratio resulted with increase in encapsulation efficiency. Actual drug contents of formulations were between 15-28%. Using the high drug:polymer ratio to prepare particles increased the actual drug contents of microspheres. These results are in accordance with literature (Orienti *et al.*, 1996).

Table 2. Mean particle size, encapsulation efficiency, actual drug content and yield of CFD- loaded microsphere formulations

Formulation	Mean particle size (μm) ± SD	Encapsulation efficiency (%) ± SD	Actual drug content (%) ± SD	Yield (%) ± SD
F1	.46 ± 0.23	48.94 ± 1.26	24.47 ± 1.45	92.27 ± 3.66
F2	.04 ± 0.12	50.85 ± 0.95	16.93 ± 1.18	94.14 ± 1.25
F3	.16 ± 0.13	42.67 ± 1.58	21.34 ± 2.77	94.46 ± 1.14
F4	.84 ± 0.09	45.75 ± 2.21	15.24 ± 2.86	93.15 ± 1.65
F5	.36 ± 0.05	55.14 ± 0.65	27.54 ± 0.89	91.72 ± 2.64
F6	.44 ± 0.15	59.50 ± 1.65	19.81 ± 2.27	90.19 ± 1.61
F7	.71 ± 0.21	51.63 ± 1.28	25.82 ± 1.56	91.55 ± 1.75
F8	.50 ± 0.09	54.38 ± 2.36	18.11 ± 2.39	93.64 ± 2.27
F9	.27 ± 0.11	46.74 ± 1.46	23.37 ± 2.14	92.15 ± 3.38
F10	.87 ± 0.15	49.90 ± 1.72	16.62 ± 2.49	90.94 ± 1.45
F11	.13 ± 0.06	44.12 ± 2.15	22.06 ± 1.75	91.75 ± 2.33
F12	.00 ± 0.06	45.34 ± 2.53	15.10 ± 0.76	92.58 ± 1.72
F13	.13 ± 0.08	56.62 ± 1.25	28.31 ± 2.62	94.85 ± 2.58
F14	.88 ± 0.20	58.46 ± 1.41	19.47 ± 1.52	92.44 ± 2.46
F15	.04 ± 0.23	50.81 ± 0.75	25.41 ± 1.34	93.39 ± 3.16
F16	.04 ± 0.16	54.81 ± 2.06	18.25 ± 1.93	94.55 ± 2.53

In vitro drug release

Figures 3-6 display the release profile of CFD from chitosan microspheres. The highest dissolution rate was obtained with F11 while the slowest was F6.

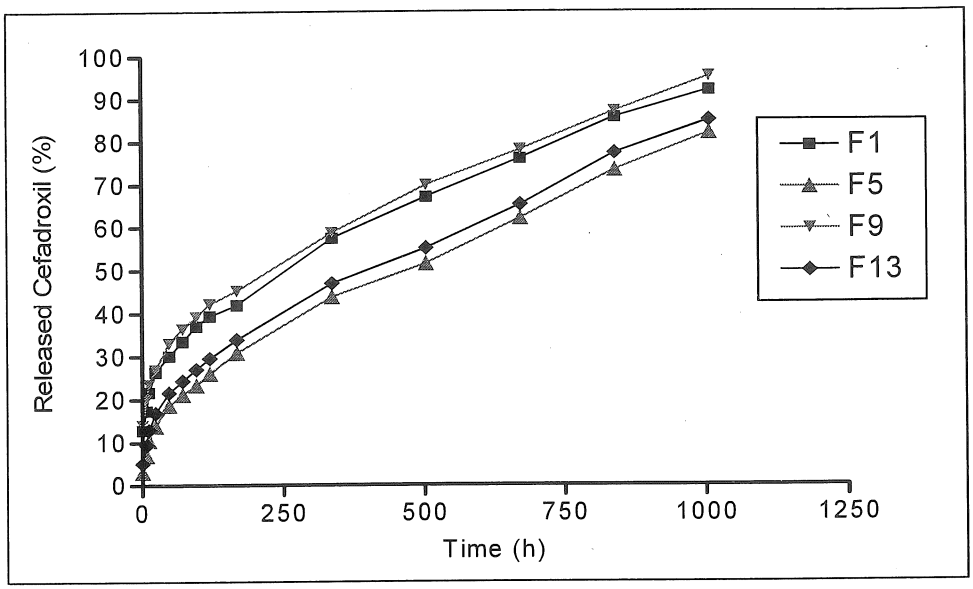

Figure 3. *In vitro* drug release profile of F1, F5, F9 and F13 formulations

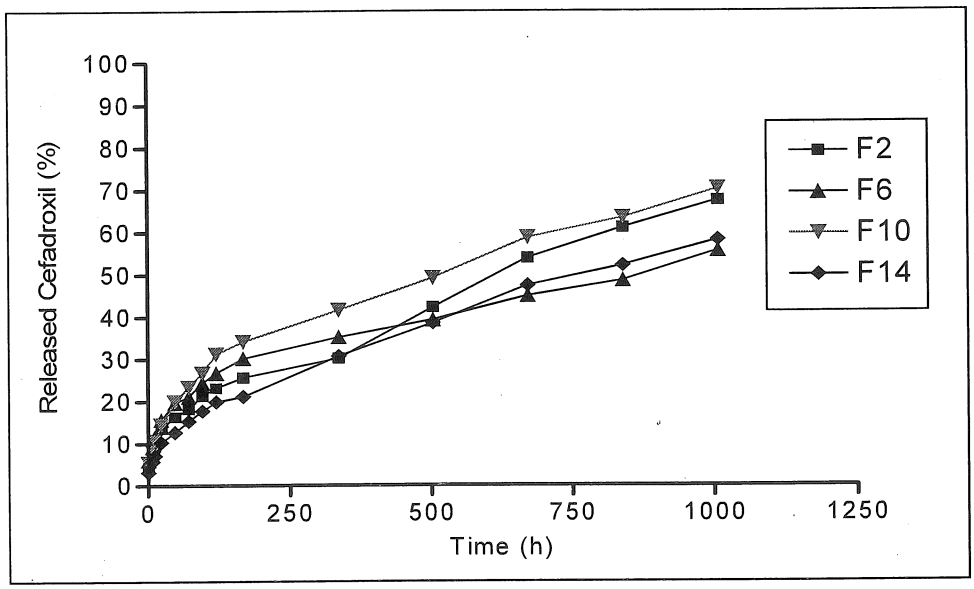

Figure 4. *In vitro* drug release profile of F2, F6, F10 and F14 formulations

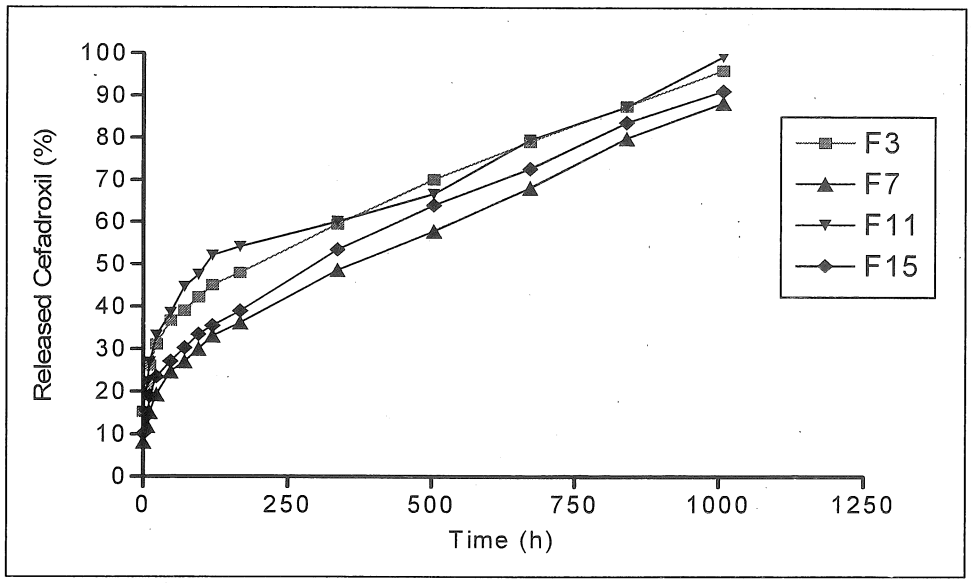

Figure 5. *In vitro* drug release profile of F3, F7, F11 and F15 formulations

Early studies reported that the drug release from polymeric microspheres was affected by the particle size and drug:polymer ratio (Sanders *et al.*, 1986; Kim *et al.*, 1998; Sung *et al.*, 1998). In this study formulation factors showed no significant effect on microsphere size. This result is in accordance with literature (Özbaş-Turan *et al.*, 2003).

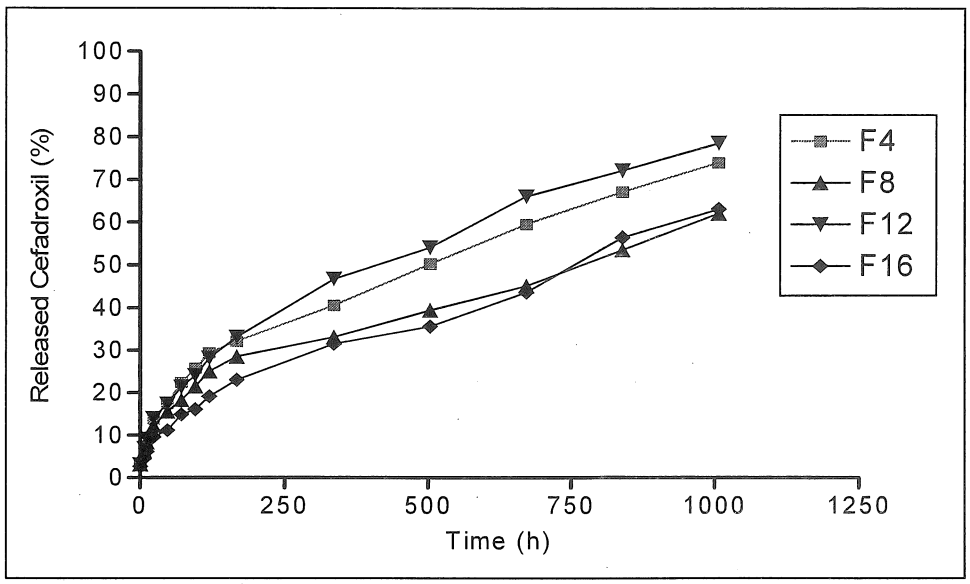

Figure 6. *In vitro* drug release profile of F4, F8, F12 and F16 formulations

In vitro drug release decreased with the increase in chitosan concentration and glutaraldehyde amount while increase in drug release resulted with the increase in drug:polymer ratio. Özbaş-Turan et.al. (2003) reported that *in vitro* drug release from microspheres was dependent on drug amount and chitosan concentration.

According to *in vitro* dissolution studies, CFD loaded microspheres showed sustained effect up to 6 weeks. Kinetically evaluation of CFD release from microspheres fitted to Higuchi model (Table 3) (Higuchi, 1963).

Table 3. Kinetic evaluation of drug release data of microsphere formulations

Formulation	Zero order	First order	Higuchi
	r^2	r^2	r^2
F1	0.9462	0.9811	0.9974
F2	0.9678	0.9863	0.9817
F3	0.9333	0.9401	0.9935
F4	0.9338	0.9847	0.9948
F5	0.9646	0.9834	0.9940
F6	0.8919	0.9420	0.9819
F7	0.9611	0.9722	0.9939
F8	0.9294	0.9655	0.9857
F9	0.9437	0.9562	0.9978
F10	0.9174	0.9755	0.9928
F11	0.9111	0.8701	0.9925
F12	0.9424	0.9928	0.9988
F13	0.9600	0.9809	0.9946
F14	0.9642	0.9901	0.9955
F15	0.9550	0.9755	0.9962
F16	0.9700	0.9802	0.9818

r^2 is the determination coefficient

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Özet

Bu çalışmada basit koaservasyon yöntemi kullanılarak Sefadroksil (CFD) içeren kitozan mikroküreleri hazırlandı. İlaç-polimer oranı, kitozan konsantrasyonu, glutaraldehit miktarı ve karıştırma hızı gibi çeşitli formülasyon parametrelerinin mikroküre oluşumuna etkisi araştırıldı. Mikrokürelerin partikül büyüklüğü, morfolojik özellikleri, termal özellikleri, enkapsülasyon etkinliği ve ayrıca mikrokürelerden *in vitro* ilaç salım hızı tayin edildi. Çalışmada 4.7-9.8 μm boyutunda mikroküreler elde edildi ve karıştırma hızının, mikrokürelerin partikül boyutunu anlamlı derecede etkilemediği görüldü. Tüm mikroküre formülasyonlarında verimin % 90 dan fazla olduğu saptandı. Enkapsülasyon etkinliği % 42-59 arasında bulundu ve ilaç-polimer oranının azalmasına bağlı olarak mikrokürelerin enkapsülasyon etkinliği artış gösterdi. DSC analizi sonucu, mikrokürelere yüklenen CFD in kristal formunda bulunduğu saptandı. *In vitro* ilaç salım çalışmaları sonucunda, mikrokürelerden ilaç salımının 6 haftaya kadar devam ettiği görüldü. Kitozan konsantrasyonunun ve glutaraldehit miktarının artışına bağlı olarak ilaç salımının yavaşladığı gözlendi. Kinetik değerlendirme sonucunda mikrokürelerden ilaç salımının Higuchi kinetic modeline uyduğu saptandı.

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