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Formulation and Evaluation of Porous Tablets for Mucosal Vaccination for *E. coli* and *Salmonella typhi* Infection in Travelers

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ABSTRACT

An attempt was made to prepare the porous tablet for mucosal vaccination for *E.coli* and *Salmonella typhi* in travelers Ciprofloxin hydrochloride pellets were prepared by using Avicel PH 101 (microcrystalline cellulose) and sodium chloride (NaCl) by extrusion/spheronization technique for controlled release. Solid, porous, discrete, reproducible pellets were obtained sieve analysis data indicated that the size of prepared pellets was in the range of 1136 to1242µm. The yield of the pellets was up to 96%. Scanning electron microscopy (SEM) revealed that prepared pellets were spherical, have dent surfaces with pores on the surface. Pellets were free-flowing with good packaging properties. The percentage encapsulation of pellets was found to be from 93.50 to 96.19. Studies such as drug loading and *in-vitro* drug release indicated F3 as an optimized formulation. Formulation F3 showed 91.4% drug release up to 24h. It was observed that there was no significant release of drug in gastric pH. It is concluded that Ciprofloxin hydrochloride was a good drug for the treatment of *E. coli* and *Salmonella typhi*

INTRODUCTION

Ciprofloxacin hydrochloride a synthetic antibiotic belongs to the family of fluoroquinolones, widely used as an anti-infective agent in the management of respiratory tract infection, urinary tract infections including infection of the bones and joints and endocarditis, gastroenteritis, malignant otitis, cellulites, and anthrax, etc(1). For the optimum therapeutic effect, the drug must be released quickly from the conventional tablet dosage form, dissolved in the aqueous medium, and absorbed from the gastrointestinal tract to get proper therapeutic efficacy. Retardation of the release of drugs from the tablet may lead to a sub-therapeutic level of the drug in plasma resulting in the delayed onset of action or short duration of action or no therapeutic action. Moreover, the sub-therapeutic level of antibiotic in the body might be the reason for the development of drug resistance, a major problem of antibiotics (2, 3).

The majority of pathogens conquer the body via one or more of the mucosal routes. Oral, nasal, pulmonary, and urinogenital routes are the most common passageways for the entry of infectious pathogens into the human host. Therefore, the importance of generating a first-line of defense at the site of entry has been well recognized. To have ample mucosal protection, several factors can sway the efficiency of vaccines, and amongst them, the most critical factor in mucosal vaccine effectiveness is the route of administration and potential for the antigen to be processed by the antigen-presenting immune cells, such as macrophages and dendritic cells. At present, most vaccines are administered via the parenteral route or other invasive routes. It is critically important to examine the development of mucosal vaccination strategies that can effectively trigger systemic as well as mucosal immunity (4).

Recent years a wide variety of newer oral drug delivery systems like controlled /Sustained release dosage formed are designed are evaluated to overcome the limitation of conventional oral therapy. These products can maintain steady drug plasma levels for extended periods, as a result, the variations of the drug levels in the blood are prevented and minimized drug-related side effects (5).

Porous pellets allow the incorporation of large drug fraction (deposited inside the porous structure or layered on the surface of pellets). To this end, pellets were using suitable polymer and pore-forming agents. After the preparation of pellets, pores in the pellets were created by aqueous extraction of pore-forming agent (NaCl)(6). In the present study porous pellets of ciprofloxacin hydrochloride were prepared by using extrusion and spheronization techniques.

MATERIALS AND METHODS

MATERIALS

Ciprofloxin hydrochloride was obtained from M/s Yarrow chemicals Mumbai, India. It is a white to almost white crystalline powder with a bitter taste. Avicel PH 101 (MCC) was procured from Yarrow Chemicals, Mumbai, India. It is a white colorless, Odorless, tasteless, and crystalline powder. Sodium Chloride from Karnataka fine chemicalsBangalore. Solvents and chemicals were of analytical grade.

METHODS:

- **Preparation of pellets**

The powdered Avicel PH 101 (MCC) and Sodium chloride (NaCl) passed through a 40 mesh sieve. The powders were granulated with water to get a good dough mass of extrudable consistency. The volume of the binder required was noted and the quantity of binder used was calculated. The wet mass was extruded into short cylinders using a cylinder roll type gravity feed extruder with a roller speed setting of 100rpm. A granulating cylinder with 1.0mm pore size was used and extruders were obtained. Spheronization of the extrudates was carried out in the spheronizer using a serrated plate. The spheronization speed was varied from 300 rpm to 1500 rpm and spheronization time was varied from 2 min to 20 mins to get pellets of good sphericity. The drying of pellets was carried out in a tray drier (6).

- **Drug loading:**

Dried pellets were collected and NaCl fraction was removed from the pellets by aqueous extraction: 30 g of pellets were placed on a 500 ml of bottle top filter (membrane filter); the filter was placed on 2 liters flask & connected to a vacuum pump. An aliquot of 2L of water was poured onto the filter in portions of 250 ml to extract the NaCl fraction. Later pellets were oven-dried at 40℃.

The drug was loaded by immersing the pellets into the drug solution. It was done by immersing 1 g of pellets into 2.0% of Ciprofloxacin HCl in water for 24 hrs. Pellets were collected & oven-dried at 40℃. (7)

Formulation of Pellets:

MCC and NaCl in different rations were used to make pellets. Water is used as a binder. The quantity of binder used was sufficient to maintain loss on drying.

Table No. 1: Formulation Chart

Formulation code	Drug %	MCC %	NaCl %
F1	2.0	90	7.5
F2	2.0	80	17.5
F3	2.0	70	27..5
F4	2.0	60	37.5
F5	2.0	50	47.5
F6	2.0	40	57.5

Characterization of Pellets:

- **Particle size analysis:**

The particle size of prepared pellets was measured using a Malvern Mastersizer 2000 version 5.1 (Malvern, UK). The drug-loaded ciprofloxacin hydrochloride pellets were dispersed on 1:20 with water and measured at a temperature of 37ºC (8, 9).

- **Micromeritic Properties**.

Tap densities of the prepared pellets were determined using Tap Densities Tester and the Percentage Carr's index was calculated.

a. Angle of repose

The angle of repose was assessed to know the flowability of pellets, by fixed funnel method. A funnel with the end of the stem cut perpendicular to its axis of symmetry was securely arranged above the graph paper of height which was placed on a flat horizontal surface. Ciprofloxacin Hydrochloride pellets were carefully poured through the funnel until the apex of the conical pile just reaches the tip of the funnel. The radius (r) and height of the pile (h) were then determined. The angle of repose (θ) for samples were calculated using the formula:

$$\textbf{Angle of repose } (\theta) = \tan^{-1}(h/r)$$

Angle of repose represents whether the given sample was free-flowing or not. The mean of three determinations was to calculate the angle of repose from each formulation (10).

b. Compressibility

Carr's index is a dimensionless quantity, which proved to be useful to the same degree as the angle of repose values for predicting the flow behavior. Apparent bulk density was determined by pouring the bulk samples into a graduated cylinder. Tapped density was determined by placing a graduated cylinder containing a known mass of powder on a mechanical tapper apparatus (Electro lab tap density tester). Samples were tapped until no further reduction in the volume of samples was observed. Carr's index is calculated using the formula given below and the relationship between compressibility and flow property is shown in the table. The mean of three determinations was used to calculate the compressibility index from each of the formulation (11).

Carr's index = (Tapped density- Bulk density)/ Tapped density

Scanning Electron Microscopic studies:

SEM photographs were taken with a scanning electron microscope Model Joel-LV-5600, USA, at the required magnification at room temperature. The photographs were observed for morphological characteristics and to confirm the spherical nature of pellets (12).

Evaluation of Pellets:

- **Percentage Yield:**

Determining whether the preparation procedure chosen for incorporating a drug into the polymers is efficient and is of prime importance. The raw materials, amount of compound, MCC and other process parameters are deciding factors for the yield of the product during the preparation of pellets(13).

The yield was determined by weighing Ciprofloxacin HCl pellets and then finding out the percentage yield concerning weight of input materials i.e, weight of drug & polymers used. The formula for calculation of % yield is as follows;

% Yield= Weight of Pellets X 100 /Weight of drug + weight of Polymers

- **Drug loading and encapsulation efficiency:**

Drug loading is important concerning release characteristics. Generally, increased drug loading leads to an acceleration of drug release. Drug entrapment efficiency represents the proportion of the initial amount of drug, which has been incorporated into the pellets.

100 mg of Ciprofloxacin HCl were weighed and transferred to 100 ml volumetric flask and diluted up to the mark. Further 1ml of this solution is diluted to 10mlabsorbance was measured at 274nm. The drug content was calculated by using the formula:

$$\text{Amount of drug} = \frac{\text{Conc. From standard graph X dilution factor}}{1000}$$

Percentage encapsulation efficiency to found out by calculating the amount of drug present in 100mg of pellets. It is further calculated by using formula:

$$\%\text{Encapsulation Efficiency} = (b)/a \times 100$$

Where a is the theoretical drug content and bis the drug entrapped.

***In vitro*- Drug release studies**

The *in-vitro* release of the drug from the pellets was carried out on in basket type dissolution tester USP XXIII TDT, type II with autosampler containing 900ml of pH1.2 buffer for the first 2hrs and 7.4pH phosphate buffer for next 22hrs. The volume of the dissolution media was maintained at 900ml with constant stirring (100rpm) and the temperature of the bath was maintained at 37±0.5℃. Aliquots (10ml) of dissolution media were sampled at specified time points and replaced with fresh media immediately after sampling. Samples were analyzed for drug content by spectrophotometrically. Dissolution studies were carried out for all the batches of the prepared formulations (06 batches) and commercial formulation, the details of which are given in the Fig 3 (14).

RESULTS AND DISCUSSION

RESULTS

Table No. 2: Micromeritic Properties and Particle Size Analysis

Formulation Code	Average Size(µm)	Angle of repose θ°	Tapped Density (g/cm^2)	Granule density (g/cm^2)	Carr's Index (%)	Friability (%)
F1	1136±0.56	26.54±0.92	0.84±0.64	1.06±0.88	9.12±0.32	0.53±0.78
F2	1189±0.45	25.42±0.25	0.86±0.92	1.07±1.78	8.79±0.99	0.52±0.45
F3	1242±0.23	23.45±0.88	0.90±1.43	1.05±1.45	9.39±0.53	0.43±0.82
F4	1239±0.55	26.30±0.65	0.89±1.01	1.05±0.96	8.93±0.98	0.47±0.36
F5	1211±1.02	25.25±0.46	0.83±0.55	1.04±0.72	8.76±1.76	0.45±0.78
F6	1229±0.92	25.98±0.74	0.83±0.82	1.07±0.81	8.69±2.01	0.49±0.22

Standard deviation, n=3

Table No. 3: Optimization of process parameters for pelletization

Parameters	**Formulation**	**Parametric Value**	**Description of pellets**
MCC:NaCl (w/w)	F1	90:10	Road Shape and Brittle
	F2	80:20	Egg Shape and brittle
	F3	70:30	Spherical and hard
	F4	60:40	Spherical and brittle
	F5	50:50	Semi Spherical and Brittle
	F6	40:60	Semi Spherical and hard
Spheronization Speed (rpm)	F3	300	Road shape
		700	Egg Shape
		1000	Semi spherical
		1250	Spherical
		1500	Semi spherical
Spheronization Speed (time)	F3	2	Road shape
		5	Egg Shape
		10	Semi spherical
		15	Spherical
		20	Semi spherical

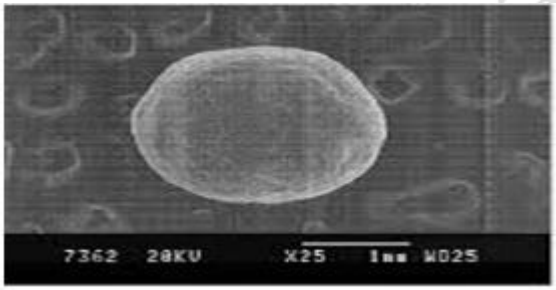
Tablet No. 4: Result of % yield of pellets formulations F1 to F6

Sr. No.	Formulation Code	% Yield			
		Trail 1	Trail II	Trail III	Mean ± S.D*
1	F1	89.2	89.5	89.1	89.33±0.1527
2	F2	93.1	92.4	93.6	93.03±0.6027
3	F3	95.7	97.9	96.1	96.5±1.1718
4	F4	95.8	92.9	93	93.9±1.6462
5	F5	93.6	95	94.1	94.2±0.79094
6	F6	96.9	93.8	94	94.9±1.734

Table No. 5: Drug Loading and Encapsulation efficiency

Formulation	Drug Loading (%)	Encapsulation Efficiency (%)
F1	15.41±0.23	93.50±0.74
F2	16.98±0.65	95.69±0.83
F3	18.32±0.44	96.19±0.33
F4	17.61±0.76	95.68±0.46
F5	17.12±0.89	94.89±0.64
F6	18.11±0.43	95.10±0.11

Standard Deviation n=3



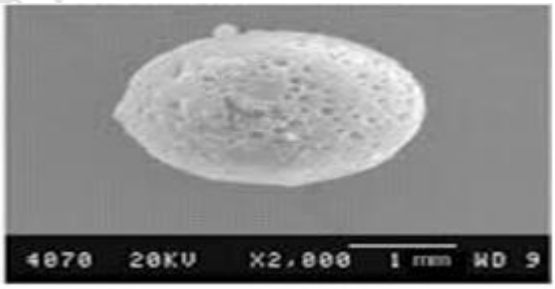


Figure No. 1: SEM of Nonporous pellet **Figure No. 2: SEM of porous pellet**

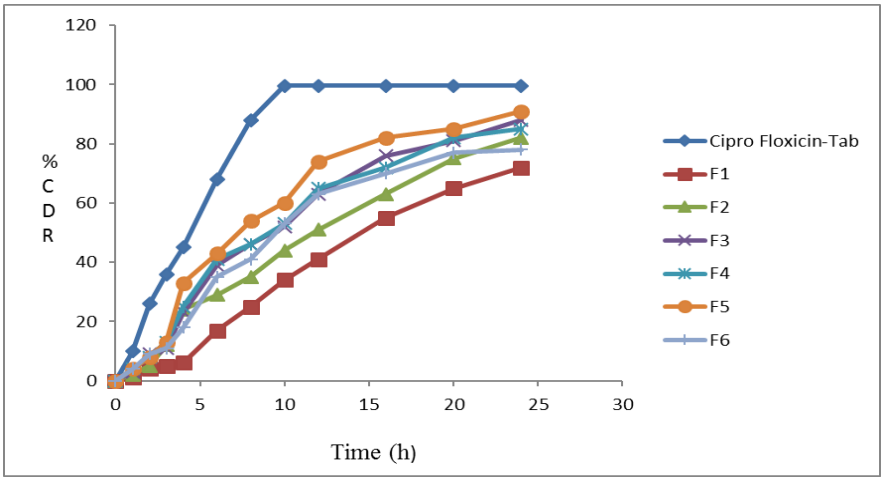


Figure No. 3: % Cumulative drug release (F1 –F6) and Ciprofloxin hydrochloride tablet

DISCUSSION

Evidence has been proved in recent years Avicel PH 101 possess physical properties and behavior suitable to prepare gastro-resistant, biocompatible, biodegradable porous pellets to release the entrapped drug in the intestinal lumen. In the present study the extrusion/ spheronization method was optimized by using MCC, NaCl to entrap the drug.

The pellets were prepared by using Avicel PH 101, as polymer and sodium chloride (NaCl), as pore-forming agent by extruder/spheronization technique. The technique was optimized using the parameters for pelletizations are shown in table when the ratio of MCC was 70% w/w produces spherical and hard pellets, suitable for pharmaceutical uses. But when the ratio of MCC was 90%, 80%, 70%, 50% and 40% w/w produces rod-shaped, egg-shaped, and semi-spherical and brittle pellets respectively. These pellets are not suitable for pharmaceutical purposes. In the present study, it was found that the ratio of MCC was 70% w/w resultant pellets did not have any surface irregularities and non-aggregated.

An attempt was made to prepare porous pellets by using 10,20,40,50,60,70 w/w of NaCl as the pore-forming agent fail to produce the required pores in the porous pellets. The increased and decreased NaCl responsible for the shrinking of the pore in the pellets. Maximum drug load was obtained, when the optimum ratio of 30% w/w NaCl was used as pore-forming agent. Produces suitable pore to entrap more amount of drug.

In the present study, it was found that optimum spheronization speed was found to be 1250rpm to produce spherical pellets. It was observed that with increase in stirring speed from1250 to 1500 there was a decrease in the average size of the pellets and produces semi-spherical pellets. When the stirring speed was 1000rpm, 700rpm and 300rpm produces semi-spherical, egg-shaped and rod shaped pellets respectively. It was also found that optimum stirring time was found to be 15min to produce spherical pellets. When the stirring time was 20 min, there was decrease in yield and produces semi-spherical pellets. When the stirring time was 10 min, 5 min, and 2min. It was observed that some amount of wetted mass adheres to the spheronizer resulting in lower recovery of yield and produces semi-spherical, egg-shaped, and rod-shaped pellets respectively. Repeat batches treated at an optimized rate mentioned above to produce reproducible sizes, showing that spheronization speed and stirring time were well controlled(7). In the present study, to produce the spherical porous pellets, an optimum drug concentration 2.5%w/v was used. It was found that higher the

amount of drug will show the presence of crystals on the surface of pellets were unsuitable for pharmaceutical use. Mutiparticulate systems should possess the required ad better micrometric properties the obtained data average size, angle of repose (θ). Tapped density, granular density, % Compressibility index (CI) along with related parameters are presented in the table the values of θ° and CI ranged from 23.45 to 26.54 and 8.69 to 9.39 respectively indicating that the obtained values were well within the limits. The result clearly shows that the prepared pellets have reasonably good flow potential. The values of tapped density ranged from 0.83 to 0.90 g/cm^2. The density difference between the formulations is negligible and the density values of formulations were well within the limits, indicating that the prepared pellets were non-aggregated and spherical. In this study, the sample was prepared by placing the formulation F3 samples in pH 7.4 buffer solution for 24hours followed by drying the samples at 30°C for 24hours. Scanning electron microscopy revealed that smooth surface with its optimal, spherical shape and spherical pellets with microporous nature and surface dents. (Fig.1 and Fig.2).The percent of drug loading in the formulation was found to be in the range of 15.41±0.23 and 18.32±0.44 %. The percentage encapsulation efficiency was found to be 93.50±0.74 and 96.19±0.33% the results obtained were shown in Table 4, the drug loading and drug entrapment efficiency increase with an increase in the polymer concentration. The decrease in the entrapment of drug content in the product may be due to the decrease in pore size and concentration (13). *In vitro* drug release profile showed pellets in both media indicates that the concentration of the polymers and decrease in pore formed decreases ciprofloxacin hydrochloride from the pellets. The increase in the concentration decreases the drug release in an immediate release of the drug. It was observed that there is no significant release of drug at gastric pH from pellets. In vitro drug release from formulation F1 to F6 was found to be 72.22 to 91.41 in the intestinal environment as shown in Figure 3. The release kinetics mainly depends on the concentrations of the polymers used, increase in the polymer concentration results in the controlled release of the drug from the pellets (14). The in vitro drug release was considerably retarded from pellets as compared with the marketed tablet of ciprofloxacin hydrochloride-100mg.

CONCLUSION

The objective of the study was to prepare pellets by the process of extrusion and spheronization and further the pellets were made porous by removing NaCl by aqueous extraction method. The drug was loaded in pellets by immersion technique. As the system

comes into gastrointestinal environment it forms micropores on the surface of the pellets. Drug release profiles were studied through a pore formed by the addition of different concentrations of pore-forming agent, then desired results were obtained via microspores formed in pellets. The in vitro drug release profile of the formulation F3 showed that the release of the drug was more controlled than the marketed tablet. The ciprofloxacin hydrochloride was a good drug for the treatment of *E. Coli* and *Salmonella typhi.*

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