Research Article

FORMULATION AND EVALUATION OF NORFLOXACIN SUSPENSION WITH β-CYCLODEXTRIN COMPLEXATION

Madhulita Panda[1*], Ganesh Patro[2], Amol Malpani[3], M. E. Bhanoji Rao[3]
[*1]College of Pharmaceutical sciences, Mohuda, Berhampur, Orissa-760001, India.
[2]Sri Venkateswara college of Pharmacy, Etcherla, Srikakulam, Andhra Pradesh, India.
[3]Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India.
***Corresponding author's E-mail:** madhupanda06@rediffmail.com

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ABSTRACT

The present study is aimed the formulation and evaluation of norfloxacin suspension with β-cyclodextrin complexation. The suspension was done by using different formulations of norfloxacin and β-cyclodextrin complexes (1:0.5, 1:1 and 1:1.5). These formulations were evaluated for sedimentation volume, redispersibility, pH measurement, viscosity measurement, particle size, and drug content estimation at various time intervals for 3 months. It also includes DSC studies in order to assess the complexation with β-cyclodextrin. The results of the study indicated inclusion of β-cyclodextrin in the form of complex in suspensions improved the physical stability. Higher the concentration of β-cyclodextrin, better is the stability. In this study, drug-β-cyclodextrin in the ratio of 1:1.5 was found to be optimum.

Keywords: Norfloxacin, β –cyclodextrin (CDs), kneading, stability, Sodium Carboxy Methyl Cellulose (sodium CMC).

INTRODUCTION

Norfloxacin, l-ethyl-6-fluoro-1,4-di-hydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid, is a synthetic antibacterial fluoroquinolone[1]. Quinolones belongs to synthetic class of antimicrobial agents with potent antimicrobial activity which are effective orally and parentally for a wide variety of infectious diseases[2]. It is active on both actively dividing as well as dormant bacteria by inhibiting bacterial DNA gyrase. It is effective in the treatment of urinary tract infections, gonococcal urethritis and infectious diarrhea[3]. The norfloxacin is commercially available only as tablets and capsules may be because of its bitter taste and instability in liquids[4]. A number of patients, especially pediatric and geriatric patients, have difficulty in swallowing solid dosage forms hence formulation of a suspension will be most suitable but product may not be physically and chemically stable. Norfloxacin is a hydrophilic fluroquinolone with unique physiochemical properties such as low water solubility and partition coefficient[5-7]. The poor aqueous solubility of norfloxacin gives rise to difficulties in pharmaceutical formulations for oral or parenteral delivery, which may lead to variable bioavailability. Cyclodextrin (CDs) are able to form inclusion complexes with poorly water-soluble drugs. Cyclodextrin (CDs) are able to form inclusion complexes with poorly water-soluble drugs. They are often depicted as hollow truncated cones with primary and secondary hydroxyl groups orientated outwards. As a result, CDs have an electron rich hydrophobic internal cavity and a hydrophilic exterior[8]. This unique cavity enables CDs to accommodate a wide range of non-polar molecules via the formation of reversible non-covalent inclusion complexes. CDs not only offer protection to the encapsulated molecule from the outer environment but also improve properties such as bioavailability, stability, solubility, dissolution rate, bioavailability and taste masking of the drug[9-11]. Cyclodextrin can also be used to prevent drug-drug and drug additive interactions, convert liquid drugs into microcrystalline powder, decrease volatility, modify gastrointestinal or ocular irritation and mask of objectionable taste or odor of drugs[8]. Present study shows the effect of β -cyclodextrin on physical stability of norfloxacin suspension which also includes formulation and evaluation.

MATERIALS AND METHODS

Norfloxacin (Acto pharmaceuticals ltd, Warangal), β-cyclodextrin, sodium carboxy methyl cellulose (sodium CMC), sorbitol, methyl paraben sodium, and propyl paraben sodium (Loba chem., Mumbai), sucrose (S.D. fine chemicals, Mumbai), tween 80 and acetone (Merck, Mumbai).

Preparation of binary mixtures of norfloxacin/β–cyclodextrin

Various techniques for the preparation of drug- β-cyclodextrin complexes include co-precipitation[12], slurry complexation (kneading method) [13], paste complexation, damp mixing and heating, extrusion, dry mixing, neutralization, freeze drying and slugging methods.

Complexation with β-cyclodextrins by kneading technique [13]

The norfloxacin and β-cyclodextrins complexes (1:0.5, 1:1 and 1:1.5) were prepared by kneading technique. In this method required amount of drug and β-cyclodextrin were taken and transferred to a mortar pestle. The mixture size was reduced by continuous stirring with pestle. Water-ethanol mixture (3:1) ratio was added to the above physical mixture and continuously stirred until the slurry

mass was formed. Slurry mass was collected and dried in a hot air oven for 2 hrs at 50°C, dried mass was collected and further dried in desiccators over silica gel for 24 hrs to remove all the excess residual solvents. The dried mass was collected and passed through 60 # mesh, and packed it in a closed container.

Preparation of suspensions

Suspensions containing 10mg/ml of norfloxacin were prepared as per the formula given in **Table 1** in about 100 ml of purified water, the required amount of suspending agent (sodium CMC)[14] was added and kept overnight for proper hydration. This solution was used as the vehicle in the preparation of the suspension. Accurately weighed quantity of the drug was distributed in the vehicle. Tween 80 was added to above dispersion. The slurry concentrate of the drug was mixed gently for 15 min. Other ingredients like sorbitol, methyl paraben and propyl paraben were added and the volume was made up with the water. The prepared suspensions were homogenized and transferred to the final containers.

Table 1: Optimized Formulations of Norfloxacin oral suspension

Ingredients	Formulations (gm)			
	F1 (Control)	F2 (1:0.5)	F3 (1:1)	F4 (1:1.5)
Norfloxacin	14	14	14	14
β-cyclodextrin	---	7	14	21
Sodium CMC	6.750	6.750	6.750	6.750
Tween 80 (0.2%)	1.80	1.80	1.80	1.80
Methyl Paraben	0.9	0.9	0.9	0.9
Propyl Paraben	0.180	0.180	0.180	0.180
Sorbitol	135	135	135	135
Water	q.s.	q.s.	q.s.	q.s.
Total (ml)	**900**	**900**	**900**	**900**

EVALUATION

Sedimentation Volume[15, 16]:

Sedimentation volume (F) is nothing but a ratio of the final volume of sediment (V_u) to the original volume of sediment (V_0) before settling. 50ml of each suspension were transferred to 50 ml. measuring cylinders and the volume of sediment formed was noted after 24 hr, 2 weeks, 3weeks, 1 month, 2 months and 3 months. The sedimentation volume (F) was calculated using the formula:

$$F = V_u / V_o$$

Redispersibility

The bottles containing suspension were held up right between the fingers and rotated clockwise upside down through 180^0 in a semicircular path and back in the anti-clock wise direction (one cycle). This process was repeated continuously until the sediment was completely redispersed.

pH measurement

The pH of the prepared suspensions was measured by using ELICO INDIA pH analyzer (Model LI612) by calibrating with standard buffers (pH 4.2 and pH 9.0).

Viscosity Measurement

The viscosity of the prepared suspensions was measured by Brookfield viscometer (Model: DV- I+) using spindle S-61 at 100 rpm.

Particle size measurement

The particle size of norfloxacin particles in the prepared suspensions was measured by optical microscopy using a trinocular microscope at 100x (10×10) magnification. The size of 100 particles were measured and the average particle size of was determined.

Drug content estimation

5 ml of the suspension was measured accurately and transferred into a 100ml volumetric flask. Sufficient quantity of 0.1N Sodium hydroxide was added to dissolve the drug and the volume was made up with 0.1N Sodium hydroxide. From this solution, 10 ml was taken and transferred to a 100ml volumetric flask the volume was made up to the mark with 0.1N sodium hydroxide. From this 1ml was drawn into 10 ml volumetric flask and the volume was adjusted with 0.1 N Sodium hydroxide. The absorbance of the solution was measured at 395nm on a UV-Vis spectrophotometer (ELICO SL-159) using 0.1N sodium hydroxide as a blank.

DSC Studies

The interaction between and β- cyclodextrin was characterized by differential scanning calorimetry (DSC). DSC patterns of samples were obtained with Shimadzu DSC-50 instrument using vented aluminium pans.

RESULTS

Sedimentation volume

The sedimentation volume in case of F_1 was found to be 1.0 at the end of 24 hours whereas it was 0.882, 0.944 and 0.982 in case of F_2, F_3 and F_4 (**Table 2**). After one week suspension F_1 resulted in a clumpy mass which was not pour able from the container. But the other suspensions (F_2-F_4) exhibited good flow. Out of these three formulations, with ageing it was found that, in F_2 the sedimentation volume gradually decreased from 0.882 to 0.628 whereas in case of F_3 and F_4 it had decreased from0.944 to 0.798 and from 0.982 to 0.800 respectively. This indicates that suspensions containing higher concentration of β – cyclodextrin displayed more stability than those without β – cyclodextrin.

Table 2: Sedimentation volume of different formulations of drug and β-CD

Formulations	$F = V_u / V_o$					
	24HRS	1 WK	2 WK	1 M	2 M	3 M
F_1	1	Formation of clumpy mass				
F_2	0.882	0.725	0.612	0.637	0.633	0.628
F_3	0.944	0.853	0.737	0.714	0.707	0.798
F_4	0.982	0.841	0.815	0.806	0.802	0.800

Redispersibility

Suspension F_1 didn't show any sedimentation; hence no redispersion. Good redispersibility was seen in the case of Norfloxacin suspensions containing different ratios of β – cyclodextrin. When the concentration of β – cyclodextrin was increased, redispersion was easier **(Table 3)**. In case of F_2, the number of cycles required to redisperse had decreased from 6 to 4 whereas in other two cases (F_3 and F_4), it remained constant at 4 and 3 cycles respectively.

Table 3: Redispersibility values of different formulations for 3 month period

Formulations	No. of cycles					
	24 HRS	1 WK	2 WK	1 M	2 M	3 M
F_1	----	Formation of clumpy mass				
F_2	6 Cycles	6 Cycles	5 Cycles	5 Cycles	5 Cycles	4 Cycles
F_3	4 Cycles	4 Cycles	3 Cycles	3 Cycles	4 Cycles	4 Cycles
F_4	3 Cycles	3 Cycles	4 Cycles	4 Cycles	3 Cycles	3 Cycles

pH

The pH value of F_1 was found to be 7.53 but when the drug was complexed with β - cyclodextrin the values were found to be less ranging from 6.25-6.45 **(Table 4)**. As formulation F_1 resulted in a clumpy mass determination of pH was not possible on ageing, whereas the other formulations (F_2-F_4) displayed a more or less constant pH value. This indicates that there is no chemical change on ageing.

Table 4: pH values of different formulations for 3 month period

Formulations	pH					
	24 HRS	1 WK	2 WK	1 M	2 M	3 M
F_1	7.53	Formation of clumpy mass				
F_2	6.45	6.42	6.23	6.01	6.28	6.26
F_3	6.25	6.25	6.10	6.05	6.03	6.00
F_4	6.40	6.40	6.05	5.92	6.15	6.09

Viscosity

In this case also determination of viscosity was not possible in case of F_1 due the clumpy mass. As the concentration of β – cyclodextrin increased from F_2 to F_4, a slight increase in viscosity was observed **(Table 5)**. On ageing the viscosity of F_2, F_3 and F_4 has decreased from 44.3, 50.3 and 58.5 to 35.24, 39.05 and 52.5 respectively. The values suggest that there was no significant change in viscosity on ageing which correlates with the redispersibility data. Here too, the change in viscosity in case of F_4 was less indicating that F_4 is relatively a stable formulation.

Table 5: Viscosity values of different formulations for 3 month period

Formulations	Viscosity (S – 61, 100 rpm.) in cps					
	24 HRS	1 WK	2 WK	1 M	2 M	3 M
F_1	----	Formation of clumpy mass				
F_2	44.3	46.5	39.1	36.55	37.05	35.24
F_3	50.3	48.5	44.3	42.45	40.25	39.05
F_4	58.5	56.2	58.1	54.3	55.8	52.5

Particle size measurement

Due to the clumpy mass particle size determination was not possible in case of F_1. In the case of F_2 the particle size remained constant over a period of three months, whereas in case of F_3 and F_4 there was a slight decrease in the particle size **(Table 6)**. The reason for this behavior may be attributed to solubilization of drug particles by β – cyclodextrin.

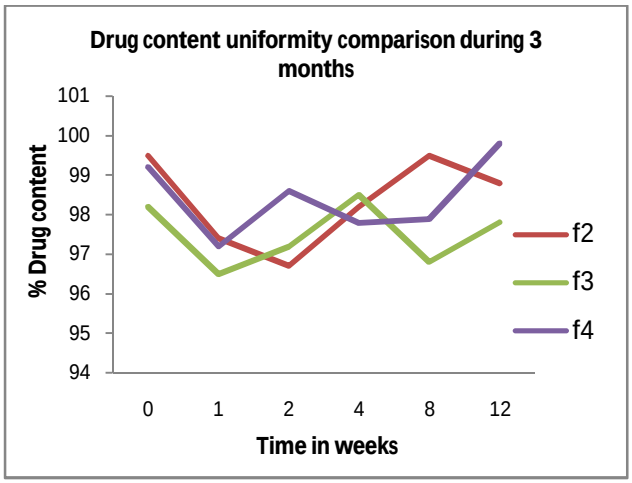
Table 6: Particle Size Determination values of different formulations for 3 month period

Formulations	24 HRS	1 WK	2 WK	1 M	2 M	3 M
F_1	-	Formation of clumpy mass				
F_2	53.16	53.92	54.95	55.23	53.15	51.05
F_3	51.30	48.7	45.07	44.77	42.42	41.20
F_4	61.33	46.05	45.0	45.94	43.78	42.05

Drug content

Assay of the drug in formulations F_2-F_4 shown 95.80% to 99.80% drug content indicates that the drug content remains within the standard limits (see **Figure 1**). Formulation F_1 forms clumpy mass during first week and thus it was not evaluated further for its drug content.

Figure 1: Drug content uniformity of three formulations in 3 months

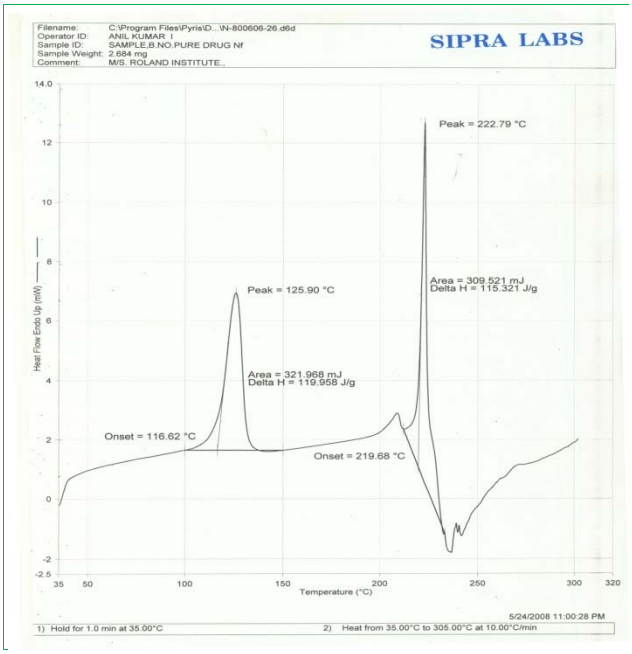


DSC studies

The thermal behaviour of pure drug norfloxacin, β – cyclodextrin and drug- β – cyclodextrin complex was studied using differential scanning calorimetry in order to

assess the formation of complex **(Fig 2)**. The DSC thermogram of Norfloxacin exhibited an endothermic peak at 222.79°C corresponding to its melting point. β – Cyclodextrin has shown a broad endothermic peak at 114.76°C, corresponding to its dehydration. The thermogram of the binary mixture contained only the peak of β – cyclodextrin, whereas the drug peak has almost disappeared. This indicates that drug has complexed totally. As Norfloxacin is a slightly soluble drug, complexation might have occurred completely.

Figure 2: DSC thermogram of norfloxacin / (β-CD) complex



DISCUSSION

Suspension F_1 was formulated employing drug and suspending agent, whereas suspensions F_2, F_3 and F_4 were formulated by employing drug, suspending agent and β -cyclodextrin. The drug and β -cyclodextrin complexes in F_2, F_3 and F_4 were in the ratio of 1:0.5, 1:1 and 1: 1.5 respectively. These formulations were evaluated for various quality parameters to determine their stability such as sedimentation volume, pH, viscosity, redispersibility, particle size and drug content for 3 months time in regular intervals (see above **Table 2, 3, and 4**). All formulations contain sodium CMC as the suspending agent. Four suspension formulations were prepared containing sodium CMC in four different concentrations (0.25, 0.5, 0.75 and 1 %w/v). These were studied for 48 hours and evaluated for sedimentation volume and redispersibility. The values are presented in **Table 7.** From the results, it is concluded that, suspension containing 0.75%w/v sodium CMC shown highest sedimentation volume and less number of cycles for redispersibility. Hence, sodium CMC in the concentration of 0.75%w/v was used in all the suspensions for further studies.

Table 7: Effect of sodium CMC on the physical stability of suspensions

Formulations (% Na CMC)	Sedimentation Volume (F)	Redispersibility (no. of cylces)
0.25%	0.12	4
0.5%	0.24	5
0.75%	0.46	3
1.0%	0.32	4

CONCLUSION

The present study was carried out to study the effect of β -cyclodextrin on the physical stability of suspensions. Norfloxacin were selected as model drugs and sodium CMC was used as the suspending agent and was used in the concentration of 0.75% w/v in all the formulations as it had shown good results. Inclusion complexes of drug and β-cyclodextrin were prepared in three different concentrations (1.0:0.5, 1.0:1.0 and 1: 1.5) by kneading method[15]. All the suspensions formulated by employing drug-β-cyclodextrin binary systems have shown improvement in physical stability when compared to control (suspension prepared without β-cyclodextrin). The suspensions with β-cyclodextrin exhibited good redispersiblity within a 3-4 cycles during the storage, whereas, more number of cycles are required in case of norfloxacin suspensions without β-cyclodextrin. The results of the study indicated inclusion of β-cyclodextrin in the form of complex in suspensions improved the physical stability. Higher the concentration of β-cyclodextrin, better is the stability. In this study, drug-β-cyclodextrin in the ratio of 1:1.5 was found to be optimum.

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