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ENHANCEMENT OF BIOAVAILABILITY OF NORFLOXACIN MUCOADHESIVE SUSPENSION BY USING DIFFERENT GRADES OF CARBOPOL

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Key words:

Bioavailability, Scintigraphy, Mucoadhesive suspension, Norfloxacin, Laser diffractometer, Carbopol polymers

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ABSTRACT: Norfloxacin is slightly soluble in water and is a synthetic antimicrobial fluoroquinolone. Absorption of Norfloxacin is slightly impaired when it is administered with food as evidenced by a reduction of approximately 30% in the peak serum concentration. In the present study concept of mucoadhesive suspension is introduced to overcome the limitation of drug that has poor oral bioavailability and to increase the residence time of drug by bringing the drug in the vicinity of absorption window by formulating it in mucoadhesive drug delivery system. An attempt has been made to increase the bioavailability of Norfloxacin by formulating mucoadhesive suspension in different Polymers. Increase in time, speed of homogenization decreases the particle size and increases the zeta potential. Batches formulated with soyalecithin, antinucleating agents, showed decreased particle size. The comparative studies of dissolution studies showed, 99.55% of processed suspension released in 10Min. as compared to 101.63% released in 40Min. for marketed suspension respectively. The reason for increased percent release of drug in short time (10Min.), for processed suspension is, more time, speed and cycles of stirrer. These variables lead to increase in surface area of particle. It was found that the various parameters for bioavailability were enhanced by Carbopol 971P.

INTRODUCTION: Norfloxacin,1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid, is a synthetic antimicrobial fluoroquinolone with bioavailability of 30-40%. It is effective in the treatment of urinary tract infections, gonoccocal urethritis, and infectious diarrhea [1, 2]. A number of patients, especially pediatric and geriatric patients, have difficulty swallowing solid dosage forms. Therefore, a liquid dosage form of norfloxacin is needed. Since Norfloxacin is a solid and is slightly soluble in water [3], a suspension dosage form is the most suitable if the product is physically and chemically stable [4, 5].

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The purpose of this study was to formulate a mucoadhesive suspension of Norfloxacin and to evaluate the bioavailability of various formulation using different polymers.

MATERIALS AND METHODS:

Norfloxacin sample was gifted by Hindustan Antibiotics, Pune. Pluronic F68, Soya-lecithin were purchased from Sigma Aldrich, HPMC K100M, PVP, Microcrystalline cellulose, carbopol 971P, carbopol 974P, carbopol 934P, polycarbophil were purchased from CDH, New Delhi. All the excipients and solvents were of Analytical grades and distilled deionized water was used in all the experiments.

Formulation of Norfloxacin mucoadhesive suspension: [6, 7]

Optimization of ingredients:

Saturated solution of drug was prepared by dissolving 4G of drug in 9 Ml of acetic acid. Pluronic F68 was added in different quantities from 0.1G to 0.7G with optimized quantity 0.6G. Soya-

lecithin was added in different quantities; from 0.1G to 0.3G with optimized quantity 0.3G. Antinucleating agents HPMC K100M, PVP were tried, from 0.1G to 0.7G for reducing particle size with optimized quantity of 0.6G & 0.7G respectively. Different concentrations of precipitating agent (NaOH) solutions were tried with optimized concentration of 5% NaOH solution.

Preparation of suspension:
Saturated solution of drug in acetic acid was prepared by using mechanical stirrer. Surfactants with optimized quantity of pluronic-F-68 & Soya Lecithin were added successively with stirring to get clear solution. Then antinucleating agent was dissolved in the clear solution with low RPM. The drug was precipitated out by using 5% NaOH solution.

Preparation of different batches of suspension:
Different batches of suspension were prepared. The quantity of soya lecithin, pluronic-F-68 & anti nucleating agents was kept constant, as they were already optimized (**Table 1**).

TABLE 1: FORMULA OF SUSPENSION

Sr. No.	Ingredients	Amount
1.	Suspension (optimized) K9	80 ml
2.	Polyvinylpyrolidone	80 mg
3.	Microcrystalline cellulose	80 mg
4.	Sodium citrate	660 mg

Evaluation of suspension: [8]
Evaluation of suspension was done for following parameters.

Particle size analysis:
Microscopic methods: The effect of anti nucleating agent, soya-lecithin and different variables on particle size was determined from time to time using compound microscope (45X).

Laser diffractometer:
Particle size measurements were performed using a laser diffractometer (LD) master sizer 2000 ver. 2.00 (Malvern. Instruments, Malvern, UK). The range of detection was 0.2 to 2000 µm. From LD data, diameter 10%, 50%, 90% [d(0.10), d(0.50), d(0.90)] were counted. The diameters were calculated using volume distribution.

Saturation solubility:
For determination of saturation solubility, the suspensions were stirred for 24 hour with mechanical stirrer at 200 rpm and filtered. The absorbance of supernatant was measured using, Shimadzu 1700 UV-Visible Spectrophotometer.

Zetapotential:
Zeta potential was measured by using lab scale assembled zetameter. Zeta potential is one of the important parameter for determining the stability of suspension. Increase in speed or time of homogenization lead to increase in free energy of surface i.e. increase in surface area along with charges.

Crystal habit determination:
Whether reduction in particle size affects the crystalline nature of the drug or not was studied. The pure drug and precipitated drug were used for Differential Scanning Calorimeter and X-ray diffractometer.

***In-vitro* release study:**
USP dissolution apparatus Type II was used for in-vitro release studies. The suspension (400mg) was placed in 900Ml of acetate buffer (pH 4). The temperature of dissolution medium was kept 37±0.2°C, speed of stirrer 100 rpm. Three ml of sample solution was withdrawn at 0, 2, 5, 7, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 105, 120, 135, 150, 175, 300 Min. and was replenished with fresh dissolution medium. 2Ml filtrate was diluted to 100Ml [acetate buffer pH 4]; the absorbance was taken at 277 nm. The percentage drug release was calculated taking into consideration dilution factor, slope & constant obtained from calibration curve.

Formulation of mucoadhesive suspension:
Best suspension was selected on the basis of optimum particle size, surface area, saturation solubility and zeta potential. Suspension prepared was formulated in form of mucoadhesive suspension. For formulation of mucoadhesive suspension different carbopol grades were used as they showed nearly same bioadhesion property. Various polymer gels were prepared for carbopol 971P, carbopol 974P, carbopol 934P, polycarbophil of 0.25%, 0.5%, 0.75%. Mucoadhesive suspension were prepared as per standard procedure [9].

Evaluation of mucoadhesive suspension: [10, 11, 12]

***In vitro* studies:**

Falling film technique:

The bioadhesion test was done by using falling film technique. Prepared muco-adhesive suspension (0.6Ml) was evenly placed on the intestinal section (purchased from slaughter house) & then placed over the apparatus. After 10 Min, effluent i.e. acetate buffer of pH 4 was passed over it for 5 Min. The effluent was collected and dried to determine detached particles. Thus it gave the amount of particles attached and hence the bioadhesive strength of prepared suspension.

Dissolution study:

USP dissolution apparatus Type II was used for *in vitro* release studies. The mucoadhesive suspension (400mg) was placed in 900Ml of acetate buffer (pH 4). The temperature of dissolution medium was kept to 37±0.2°C with speed of stirrer at 100 rpm. Sample (3Ml) was withdrawn at 0, 2, 5, 7, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 105, 120, 135, 150, 175, 300 Min. and replenished with fresh dissolution medium. Filtrate (2Ml) was diluted to 100Ml using acetate buffer of pH 4 and the absorbance was taken at 277 nm. The percentage drug release was calculated taking into consideration dilution factor, slope & constant obtained from calibration curve.

***In vivo* Studies:** [13]

Gamma Scintigraphy:

The mucoadhesive suspension was labeled with technetium and filled in capsule shell.

Two healthy female volunteer with average age of 24Y and average weight 50kg were administered with capsule. After administration of capsule (containing labeled mucoadhesive suspension), images were collected using gamma scinticamera lined with online computer system. For collection of images, volunteers were laid in supine position under gamma camera. Images were collected at different time intervals i.e. 0, 45, 90, 180, 300, 360Min. The study was carried out on an empty stomach.

RESULTS AND DISCUSSIONS:

Results of Particle Size Analysis Table 2

TABLE 2: PARTICLE SIZE ANALYSIS FOR DIFFERENT BATCHES OF SUSPENSION

Batch No.	d(0.1) in µ	d(0.5) in µ	d(0.9) in µ	Batch No.	d(0.1) in µ	d(0.5) in µ	d(0.9) in µ
K0	67.629	243.984	1382.119	K_6	13.170	142.030	620.829
K_1	65.113	175.312	1330.624	K_7	10.978	139.112	599.752
K_2	32.231	154.365	705.049	K_8	1.300	18.360	591.078
K_3	63.113	168.615	1116.319	K_9	1.174	16.085	434.547
K_4	18.927	143.294	637.127	K_{10}	33.512	161.984	701.432
K_5	26.906	152.017	677.564				

Effect of Time on Particle size:

Time is effective variable in particle size reduction as it is found from table. Increase in time of homogenization decreases the particle size. Batch K_5, K_6, K_7 are with same variables except time, increasing time from 5Min. to 15Min. shows decrease in particle size (**Table 3).**

TABLE 3: EFFECT OF TIME OF STIRRING ON PARTICLE SIZE.

Time	Batch No.	d(0.1) in µ	d(0.5) in µ	d(0.9) in µ
5 min.	K_5	26.906	152.017	677.564
10 min.	K_6	13.170	142.030	620.829
15 min.	K_7	10.978	139.112	599.752

Effect of speed of stirrer*:*

The results depicted from Table 4 conclude that as speed of homogenization increases, the particle size decreases. Batch K_3, K_4, K_7 are with same process variables except speed, results of these batches shows that increase in speed from 3000 to 9000 rpm, decreases the particle size (**Table 4**).

TABLE 4: EFFECT OF SPEED OF STIRRING ON PARTICLE SIZE

Speed	Batch No.	d(0.1) in µ	d(0.5) in µ	d(0.9) in µ
3000	K_3	63.113	168.615	1116.319
6000	K_4	18.927	143.294	637.127
9000	K_7	10.978	139.112	599.752

Effect of Soya lecithin:

The purpose of using soya lecithin is to decrease the particle size. The formulation should contain optimum concentration of soyalecithin to decrease particle size. Soya lecithin acts as electrostatic stabilizer, so prevents aggregation of particles. Batches formulated with and without soyalecithin K_{10} & K_5 respectively, shows that presence of soyalecithin decreases particle size (**Table 5**).

TABLE 5: EFFECT OF SOYA LECITHIN ON PARTICLE SIZE

Effect of Lecithin	Batch No.	d(0.1) in µ	d(0.5) in µ	d(0.9) in µ
With Lecithin	K_5	26.906	152.017	677.564
Without Lecithin	K_{10}	33.512	161.984	701.432

Effect of no. of cycles:

Increase in no. of cycles shows decrease in particle size. It can be seen in batches K_7, K_8, K_9 are having all the same process variables except no. of cycles. Result shows increase in no. of cycles from 1 to 3 decreases particle size (**Table 6**).

TABLE 6: EFFECT OF NUMBER OF CYCLES ON PARTICLE SIZE

No. of Cycles	Batch No.	d(0.1) in µ	d(0.5) in µ	d(0.9) in µ
One cycle	K_7	10.978	139.112	599.752
Two cycles	K_8	1.300	18.360	591.078
Three cycles	K_9	1.174	16.085	434.547

Effect of nucleation inhibitor:

Addition of antinucleating agent decreases the particle size. Batches were prepared with and without antinucleating agent i.e. batch K_5 & K_1 respectively. Results shows addition of antinucleating agent reduces particle size (**Table 7**).

TABLE 7: EFFECT OF NUCLEATION INHIBITOR ON PARTICLE SIZE

Effect of nucleation inhibitor	Batch No.	d(0.1) in µ	d(0.5) in µ	d(0.9) in µ
With HPMC K100 M	K_5	26.906	152.017	677.564
Without HPMC K100 M	K_1	65.113	175.312	1330.624

Effects of variables on surface area and saturation solubility:

Effect of speed of stirrer: Results depicted in table shows that as speed increases, the surface area and saturation solubility increases. The increase in surface area lead to increased surface curvature and as per Noye Whitney's equation, more the surface area, more will be saturation solubility. Increasing RPM from 3000 to 9000 for batches K_3, K_4, K_7 shows increase in surface area and saturation solubility (**Table 8**).

TABLE 8: EFFECT OF SPEED OF STIRRER ON SURFACE AREA AND SATURATION SOLUBILITY

Speed (rpm)	Batch No.	Specific surface area (m^2/g)	Saturation solubility (µg/ml)
3000	K_3	0.0521	49.88
6000	K_4	0.2721	65.93
9000	K_7	0.4520	86.49

Effect of time of stirring:

It is observed, increase in time of homogenization lead to increase in surface area and saturation solubility. When time is increased from 5Min to 15Min for K_5, K_6, K_7 respectively K_7 resulted in higher specific surface area and saturation solubility (**Table 9**).

TABLE 9: EFFECT OF TIME OF STIRRING ON SURFACE AREA AND SATURATION SOLUBILITY

Time	Batch No.	Specific surface area (m^2/g)	Saturation solubility (µg/ml)
5 min.	K_5	0.2342	65.28
10 min.	K_6	0.3864	80.54
15 min.	K_7	0.4520	86.49

Effect of no. of cycles:

Increase in the no. of homogenization cycles from one to three increases specific surface area and saturation solubility. Varying the no. of cycles i.e. from one to three, for batches K_7, K_8, K_9, showed increase in surface area and saturation solubility (**Table 10**).

TABLE 10: EFFECT OF NO. OF CYCLES ON SURFACE AREA AND SATURATION SOLUBILITY

No. of Cycles	Batch No.	Specific surface area (m^2/g)	Saturation solubility (µg/ml)
One cycle	K_7	0.4520	86.49
Two cycles	K_8	0.6473	90.53
Three cycles	K_9	1.56131	98.72

Zeta potential:

As speed and time of homogenization increases, the value of zeta potential increases.

Effect of speed of stirrer on zeta potential:

As speed of mechanical stirrer is increased from 3000 to 9000 for batches K_3, K_4, K_7, shows increase in zeta potential. Higher speed of mechanical stirrer is responsible for decrease in particle size and creation of high energy surface. This high energy surface, is found to be responsible for increasing zeta potential.

Effect of time of stirring on zeta potential:

As time of homogenization is increased from 5 to 15Min. for batch K_5, K_6, K_7, shows increase in zeta potential. The high energy surface is responsible for increase in zeta potential when time increase from 5 to 15Min.

Effect of number of cycles on zeta potential:

As number of cycles increased from 1 to 3 cycles for batch K_7, K_8, K_9 shows increase in zeta potential. The high energy surface is found responsible for increase in zeta potential when time increase from 1 to 3 cycles.

Dissolution studies:

The comparative studies shows, 99.55% of processed suspension released in 10Min. as compared to 101.63% released in 40Min. for marketed suspension respectively. The reason for increased percent release of drug in short time (10Min.), for processed suspension is, more time, speed and cycles of stirrer. These variables lead to increase in surface area of particle.

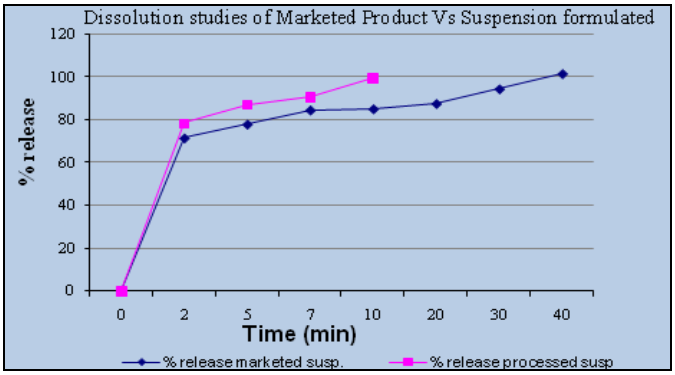

FIG. 1: % RELEASE OF MARKETED SUSPENSION AND FORMULATED SUSPENSION

Dissolution studies of suspension using different polymers:

The mucoadhesive suspension of carbopol 971P has shown 99% of drug released in 135Min. as compared to carbopol 974P released in 60Min. Carbopol 971P (**Fig. 2**: % Release of various formulated suspension)

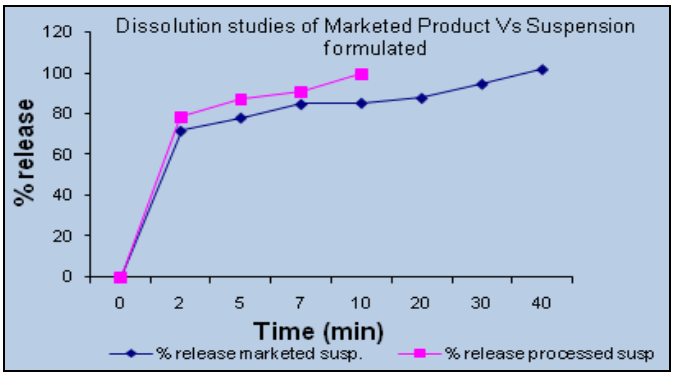

FIG. 2: % RELEASE OF VARIOUS FORMULATED SUSPENSION

In vivo Method:

Gamma Scintigraphy:

The gamma scintigraph demonstrated that mucoadhesive suspension of carbopol 971P was retained with maximum amount in stomach for absorption, for 6H, compared to carbopol 934P. As 97.49% of drug is released in 135Min from carbopol 971P, the mucoadhesion of formulation in stomach for 6 hrs is beneficial because of increased residence time of the formulation.

CONCLUSIONS: Polymers with greater adhesive force, higher viscosity, higher shear stress value shows better bioadhesion, both *in vivo* & *in vitro*. Carbopol 971 is with best bioadhesion property of all the carbopol grades.

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