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Mucoadhesive microspheres containing gentamicin sulfate for nasal administration: preparation and in vitro characterization

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Abstract

In this study, suitable microsphere formulations were designed in order to provide the absorption of a high polar drug through nasal mucosa. For this purpose, gentamicin sulfate (GS) was chosen as a model drug and used at different drug/polymer ratios in the microsphere formulations. The microspheres were prepared by spray drying technique. Hydroxypropyl methylcellulose was used as a mucoadhesive polymer in the formulations to increase the residence time of the microspheres on the mucosa. Sodium cholate was added into the formulations for increasing the absorption of GS through nasal mucosa. The in vitro characteristics of the microspheres were determined. The microspheres were evaluated with respect to the particle size, production yield, encapsulation efficiency, shape and surface properties, drug–polymer interaction, mucoadhesive property, in vitro drug release and suitability for nasal drug delivery.

Keywords: Mucoadhesive microspheres; Nasal drug delivery; Gentamicin sulfate; Sodium cholate; Hydroxypropyl methylcellulose; Spray drying

1. Introduction

Nasal drug delivery is an interesting way and an alternative to the parenteral route for systemic drug delivery. The nasal cavity as a site for the systemic absorption of drugs has some advantages which include relatively large surface area, porous endothelial basement membrane, highly vascularized epithelial layer, high total blood flow per cm^3, avoiding the first pass metabolism and easy access [1]. However, there are some problems such as mucociliary clearance and low permeability of the nasal mucosa to some drugs that have a large influence on the efficiency of the nasal absorption of drugs [2].

Nasal mucociliary clearance is one of the most important limiting factor for nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively rules out sustained nasal drug administration. However, mucoadhesive preparations have been developed to increase the contact time between the dosage form and mucosal layers of nasal cavities thus enhancing drug absorption [3,4]. Illum et al. [5] introduced mucoadhesive microsphere systems for nasal delivery and characterized them well. The microspheres form a gel-like layer, which is cleared slowly from the nasal cavity, resulting in a prolonged residence time of the drug formulation.

In the preparation of mucoadhesive microspheres, some semi-synthetic polymers such as cellulose derivatives can be used. Hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC) and carboxymethylcellulose sodium (CMC Na) have all been investigated for nasal drug administration [6–8].

Another important limiting factor in nasal application is the low permeability of the nasal mucosa for the drugs with polar and high molecular size. It seems to be necessary to consider an absorption enhancement mechanism for co-administration of drugs with either mucoadhesive polymers or penetration enhancers or combination of the two [9–11].

Several enhancers such as surfactants [12], bile salts [13], phosphatidylcholine [14], cyclodextrin [15] and

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fusidic acid derivatives [16] have been used in nasal drug delivery systems. Bile salts are the most widely used for the optimization of nasal absorption. Duchateau et al. [17] studied the effect of bile salts on the nasal absorption of gentamicin. They found that without using these compounds, gentamicin was not absorbed. Sodium cholate (SC) and sodium taurodexycholate were determined the most active absorption promoters which enhanced the absorption in the ratios of 41 and 34%, respectively. Considering the results of the studies in the literatures, SC was selected and used as a penetration enhancer in this study.

The aim of the present study was to design suitable microsphere formulations that enable absorption through nasal mucosa for a highly polar aminoglycosidal antibiotic, gentamicin sulfate (GS) and also to investigate the in vitro characterization of the prepared formulations. The microsphere system was prepared by spray drying technique. In the microsphere formulations, HPMC was used as a mucoadhesive polymer. In our preliminary studies, the GS microspheres were tried to be prepared by using different kinds of mucoadhesive polymers; namely HPC, HPMC, CMC Na and Chitosan with spray drying technique. Considering the results of this technique, HPMC and CMC Na were selected due to easiness of application. In our previous study [18] CMC Na, and in this study HPMC were chosen and used in the preparation of GS microsphere system.

2. Experimental

2.1. Materials

GS was supplied from Deva Pharm. Co., Turkey. HPMC (1% w/v aqueous solutions, 100 cp, 25 °C) and SC were obtained from Sigma Chemical Co., USA.

2.2. Preparation of the microspheres

The polymer solution was prepared by dissolving the polymer in distilled water at a concentration of 1% (w/v) at room temperature (r.t.). GS was dissolved at different percentages in the polymer solutions (Table 1). SC was added into the solution at the amount of 1% (w/w) of the total amount of polymer and drug. Stirring the final solution of the polymer, drug and penetration enhancer, was sprayed through the nozzle (0.7 mm diameter) of a spray dryer (Büchi, type 190, Switzerland). The experimental parameters of the process were set as follows: inlet temperature; 150–155 °C, outlet temperature; 95–97 °C, aspirator setting; 10, pump setting; 2–5 ml/min, spray flow; 700 N l/h. Microspheres were collected into the final bottom vessel of the spray dryer and then harvested and kept under vacuum for 24 h.

Table 1
Composition of the microsphere formulations

Formulation code	Drug/polymer ratio	GS (% w/w)	HPMC (% w/w)
F1	4:1	80.00	20.00
F2	2:1	66.70	33.30
F3	1:1	50.00	50.00
F4	1:2	33.30	66.70

2.3. Characterization of the microspheres

2.3.1. Particle size analysis

Particle size analysis was performed on GS microspheres suspended in ethanol and sonicated for 5 min. The samples were analyzed by a Malvern Mastersizer S laser diffraction spectrometer.

2.3.2. Scanning electron microscopy (SEM)

Scanning electron microscopy (JEOL JMS-840A) was used to examine the shape and surface morphology of the HPMC microspheres. Samples of microspheres were dusted onto double sided tape on an aluminium stub. The stubs were then coated with gold using a cold sputter coater (Polaran E 5100) to a thickness of 400 Å. The samples were imaged using a 25 kV electron beam.

2.3.3. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (Netzsch Geratebau DSC 204) was carried out on pure drug, raw polymer, blank microspheres, and drug loaded microspheres (1:1 drug/polymer ratio). Samples (5 mg) were accurately weighed into aluminium pans and then sealed. The DSC runs were conducted over a temperature range 20–300 °C at a rate of 10 °C/min using nitrogen flow.

2.3.4. Drug loading

Samples from each batch of microspheres were dissolved in a phosphate buffer solution (pH 7.4) and the actual drug content was determined by first-derivative UV spectrophotometer (Shimadzu UV, 1601). Encapsulation efficiency was calculated from the ratio of actual to theoretical drug content and expressed as a percentage.

2.3.5. Mucoadhesive property

The bioadhesive properties of microspheres were determined by an adapted method described by Rango-Rao and Buri [19]. The principle of this test is based on simulating a biological flow by washing of a mucous membrane covered with the product to be tested. A freshly cut 5 cm long piece of intestine of rabbit was obtained and cleaned by washing with isotonic saline solution. Accurately weighed 125 mg of microspheres were placed on mucosal surface, which was fixed over a polyethylene support. The intestinal piece was maintained at 90% relative humidity and r.t.

for 15 min in a desiccator. The intestine was thoroughly washed with phosphate buffer solution (pH 7.4) at r.t. at the rate of 5 ml/min using a peristaltic pump. The concentration of the drug in the collected perfusate was first-derivative UV spectrophotometrically determined. The microsphere amount corresponding to the drug amount in perfusate was calculated. The adhered microsphere amount was estimated from the difference between the applied microsphere amount and the flowed microsphere amount. The ratio of the adhered microspheres to the applied microspheres was computed as per cent mucoadhesion.

2.3.6. *In vitro drug release*

The experimental conditions of drug release experiments were similar to those encountered in the nasal cavity. The in vitro drug release test of the microspheres was carried out using an apparatus called Franz diffusion cells. This apparatus was designed to imitate the nasal cavity and it was consisted of a donor and receptor compartments [20,21]. A dialysis membrane (cut-off Mw 12 000) was used to keep the microspheres on the donor side and it allowed free diffusion of GS to the receptor compartment containing phosphate buffer solution (pH 7.4). The volume of the receptor compartment was 20 ml. This volume is similar to that of a nasal cavity. The temperature of the receptor medium was adjusted to 37 ± 1 °C and maintained at that temperature by a peristaltic pump. The content of the receptor compartment was continuously stirred with a magnetic stirrer. Samples of a 0.5 ml were withdrawn from the receptor compartment at hourly intervals for 5 h and replaced with the same amount of fresh buffer solution.

The data obtained from the drug release studies were evaluated kinetically using a computer program written for this purpose [22].

Table 2
Characterization of the spray dried microspheres

Formulation code	Theoretical drug content (% w/w)	Actual drug content (% w/w) (mean ± SD)	Encapsulation efficiency (%)	Production yield (%) (mean ± SD)	Particle size (median (μm)) (mean ± SD)
F1	80.00	76.80 ± 2.47	96.00	52 ± 2.37	13.38 ± 2.17
F2	66.70	62.00 ± 4.62	93.00	58 ± 2.40	18.12 ± 1.86
F3	50.00	48.50 ± 3.95	97.00	66 ± 5.25	24.70 ± 1.23
F4	33.30	31.30 ± 1.83	94.00	51 ± 7.63	22.13 ± 2.09

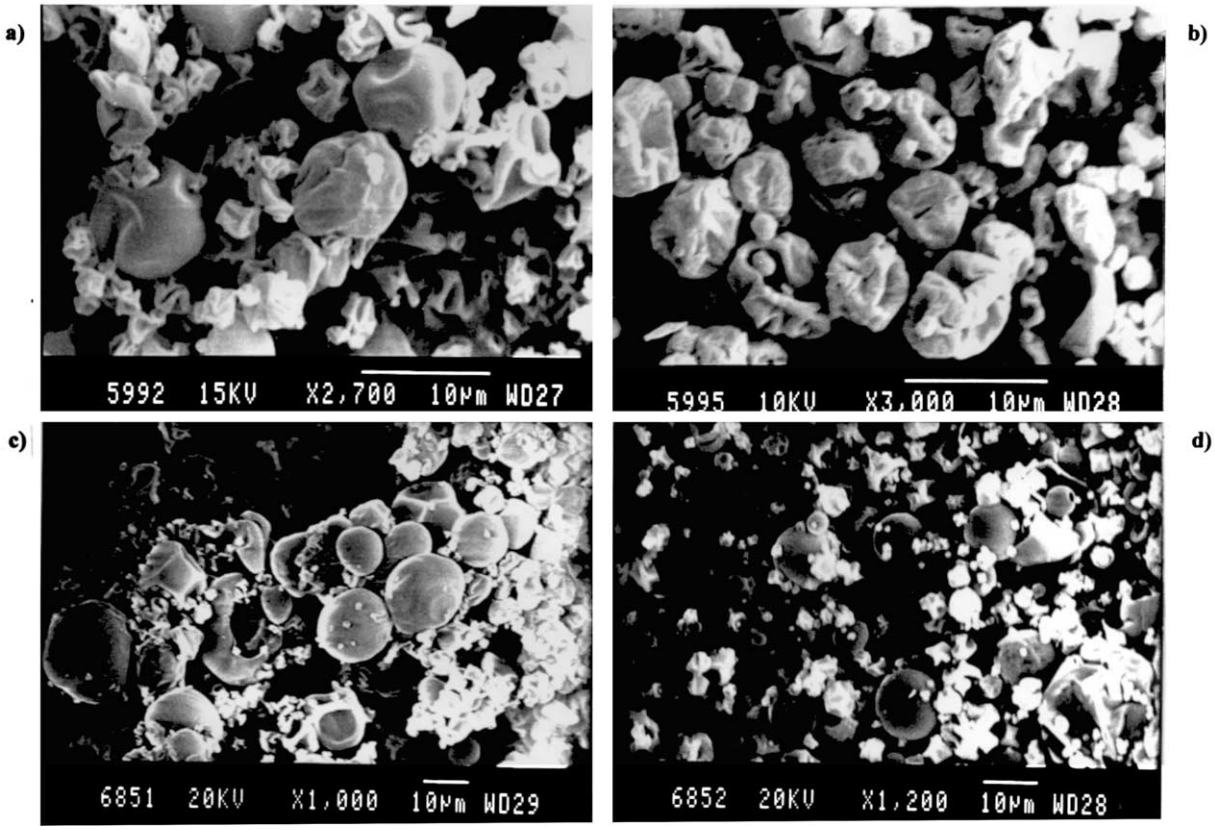

Fig. 1. Scanning electron micrographs of HPMC microsphere formulations, (a) 4:1 (F1); (b) 2:1 (F2); (c) 1:1 (F3); (d) 1:2 (F4).

3. Results and discussion

3.1. Preparation of the microspheres

GS loaded microspheres were produced with a high drug encapsulation efficiency (Table 2). Encapsulation efficiency ranged from 93 to 97%. Spray drying technique is generally characterized by high drug encapsulation efficiency [23]. The yield of production ranged from 51 to 66% (Table 2). Similar percentages were obtained in some studies on spray drying technique [24,25]. This relatively low percentage depends on the technical characteristics of the spray dryer; much of the spray dried powder adhering to the cyclone walls is lost during spray drying.

Considering the high encapsulation efficiency and sufficient production yield, it can be concluded that spray drying method is a simple and suitable technique for producing GS loaded microspheres.

3.2. Characterization of the microspheres

The particle size of each microsphere formulation coded F1, F2, F3, F4 was reported in Table 2. Median sizes of the microsphere formulations ranged from 13.38 to 24.70 μm. Such particles were considered to be suitable for nasal administration by insufflation [3,26].

SEM micrographs of the microsphere formulations coded F1 and F2 were reported in Fig. 1a and b, respectively. The microspheres exhibited irregular shape and crumpled surface. They seemed to be hollow microspheres, which collapsed during the preparation process. The micrographs belong to the formulations coded F3 and F4 were shown in Fig. 1c and d, respectively. The microspheres exhibited spherical shape and smooth surface. The results showed that the drug/polymer ratio affected the morphological characteristics of the spray dried microspheres extensively. As the polymer ratio increased, more spherical microspheres with smoother surface were obtained.

DSC was used to determine the thermal behavior of the pure drug, the raw polymer, blank microsphere and drug loaded microsphere. It was also used to determine the existence of possible interaction between the polymer and drug. The thermograms obtained from DSC are shown in Fig. 2. The thermogram of the pure drug, GS which is in amorphous state, is given in Fig. 2a. In the thermogram, a broad endothermic peak at 124.15 °C and three sharp peaks at 251.71, 255.02 and 260.28 °C were observed. GS is a complex mixture of the sulfates of gentamicin C_1 (melting point (m.p.) 94–100 °C), gentamicin C_{1a} and gentamicin C_2 (m.p. 107–124 °C). Some commercial samples may contain significant quantities of the minor components, gentamicin C_{2A}

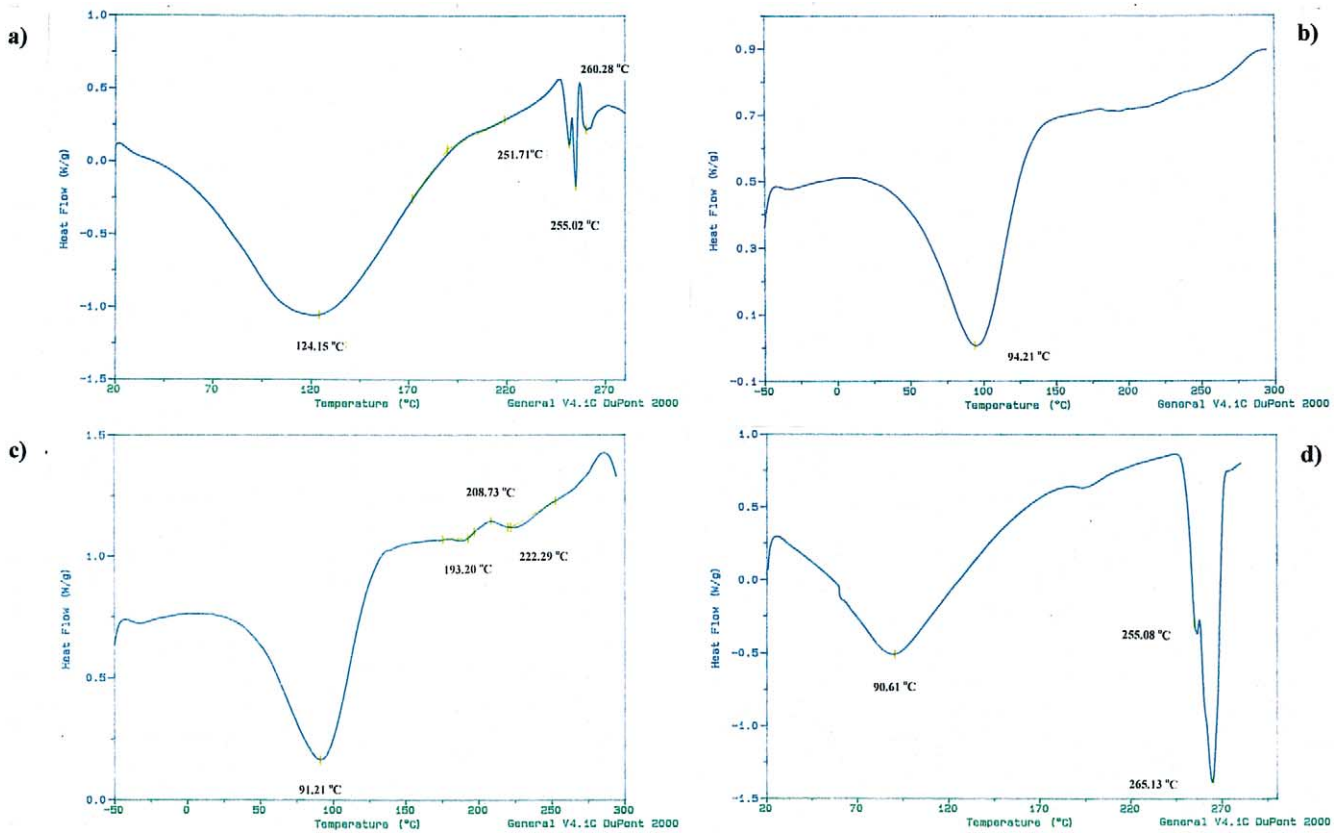

Fig. 2. DSC thermograms of (a) pure drug; (b) raw polymer; (c) blank microsphere; (d) drug loaded microsphere (1:1 drug/polymer ratio).

Table 3
Mucoadhesive properties of GS loaded microspheres ($n = 3$)

Formulation code	Mucoadhesion (%) (mean ± SD)
F1	75.20 ± 3.79
F2	78.50 ± 4.20
F3	79.40 ± 5.18
F4	82.10 ± 5.70

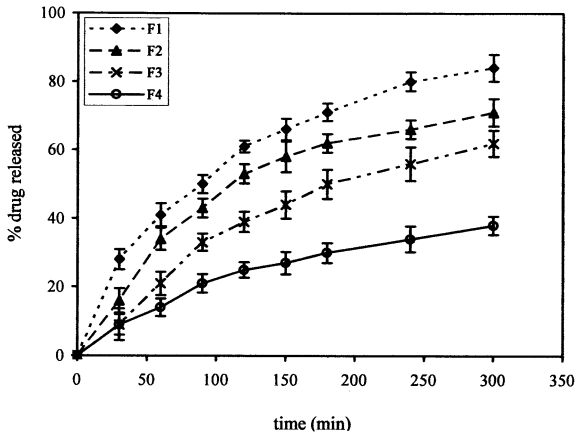

Fig. 3. In vitro release profiles of GS microsphere formulations.

and gentamicin C_{2B}. The m.p. of the C complex sulfate is given as 218–237 °C [27,28]. These peaks seem to correspond to the m.p. of the components of GS complex. The thermograms of raw polymer and blank microsphere were given in Fig. 2b and c, respectively. In the first thermogram, thermal transition at 94.21 °C can be seen which is attributed to the glass transition temperature (Tg) of the polymer. After processing of the polymer, its Tg was found as 91.21 °C (Fig. 2c). During the spray drying process, dissolving and heating of the polymer probably cause the Tg to decrease to 90.21 °C, but process does not affect the nature of the polymer. The thermogram of the drug loaded microsphere was given in Fig. 2d. The Tg of the polymer was observed at 90.61 °C and endothermic peaks at 255.08 and 265.13 °C were observed as the m.p. for the drug. The evaluation of the thermograms obtained from DSC revealed no interaction between the polymer and the drug in the microspheres.

Percentages of mucoadhesion were given in Table 3. The results showed that the microspheres had good mucoadhesive properties and could adequately adhere on nasal mucosa. The results also showed that with increasing polymer ratio, the higher mucoadhesion percentages were obtained. Since the highest percentage (82%) was obtained with F4 formulation, it can be thought the best one with good mucoadhesive property.

In vitro release profiles of GS microsphere formulations are shown in Fig. 3. The percentage of the drug released in 5 h was not the same for all formulations. The release profiles of the formulations coded F1 (drug/polymer ratio 4:1) and F4 (drug/polymer ratio 1:2) were the highest and the lowest, respectively. This result shows that drug/polymer ratio affects the release rate of GS. As the drug amount increases, the drug releases faster. Conversely, as the polymer amount increases, the drug releases slower.

The release data obtained were evaluated kinetically by zero order, first order and Higuchi model. According to the determination coefficients (r^2) release data was best characterized by Higuchi model suggesting a similarity to release from a matrix (Table 4). A linear graph was obtained by plotting the percentage of the drug released versus the square root of time. These profiles showed that drug release obeys Higuchi diffusion controlled model (Fig. 4). The results obtained from the computer program were also supported by these profiles. When the microspheres are immersed into the buffer solution, they swell by absorbing water into their matrix and form a gel diffusion layer. This layer hinders the outward transport of the drug producing a diffusion controlled release effect [29].

4. Conclusion

Spray drying is a rapid and simple technique for producing GS loaded microspheres. They were produced with sufficient production yield, high drug encapsulation efficiency and reproducible from batch

Table 4
Release parameters of GS from microsphere formulations

Kinetic model		Formulation			
		F1	F2	F3	F4
Zero-order	k_0	31.7880	28.4904	22.1730	8.7879
	r^2	0.9771	0.8626	0.9223	0.9360
First-order	k_1	0.2729	0.2255	0.1895	8.406×10^{-4}
	r^2	0.9893	0.9258	0.9745	0.9592
Higuchi model	k	4.1821	4.4932	4.4648	2.4983
	r^2	0.9962	0.9464	0.9811	0.9821

r^2, Determination coefficient; k_0 (mg/h); k_1 (per h); k (mg/h per cm^2), rate constants and units.

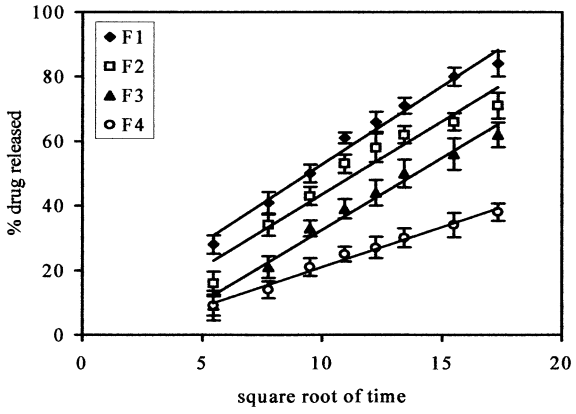

Fig. 4. Percentage of the drug released as a function of the square root of time.

to batch. All the microspheres were at a suitable size and had good mucoadhesive property for nasal administration. The hydrophilic polymer, HPMC and the microsphere system achieved to modify the in vitro release of GS. It was concluded that the microspheres prepared were suitable with respect to the in vitro characteristics for in vivo studies.

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