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Nasal administration of ciprofloxacin HCl loaded chitosan microspheres: *in vitro and in vivo* evaluation

Siprofloksazin HCl yüklü kitozan mikroküre lerinin nazal verilişi: *in vitro* ve *in vivo* değerlendirme

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Abstract

The aim of this study was to evaluate ciprofloxacin hydrochloride-loaded chitosan microspheres for nasal administration. Microspheres were prepared by spray drying method and evaluated with respect to the particle size, morphological properties, drug-polymer interaction, production yield, drug content, encapsulation efficiency, *in vitro* drug release and kinetic assessment and *in vivo* bioavailability. The particle size of microspheres prepared ranged from 3.3 to 6.7 μm. The microspheres showed spherical shape and smooth surface. For all formulations, drug loading capacity and microsphere yield were higher than 74% and 38%, respectively. Based on *in vitro* evaluation of microspheres, the most suitable formulation has chosen for *in vivo* nasal application to rats. *In vivo* studies showed that, absolute bioavailability of CIPRO formulations (oral solution, nasal solution and nasal microsphere suspension) were found as 8.57%, 15.7% and 32.9%, respectively. According to the obtained data, CIPRO-loaded chitosan microspheres prepared with spray-drying method are able to prove sustained release and could be use via nasal route as an alternative to oral route.

Key words: Chitosan, ciprofloxacin hydrochloride, microsphere, nasal drug delivery, spray drying method.

Introduction

Ciprofloxacin hydrochloride (CIPRO) is a wide-spectrum fluoroquinolone anti-bacterial agent (Sweetman 2002). With its broad spectrum against aerobic gram-negative and gram-positive bacteria as well as some anaerobic bacteria and ability to achieve therapeutic concentrations in most body fluids and tissue, CIPRO has used to treat bacterial diseases, widely. It is currently available in tablet, parenteral infusion and ophthalmic forms (Davis et al. 1996). There are numerous investigations on different formulation of CIPRO such as gels (Özsoy et al. 2000), microspheres (Martinez et al. 1997), microparticles (Owusu-Ababio and Rogers 1996, Orhan et al. 2006), nanoparticles (Page-Clisson et al. 1996, Fawaz et al. 1997), liposomes (Gürsoy and Senyücel 1997) and implants (Désévaux et al. 2002a, b; Ramchandani et al. 1998).

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In recent years, the nasal cavity has been widely investigated as a potential site for non-invasive systemic drug delivery. The nasal cavity would provide of distinct advantages in term of a large surface area for absorption with a subepithelial layer that highly vascularised and high permeable mucosa compared to gastrointestinal tract mucosa (Özsoy 2008). However, there are some problems such as normal defence mechanisms of nasal cavity that mucocillary clearance and ciliary beating. Due to these mechanisms drug contact time with nasal mucosa is reduced therefore absorption of drug decreases. To overcome these problems, recently studies focused on the developing bioadhesive formulations using bioadhesive polymer for effective nasal delivery (Soane et al. 1999, Vasir et al. 2003, Yıldız et al. 2005).

Due to bioadhesive polymers are capable of interacting with nasal mucosa, the mucociliary clearance rate and ciliary beating slow down so contact time of formulation with nasal mucosa is extended thus absorption of drug is improved. Chitosan has been extensively examined as an ideal candidate in the pharmaceutical application with being hydrophilic, biocompatible, bioadhesive and biodegradable polymer of low toxicity. Chitosan is a cationic polysaccharide, derived by the deacetylation of chitin. Chitosan is positively charged due to its amino groups and able to interact strongly with the negatively charged surface (Lehr et al. 1992). Nasal epithelium is covered by mucus layer and one of the components of mucus is glycoprotein which has negatively charged by sialic acid residues (Illum 1992).

So there is strong interact chitosan with the nasal epithelial cells. In addition, chitosan has been shown to increase the paracellular transport of polar drugs by transiently opening the tight junctions between the epithelial cells (Artursson et al. 1994).

Recently, with their small size and efficient carrier character, microspheres are used for drug delivery systems. Coupling of bioadhesive properties to microspheres, bioadhesive microspheres have efficient absorption and enhanced bioavailability of the drugs due to both a much more intimate contact and longer contact time with the mucus layer. When drugs are administered by nasal route with bioadhesive microspheres, mucociliary clearance of drugs decreases due to adhering to nasal mucosa (Vasir et al. 2003).

The spray drying technique is easy and reproducible to obtain microspheres. Essentially, research has revolved around the application of spray drying method to different polymer and drugs to prepare microparticular systems. Chitosan microspheres (Somavarapu et al. 1998, Ganza-Gonzales et al. 1999) had been prepared with spray drying technique for nasal route in this manner.

In this study, CIPRO-loaded microspheres were prepared using chitosan with spray-drying method. The surface morphology of microspheres, the influence of drug-polymer ratio and crosslinking agent concentration on the formation of microspheres, drug loading capacity, particle size and *in vitro* release studies were investigated. The formulation which fitting Higuchi's kinetic model was selected for *in vivo* study via nasal route. After nasal administration of selected formulation to rats, the concentration of CIPRO in collected plasma samples was determined using microbiological method. Pharmacokinetic parameters were calculated and compared with that of CIPRO formulations administrated different route (intravenous, nasal and oral).

Materials and Methods

Materials

Ciprofloxacin hydrochloride was obtained from Yeni Drug Company (Turkey). Chitosan (high molecular weight, deacetylation degree >75% from Aldrich (Germany). Glutaraldehyde (25 % v/v aqueous solution) from Merck (Germany); bovine serum albumin from Sigma (Germany); Iso-Sensitest Agar from Oxoid (United Kingdom).

Preparation of microspheres by spray-drying: 3^2 factorial design (Bolton 1990) was used to study the effect of two variables at three levels as shown in the Table 1.

Table 1. Application of 3^2 factorial design.

Independent variables	**Levels**		
Polymer :drug ratio	3:1	3:1.5	3:3
Concentration of glutaraldehyde % (v/v)	0.00	0.15	0.30

The independent variables in the 3^2 factorial designs were polymer: drug ratio and concentration of crosslinking agent. In this respect, the microspheres were prepared with polymer: drug ratios (3:1, 3:1.5 and 3:3) and amount of glutaraldehyde solution (%0.00, %0.15, %0.30). Chitosan concentration was selected 0.5% w/v, according to literature He et al. 1999. Briefly, different amount of CIPRO was dissolved in the chitosan solution (0.5 % w/v chitosan solution in (1% v/v) acetic acid). Glutaraldehyde was added at different percentages into the polymer and drug solution and applied ultrasonication to obtain homogeneous solution. The prepared solution was sprayed through the nozzle of a spray-dryer (Büchi, 190 Mini Spray Dryer, Switzerland). The process conditions were set as follows: Inlet temperature 130-132°C, outlet temperature 85-90°C, aspirator setting 100% capacity, pump setting 4 mL/min, spray flow rate 600 NL/h and nozzle diameter 0.7 mm. Microspheres were collected and weighted to determine production yield with following Equation 1:

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

Each formulation was carried out in triplicate. As shown in Table 2, drug-loaded microspheres (F1-F9), nine formulations, were obtain depend on the 3^2 factorial design. Three drug-free microspheres were prepared to comparison as B1, B2 and B3 code containing 0.00%, 0.15% and 0.30% of glutaraldehyde, respectively.

Infrared (IR) spectroscopy: The IR spectrums of CIPRO, and drug loaded microsphere formulations (F3 and F6) were obtained with KBr pellets using FT-IR spectrometer (Perkin Elmer, 1600 Model 615).

Differential scanning calorimetry (DSC): Thermal analysis using a DSC method were performed on CIPRO, Chitosan and crosslinked of the drug-free microspheres (B3), crosslinked drug loaded microspheres (F6), employing a Mettler Toledo DSC 822^e. Samples (5 mg) were accurately weighed into aluminium pans and sealed. All samples were run at a heating rate of 15°C/min over a temperature range 25-450°C in atmosphere of nitrogen.

Morphology and particle size studies: Particle size analyses were performed on chitosan microspheres by Malvern MetaSizer (Malvern Instruments, Malvern, UK). The results are the average of three analyses. The size of microspheres is expressed as d_{50} representing the size below, which 50 % by weight of sample. The values (d_{50}) were expressed for all formulations as mean size range. For morphology and surface characteristics, prepared microspheres were coated with gold under an argon atmosphere at room temperature and then the surface morphology of the microspheres were studies by scanning electron microscopy (SEM), JEOL 840A JXA (USA).

Actual drug content and encapsulation efficiency: Weighted samples of CIPRO-loaded microspheres (60 mg) were dissolved in 100 mL 0.1N HCl under ultrasonication for 4 h at 30°C. The samples were filtered using 0.2 μm membrane filter and absorbances of samples were analysed at 277 nm using

spectrophotometer. Actual drug content (AC) and encapsulation efficiency (EE) were calculated using Equations 2 and 3, respectively. All analyses were carried out in triplicates.

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

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

Where M_{act} is the actual SF content in weighed quantity of microspheres, M_{ms} is the weighed quantity of microspheres and M_{theo} is the theoretical amount of CIPRO in microspheres calculated from the quantity added in the process.

Solubility studies: The volume of the dissolution medium for in vitro studies was determined taking into account that "sink condition" plays role on dissolution studies. In this respect, the solubility studies of the CIPRO in phosphate buffer (pH 7.4) were investigated. For each medium 500 mg CIPRO was accurately weighed, added 15 mL of each solution and placed in shaker incubator (Memmert- Germany) thermostated at 37±1°C at 120±5 rpm for 24 h. Samples were withdrawn after 24 h, filtered from membrane filter and analysed using spectrophotometer (Shimadzu UV-visible Spectrophotometer UV-1601, Japan) at 271 nm. Solubility studies were performed in triplicate.

In vitro drug release: In vitro release profiles of CIPRO from prepared chitosan microspheres were examined in phosphate buffer (pH 7.40±0.02) preferred as physiological medium. 10 mL of dissolution medium were put into tube and 10 mg CIPRO-loaded microspheres were suspended in this solution. The tubes were placed in shaker incubator (Memmert- Germany) thermostated at 37±1°C at 120±5 rpm. At scheduled time intervals (30, 60, 120, 240, 360, 480 and 600 min), the tubes were taken and centrifuging at 5000 rpm for 5 minute; 200 µl samples were withdrawn and replaced with fresh medium. The tubes were stirred with vortex and placed in shaker incubator. The samples were diluted with same buffer solution and analyzed spectrophotometrically at 271 nm. *In vitro* release studies for all formulations were done in triplicate.

Kinetic assessment: Drug release mechanism was investigated in comparison with models according to the equations of zero order and first order (Martin 1993) and Higuchi's square root of time (Higuchi 1963). The following plots were made: Q_s vs. t (zero order kinetic model); log (Q_0 - Q_t) vs. t (first order kinetic model), and Q_t vs. square root of t (Higuchi's square of root kinetic model), where Q_t is the percentage of drug released at time t and Q_0 is the initial amount of drug.

The release constants (k), correlation coefficients (r) and sum the weighed square deviations (SWSD) were calculated by means of a computer programme for the kinetic assessment of the dissolution data (Ege et al. 2001). The percent of released drug were input. The programme fits these dissolution data to kinetic models and prints kinetic parameters, together with goodness of fit.

In vivo studies, Animal studies: All experiments were done according to 3R (reduction, replacement, refinement) rule. With the aim of the *in vivo* studies, Wistar albino rats were selected. It has been established that the rat is an excellent animal model to study nasal absorption of drugs (Hussain 1998).

Wistar Albino rats of both sexes with weight of 200-300 g were fasted during 18 hours and weighted before administration of the formulations. The *in vivo* studies were carried on total 20 rats. Four rats were randomly selected for each formulation. Intravenous (i.v.), nasal and oral solutions were applied within 24 hours after preparation whereas drug loaded and drug-free microsphere suspension were applied as soon as possible after preparation. Volume of the formulation administered to rats was adjusted according to the weight of animals. All administrations were applied to animals under light ether anesthesia.

Formulations as below described were administered to rats:

a) i.v. solution of drug was prepared by adding isotonicity agent (sodium chloride) calculated according to freezing point depressions method (Martin 1993) and applied by sterile filtration using 0.22 µm

membrane filter. The administered dose of i.v. solution of CIPRO was 20 mg/kg. The i.v. solution of drug was injected to tail vein of the rats.

b) Nasal solution of drug was obtained by dissolving CIPRO in serum physiologic and sterile filtration using 0.22 μm membrane filter. The administered dose of CIPRO in nasal solution was 20 mg/kg. The nasal solution of drug was administered into the nasal cavity of rats by applying one-half of the dosage to each nostril via micropipette.

c) Oral solution of drug was prepared in distilled water. The administered dose of oral solution of CIPRO was 50 mg/kg. The oral solution of drug was administered to rats via gavage.

d) Suspension of drug-loaded microspheres (F6), which was selected according to in vitro drug release kinetic, was prepared in phosphate buffer (pH 7.4). CIPRO concentration of microspheres applied by nasal route was 30 mg/kg. The suspension of drug-loaded microspheres was administered into the nasal cavity of rats by applying one-half of the dosage to each nostril via micropipette.

e) Suspension of drug-free microspheres (B2) was administered in order to investigate whether or not blank chitosan microspheres play a role improving microbiological effect of CIPRO, as chitosan have an antimicrobial activity (Giunchedi et al. 1998). Drug-free microspheres were suspended in phosphate buffer (pH 7.4) and administered nasally. The suspension of drug-free microspheres was administered into the nasal cavity of rats by applying one-half of the dosage to each nostril via micropipette.

Blood samples (600 μl) were collected, before administration and at 30, 45, 60, 90, 120, 180, 240, 360 and 480 min after administration from orbital sinus of rats into heparinised tubes. The plasma was separated by centrifugation at 3600 rpm for 10 min, collected and stored at -20°C until analysis. The amounts of CIPRO in the plasma samples were determined by microbiological method (agar plate diffusion technique).

Microbiological assay: The blood plasma samples taken at 0-8 hours were stored at -20°C until analysis. 50 μl of plasma samples were applied in triplicate to the reservoirs. Agar plate diffusion technique (USP 33, NF 28, 2009) was applied for the assay of CIPRO blood plasma concentration. *Escherichia coli* ATCC 8739 was used as a test organism. From the bacteria suspension, having an absorbance of 0.890 at 600 nm, 1 mL was dispersed in 100 mL of premelted Iso-Sensitest Agar, and then 20 mL of this medium were poured into petri dishes containing reservoirs of 6 mm diameter. Standard solutions of CIPRO in 0.25 - 13.00 μg/mL concentration range were prepared by dissolving CIPRO reference standard in 7% bovine serum albumin. After 50 μl of the standard solution was applied to the reservoirs, all assay plates were incubated at 37°C for 24 h. Inhibition zones were measured and corresponding concentrations were calculated. Eleven experiments were done in order to obtain for each concentration inhibition zone average. The standard curve of CIPRO was obtained by plotting concentration versus average inhibition zone on semi-log paper and standard linear equation of CIPRO was calculated.

Pharmacokinetic analysis: The maximum plasma concentration of CIPRO (C_{max}) and the time to reach this concentration (t_{max}) were determined from the individual plasma concentration-time profiles after nasal and oral administrations. Least-squares regression analysis was employed to obtain the elimination rate constant (k_{el}) and terminal elimination half-life ($t_{1/2}$) was calculated as $0.693/k_{el}$. $k_{1/2abs}$ was calculated using peeling method (Rowland and Tozer 1995) and $t_{1/2abs}$ was determined using $k_{1/2abs}$ value. The oral clearance (CL/F) was calculated from the formula Dose/ $AUC_{0-\infty}$.The dose corrected absolute bioavailabilities were calculated by comparing $AUC_{(0-\infty)\ test}$ values (nasal and oral) to $AUC_{(0-\infty)}$ (i.v.). The dose corrected relative bioavailabilities were calculated by comparing $AUC_{(0-\infty)}$ values (nasal) to $AUC_{(0-\infty)}$ (oral). Absolute and relative bioavailabilities calculated using the following equations (Eq. 4 and 5) (Ritschel 1986):

$$\text{Absolute bioavailability (\%)} = \frac{AUC_{(0-\infty)\ test}}{AUC_{(0-\infty)\ i.v.}} \times \frac{Dose_{\ i.v.}}{Dose_{\ test}} \times 100 \qquad \text{Eq. 4}$$

$$\text{Relative bioavailability (\%)} = \frac{AUC_{(0-\infty)\ nasal}}{AUC_{(0-\infty)\ oral}} \times \frac{Dose_{\ oral}}{Dose_{\ nasal}} \times 100 \qquad \text{Eq. 5}$$

The area under the plasma concentration-time curve ($AUC_{(0\text{-}8h)}$) was calculated using the TopFit 4.0 (Pharmacokinetic and Pharmacodynamic Data Analysis System for PC, G.Heinzel, R. Woloszczak, P. Thomann, Dr. Karl Thomae GmbH) and $AUC_{0-\infty}$ was calculated using the following equations (Eq. 6 and 7) (Ritschel 1986):

$$AUC_{(8h-\infty)} = C_{(8h)} / k_{el} \qquad \text{Eq. 6}$$

$$AUC_{(0-\infty)} = AUC_{(0\text{-}8h)} + AUC_{(8h-\infty)} \qquad \text{Eq. 7}$$

Statistical analysis: In vitro release data obtained from each experiment and the relationships to pharmacokinetic parameters were subjected to statistical analysis using a computer programme called GraphPad-Prism 4.0 software (USA), for a one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparisons test and the significance of the differences was evaluated by Student's t-test. $P<0.05$ was considered to be indicative of significance.

Results and Discussion

Characterisation of microspheres: Spray drying seems to promising for the preparation of chitosan microparticles with being easy, reproducible and rapid method. Therefore, CIPRO loaded microspheres were prepared using mentioned method. The yield of production is between 38-47% for all formulations (Table 2). This low yields can be attributed to both to small batch size as seen in the study of Giunchedi et al. (Giunchedi et al. 2002), and some of the liquid droplets attached inside the wall of main chamber.

When compared the IR spectrums of CIPRO alone, non-crosslinked (F3) and crosslinked drug loaded microspheres (F6), characteristic absorption intensity of CIPRO can obtained on microsphere formulations. So we deduced that all formulation include CIPRO in chemically stabile form.

The DSC thermograms of the CIPRO, polymer, and microsphere formulations are given at Figure 2, mutuality. The thermal behaviour of CIPRO is shown in the graphs presented in Figure 1. The drug presented two endothermic peaks. First peak is at 160°C, which shows water evaporation and the sharpness one at 329°C corresponds to the melting point. The decomposition process followed the melting of the drug. As presented at Fig.1, this sharpness peak was dramatically reduced in the DSC profiles of crosslinked CIPRO-loaded chitosan microsphere (F6). This shown that CIPRO was transformed into an amorphous state during the spray drying process.

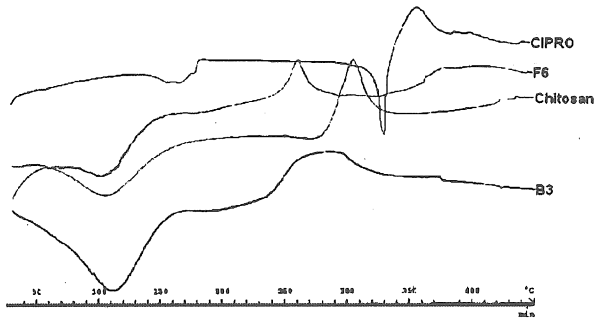

Figure 1. DSC thermograms of formulations: CIPRO, chitosan, crosslinked drug-free microspheres (B3) and crosslinked drug-loaded microspheres (F6)

Representative SEM photographs of the microspheres (F6) shown in Figure 2. SEM analyses showed that all microsphere formulations were spherical shape. The non-crosslinked drug-free microspheres have porous surface and rough character but non–crosslinked drug loaded and crosslinked drug loaded microspheres have a smooth surface.

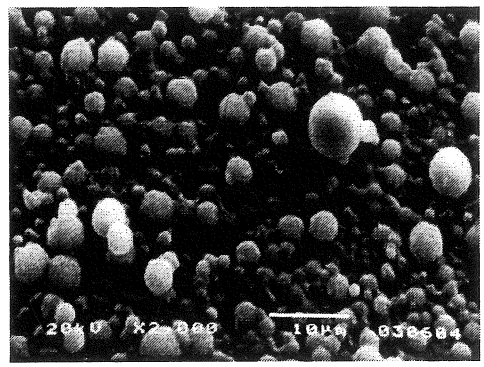

Figure 2. Representative SEM photographs of the crosslinked microspheres (F6)

The mean particle size (d_{50}) of all microsphere formulations (F1-F9) are ranged from 3.34 – 6.72 μm as reported in Table 2. As explain in the literature, if the particle size is <10 μm, then particles will be deposited in the upper respiratory tract, whereas if particle size is <0.5 μm then it will be exhaled. Particles or droplets with size between 5–7 μm will be retained in the nasal cavity and subsequently permeated (Donovan and Huang 1998). Regarding these considerations, a mean size of 3.3-6.7 μm seems to be appropriate for optimum deposition of the microparticles. Therefore, we concluded that the particle sizes of spray dried chitosan microspheres are suitable for nasal administration for systemic effect. Due to a using low crosslinking agent concentration, there are not remarkable differences in particle size between drug-loaded microspheres that are acted by crosslinking agent. Actual drug content and encapsulation efficiency of all formulation were given in Table 2. The encapsulation efficiency was above 74% in all formulations. As listed in Table 2, the encapsulation efficiency of non-crosslinked microspheres is between 94-97 % however microspheres that crosslinked with 0.15% and 0.30% crosslinking agent, are between 85–87% and 74–82%, respectively. This

finding indicated that encapsulation efficiency decreased with increase in concentration of crosslinking agent.

Table 2. Physicochemical properties of microsphere formulations.

Formulation code	Polymer: drug ratio (w/w)	Amount of crosslinking agent % (v/v)	Theoretical drug content % (w/w)	Actual drug content (%) (w/w) ± SD	Encapsulation efficiency % (w/w) ± SD	Yield (%) ± SD	*Mean particle size ± SD*
F1	3:1	0.00	25.0	23.7 ± 0.42	94.9 ± 1.66	39.2 ± 1.42	*4.16 ± 0.45*
F2	3:1.5	0.00	33.3	30.8 ± 0.37	92.7 ± 1.14	41.2 ± 1.98	*5.23 ± 0.37*
F3	3:3	0.00	50.0	48.8 ± 0.86	97.2 ± 1.79	44.6 ± 4.99	*4.83 ± 0.44*
F4	3:1	0.15	25.0	21.3 ± 0.43	85.3 ± 1.73	46.4 ± 5.25	*5.31 ± 0.89*
F5	3:1.5	0.15	33.3	28.6 ± 0.37	85.8 ± 1.12	43.2 ± 4.69	*6.58 ± 0.17*
F6	3:3	0.15	50.0	43.5 ± 0.73	87.0 ± 1.46	47.8 ± 3.90	*3.34 ± 0.03*
F7	3:1	0.30	25.0	18.6 ± 0.25	74.8 ± 1.16	41.9 ± 1.18	*6.37 ± 0.15*
F8	3:1.5	0.30	33.3	25.5 ± 0.19	76.5 ± 0.56	43.1 ± 3.66	*6.72 ± 0.19*
F9	*3:3*	*0.30*	*50.0*	*41.3 ± 0.52*	*82.7 ± 1.03*	*38.7 ± 2.96*	*5.46 ± 0.32*

Solubility studies: The solubility of CIPRO pH 7.4 phosphate buffer at 37±1°C was found being 5.46±0.20 mg/mL. According to these results, the volume of the dissolution medium for in vitro studies was adjusted "sink condition".

In vitro drug release: Figure 3-5 display the release profiles of CIPRO from chitosan microspheres. Crosslinked with 0.15% glutaraldehyde microspheres (F4-F6) showed slow release profile, however crosslinked with 0.30% glutaraldehyde microspheres (F7-F9) showed rapid release profile. Concentration of crosslinking agent also has an effect on the "burst effect"; the initial burst effect of formulations increased as crosslinking agent concentration increased. This could be attributed to some of drug accumulated on the microspheres surface due to crosslink process letting form tight matrix network. In addition, non-crosslinked microspheres could not suspended in the dissolution medium due to letting aggregate formation, so interacted of particle surface area with dissolution medium get smaller. The crosslinking process prevents aggregation in the dissolution medium, and therefore rate of drug release increase due to widening surface area that dissolution medium can interact with particle. When the drug content of microsphere increased, the amount of CIPRO released from the microspheres increased, however, the rate of released slowed down. This result may explain with swelling of chitosan microspheres in dissolution medium. When polymer: drug ratio was 3:1, there was more chitosan to swell, although it was 3:3, the amount of chitosan decreased. Due to swelling of microspheres, it is possible that drug can diffuse out matrix structure to dissolution medium. This finding indicated that when polymer: drug ratio decreased, the rate of dissolution-slowed down.

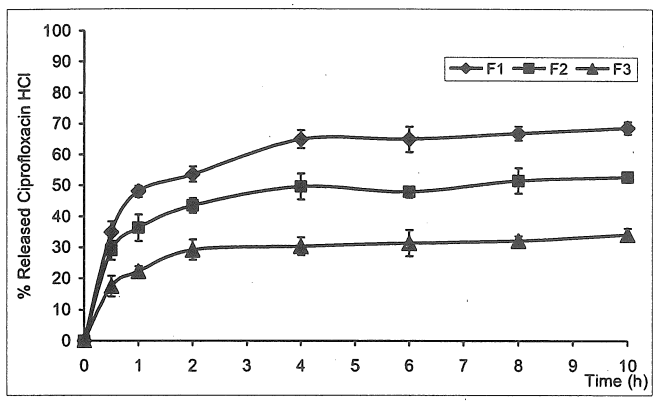

Figure 3. In vitro release profiles of CIPRO from formulation F1, F2, F3 (n=3)

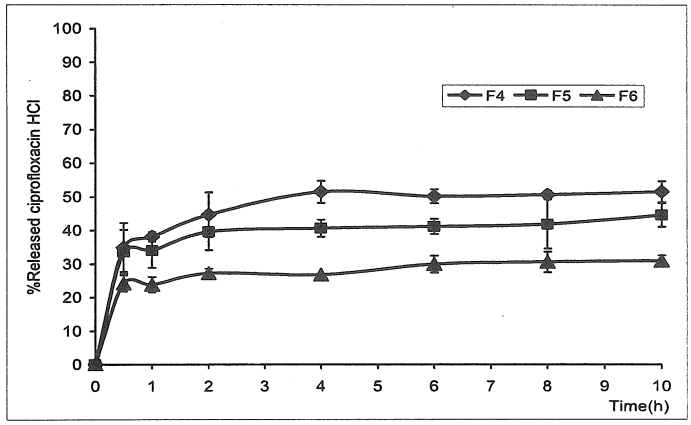

Figure 4. In vitro release profiles of CIPRO from formulation F4, F5, F6 (n=3)

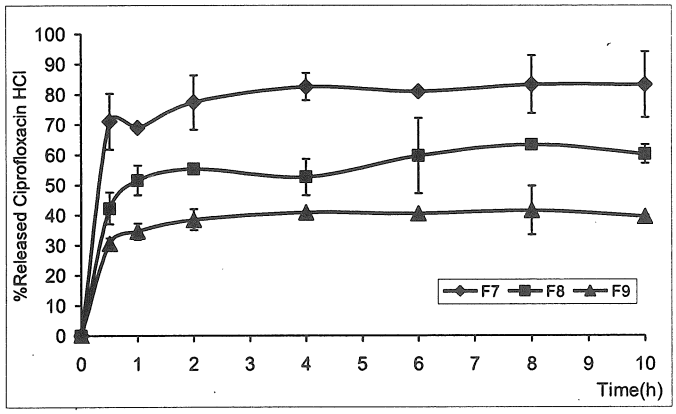

Figure 5. *In vitro* release profiles of CIPRO from formulation F7, F8, F9 (n=3)

Kinetic assessment: Obtained results from *in vitro* release studies were evaluated kinetically. Table 3 presents the correlation coefficients of formulation calculated according to zero order, first order and Higuchi kinetic models. All formulations followed Higuchi's square root of time dependent model. F6 provided an ideal drug release rate, which was confirmed by comparing all formulations correlation coefficients for Higuchi model and having the lowest SWSD. Therefore, F6 was chosen for *in vivo* studies.

Table 3. Kinetic assessments of *in vitro* drug release data.

Formulation Code	0. order		1. order		*Higuchi*	
	k_0	r	k_1	r	k_h	r
F1	2.952	0.859	0.024	0.820	12.43	*0.928*
F2	2.091	0.864	0.022	0.832	8.775	*0.929*
F3	1.399	0.848	0.023	0.811	5.888	*0.915*
F4	1.573	0.831	0.016	0.821	6.700	*0.908*
F5	1.012	0.904	0.011	0.891	4.121	*0.944*
F6	0.764	0.940 (*0.391)	0.012	0.932 (*0.392)	3.038	*0.959 (*0.067)*
F7	1.386	0.845	0.008	0.838	5.783	*0.902*
F8	1.612	0.834	0.013	0.814	6.649	*0.882*
F9	*0.793*	*0.725*	*0.010*	*0.515*	*3.528*	*0.826*

k_0, release rate constant of zero order kinetic ($mg.h^{-1}$), k_1, release rate constant of first order kinetic (h^{-1}), k_h, release rate constant of Higuchi's square root of time ($mg.h^{-1/2}$) r= correlation coefficient, *SWSW, sum of weighed squared deviation values of F6.

In vivo studies, Pharmacokinetic analysis: The mean plasma concentration-time curve of CIPRO after i.v., oral, nasal (solution and microsphere suspension) administration to rats is shown in the Figure 6 and pharmacokinetic parameters of CIPRO formulations are shown in Table 4.

The mean times to reach the peak level (t_{max}) after administration of oral and nasal solution and nasal microsphere (F6) suspension were 1.13±0.25, 0.63±0.14 and 0.75±0.00 h, respectively. The maximum plasma concentrations (C_{max}) for oral and nasal solutions were 2.82±1.06 and 1.87±0.53 μg/mL, respectively, whereas the C_{max} value for nasal microsphere administration was 1.40±0.38 μg/mL. The pharmacokinetic parameters and the values of absolute and relative bioavailabilities of four different nasal preparations of CIPRO are summarized in the Table 4. The mean AUC $_{(0-\infty)}$ after the i.v. injection of CIPRO was 41.40±6.04 μg.h/mL. The mean AUC $_{(0-\infty)}$ values of the administration of oral and nasal solution and nasal suspension of microsphere were 8.87±1.24, 6.47±2.89 and 20.4±3.33 μg.h/mL, respectively. The terminal absorption half-life ($t_{1/2abs}$) values for administration of oral and nasal solution were 0.24±0.13 and 0.15±0.08 h, respectively. The $t_{1/2abs}$ value of CIPRO in microsphere formulation was 0.09±0.02 h. The microsphere formulation's absorption rate constant (k_{abs}) 8.40±2.06 h^{-1} determined using $t_{1/2abs}$, was higher than other administrations. The terminal elimination half-life ($t_{1/2el}$) values for i.v., oral and nasal solution administration were 2.54±0.28, 2.56±0.46 and 2.88±1.04 h, respectively. Whereas the $t_{1/2el}$ value of CIPRO in microsphere formulation was 19.30±9.33 h. The elimination rate constant (k_{el}) of microsphere (F6) suspension was significantly lower than i.v., oral and nasal solution. For a drug subject to first-order kinetics, the percentage of the amount eliminated in one hour determined by k_{el} x 100 (Kayaalp 2002). Using this approach, the percentages of drug amount eliminated per hour for i.v., oral and nasal solution, and F6 microsphere suspension were 28, 28, 26 and 4%, respectively. This data show that a remarkable decrease in CIPRO elimination is observed with CIPRO microsphere formulation, compared to other formulations. This finding was supported by oral clearance (CL/F) data. The oral clearance (CL/F) values of the four applications were as follows: 0.49± 0.07 (L/h) for i.v.; 5.72±0.78 (L/h) for oral solution; 3.46±1.25 (L/h) for nasal solution; nasal microsphere suspension 1.49±0.24 (L/h). The absolute bioavailabilities (AB) of CIPRO oral and nasal

solution were 8.57±1.20% and 15.70±6.99%, respectively. The nasal microsphere suspension showed higher AB with 32.90±5.37% than oral and nasal solution. The relative bioavailabilities (RB) of CIPRO nasal solution and nasal microsphere suspension were calculated as 183±81% and 384±62%, respectively, compared to oral administration.

After administration of non-crosslinked drug-free microsphere formulation (B2) to rats a negligible activity (0.299±0.166 μg/mL whereas loaded microspheres is 0.960±0.402 μg/mL) was observed just in 30 minute, which was not taken into account in activity calculations.

These pharmacokinetic data show that CIPRO-loaded microsphere formulations are significantly different in all pharmacokinetic parameters. Nasal formulation of CIPRO-loaded microspheres showed approximately four-fold higher relative bioavailability compared to oral application. In addition, C_{max} was attained much faster with the microsphere formulation (0.75 h), compared to oral solution (1.13 h).

Table 4. Pharmacokinetic parameters of administrated CIPRO formulations to rats.

Dose of CIPRO (mg/kg)	Route	t_{max} (h)	C_{max} (μg/mL)	$t_{1/2abs}$ (h)	$t_{1/2el}$ (h)	k_{abs} (h^{-1})	k_{el} (h^{-1})	AUC $_{0-\infty}$ (μg.h/mL)	AB (%)	RB (%)	CL/F (L/h)
20	i.v.	-	-	-	2.54 ±0.28	-	0.28 ±0.03	41.4 ±6.04	100	---	0.49 ±0.07
50	Oral solution	1.13 ±0.25	2.82 ±1.06	0.24 ±0.13	2.56 ±0.46	3.64 ±1.84	0.28 ±0.04	8.87 ±1.24	8.57 ±1.20	100	5.72 ±0.78
20	Nasal solution	0.63 ±0.14	1.87 ±0.53	0.15 ±0.08	2.88 ±1.04	5.93 ±3.31	0.26 ±0.08	6.47 ±2.89	15.7 ±6.99	183 ±81.6	3.46 ±1.25
30	Nasal MS Susp.	0.75 ±0.00	1.40 ±0.38	0.09 ±0.02	19.3 ±9.33	8.40 ±2.06	0.04 ±0.02	20.4 ±3.33	32.9 ±5.37	384 ±62.6	1.49 ±0.24

MS Susp.= Microsphere suspension

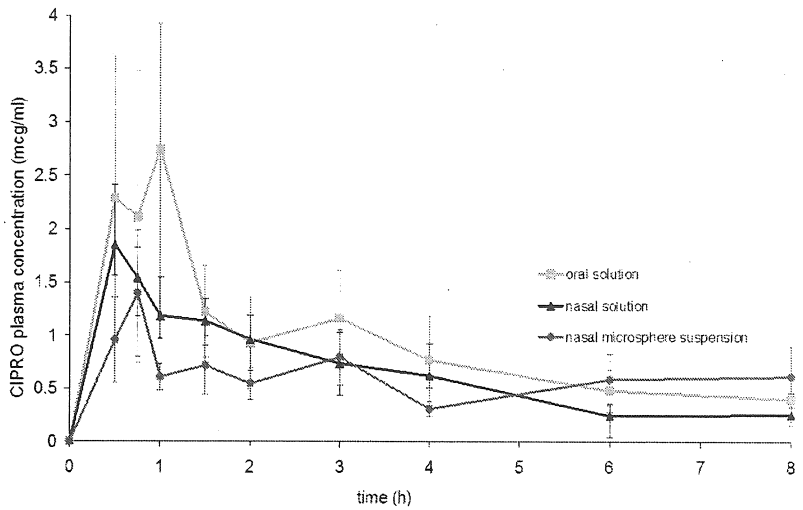

Figure 6. Comparison of plasma CIPRO level after administration CIPRO formulations

Conclusion

Spray dried method is simple, suitable and reproducible method to obtain CIPRO loaded chitosan microspheres. Experiments performed on rats showed that the nasal administration of CIPRO loaded chitosan microspheres may be used as an alternative route to the oral treatment.

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Özet

Bu çalışmanın amacı siprofloksazin hidroklorür yüklü kitozan mikroküre lerinin nazal uygulanmasını değerlendirmektir. Mikroküreler püskürterek kurutma yöntemi ile hazırlanmış ve partikül büyüklüğü, şekilsel özellikleri, ilaç-polimer etkileşimi, üretim verimi, ilaç içeriği, tutma kapasitesi, *in vitro* ilaç salımı, kinetik değerlendirme ve in vivo biyoyararlanımları açısından ele alındı. Mikrokürelerin partikül büyüklüğü 3.3 - 6.7 μm aralığında, küresel ve düzgün yüzeyli olarak elde edildi. Tüm formülasyonların ilaç yükleme kapasitesi ve mikroküre verimi sırasıyla %74 ve %38 den yüksek olarak hesaplandı. Mikrokürelerin in vitro değerlendirmeleri sonucu en uygun formülasyon sıçanlara in vivo uygulama için seçildi. In vivo çalışmalar sonucu CIPRO formülasyonlarının (oral çözelti, nazal çözelti ve nazal mikroküre süspansiyonu) mutlak biyoyararlanlarının sırasıyla %8.57, %15.7 ve %32.9 olduğu bulundu. Elde edilen veriler, püskürterek kurutma yöntemi ile hazırlanan CIPRO yüklü kitozan mikrokürelerinin uzatılmış etki sağladığını ve nazal yolun oral yola alternatif olarak kullanılabileceğini göstermektedir.

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