Acta Poloniae Pharmaceutica – Drug Research, Vol. 68 No. 2 pp. 273–278, 2011 ISSN 0001-6837
Polish Pharmaceutical Society

DEVELOPMENT AND CHARACTERIZATION OF MUCOADHESIVE NANOSUSPENSION OF CIPROFLOXACIN

VIKAS JAIN*, PRADEEP KARE, DEEPIKA JAIN and RANJIT SINGH

School of Pharmaceutical Sciences, Shobhit University,
NH-58, Modipuram, Meerut, Uttar Pradesh, 250110, India

Abstract: Mucoadhesive nanosuspension of ciprofloxacin was designed in order to improve the solubility, bioavailability and efficacy for the treatment of typhoid fever. The identity and purity of drug was established. The compatibility of drug with various excipients was ascertained by FTIR techniques, which indicated no interaction between the drug and excipients. Four different formulations were prepared by optimizing various parameters using different polymers like soya lecithin, pluronic F68, polyvinyl alcohol, and polyvinylpyrrolidone K30. Particle size and polydispersity index were determined by photon correlation spectroscopy. Average particle size of different formulations was found to be between 503–1844 nm. The ζ potential of all formulations was found to be around ± 20 Mv indicating satisfactory physical stability. Scanning electron microscopy showed that process parameters affect the crystal morphology. The promising formulations prepared from combination of soya lecithin and pluronic F68 and those based on soya lecithin alone were subjected to dissolution profile studies. The later formulation exhibited fast dissolution rate as compared to the former. Thus nanosuspension based on soya lecithin was incorporated into hydrogels prepared using different grades of carbopol 934 and 971 as mucoadhesive polymers. After 10 h, mucoadhesive nanosuspensions showed 45–56% release. The developed mucoadhesive nanosuspensions exhibited satisfactory physical stability. The studies indicated potential of these formulations as novel gastroretentive systems.

Keywords: nanoparticles, ciprofloxacin, mucoadhesive, hydrogel

Drug particles in the nanometer size range have unique characteristics that can lead to enhanced performance in a variety of dosage forms. When formulated correctly, particles in this size range are resistant to settling and can have higher saturation solubility, rapid dissolution, and enhanced adhesion to biological surfaces, thereby providing a rapid onset of therapeutic action and improved bioavailability. Scientists use nanotechnology for classical and novel drug delivery applications. Nanoparticles can be defined as structures that have at least one length dimension less than or equal to 500 nm, and exhibit novel and unique chemical, physical, or biological behavior because of their small size (1) Nanosuspension, a carrier-free colloidal drug delivery system, consists essentially of pure drug nanoparticles (100–1000 nm) and a minimum amount of surface active agent required for stabilization. By definition, drug nanocrystals are nanoparticles composed of 100% drug without any matrix material, with a mean diameter below 1000 nm. The dispersion medium can be water, aqueous solutions or non-aqueous media. Surfactants and/or polymeric stabilizers are used for the stabilization of these systems (2). Nanonization of drug powders increases the surface of the particles, leading to an increase of the dissolution velocity. Another important aspect is the increase in saturation solubility. In addition, the distance of diffusion on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient. The increased concentration gradient leads to much higher increase in the dissolution velocity as well (3).

The present study was aimed at developing mucoadhesive nanosuspension of ciprofloxacin in order to improve the solubility, bioavailability and its efficacy in treatment of typhoid fever. The mucoadhesive property was expected to increase the residence time in gastrointestinal tract for an extended period so that dosing frequency and the amount could be reduced improving the patient compliance.

* Corresponding author: e-mail: vikasjain111180@gmail.com; vikasjain111180@yahoo.co.in

Table 1. Composition of different ciprofloxacin nanosuspensions.

Contents	Formulations Code			
	F1	F2	F3	F4
Drug (%w/w)	1	2	3	4
Soya lecithin (%w/w)	0.5	1	1	-
Pluronic F68 (%w/w)	1	-	-	1
PVA (%w/w)	-	0.2	-	-
PVP K30 (%w/w)	-	0.5	-	-
Tween 80 (%w/w)	-	-	0.5	0.3

EXPERIMENTAL

Materials

Ciprofloxacin base was kindly provided as a gift sample by Ranbaxy Laboratory Ltd. Paonta sahib (H.P.) India. Pluronic F68, soya lecithin and polyvinyl alcohol (MW 30000–70000) were procured from Sigma-Aldrich, USA. Tween 80 was purchased from Qualikems Fine Chemical Ltd., New Delhi. Polyvinylpyrrolidone K-30, carbopol 971 and carbopol 934 were obtained from Central Drug House (P) Ltd., New Delhi. All excipients and solvents were of laboratory grade and double distilled deionized water was used in all experiments.

Methods

Formulation of nanosuspension of ciprofloxacin (4)

To prepare nanosuspension of a poorly soluble drug, it is preferred to start with a very fine powder. Firstly, the surfactants in different concentrations were dissolved in distilled water. The drug powder was dispersed in the aqueous surfactant solution using high speed homogenizer (Remi, RQT-124A, India) for 30 min at 20000 rpm to obtain premixed microsuspension. Ultrasound was applied to the microsuspension using ultrasonic homogenizer (Sartorius, Labsonic-M) at different intensities and times to obtain nanosuspension. Composition of different ciprofloxacin nanosuspensions are presented in Table 1.

Preparation of mucoadhesive nanosuspension (5, 6)

Hydrogel was prepared using carbopol 934 and carbopol 971 (0.5% w/w). The polymers were added to distilled water and allowed to swell for 24 h. Then, nanosuspension was incorporated into the hydrogels with the help of a pestle and mortar to obtain mucoadhesive nanosuspension.

Characterization of nanosuspensions

Particle size analysis (5, 7)

The mean particle size and the polydispersity index (PI) were determined by photon correlation spectroscopy (PCS) using Malvern Mastersizer Nano-ZS (Malvern Instruments, UK). Prior to the measurement, the samples were diluted with double distilled water to a suitable scattering intensity and redispersed by hand shaking.

ζ potential measurment (5, 7)

The ζ potential was determined by a Malvern Mastersizer Nano-ZS (Malvern Instruments, UK). It is a measure of electric charge at the surface of particles indicating the physical stability of colloidal systems. The ζ potential values higher than 30 mV indicate long term electrostatic stability of aqueous dispersions. The analysis was performed using the clear disposable ζ cell, in distilled water at 20 V/cm of field strength. All measurements were done in triplicate.

Light microscopy (5, 8)

Light microscopy was performed using photo microscope RXLr-3T (Radical, India). Each sample was investigated in triplicate.

Scanning electron microscopy (SEM) (5, 9)

The size and shape of ciprofloxacin nanosuspension were also determined by SEM. The nanosuspensions were spread on a sample holder and dried using vacuum. They were subsequently coated with gold (JFC 1200 fine coater, JEOL, Japan) and examined using scanning electron microscope (JSM 6301F, JEOL, Japan).

***In vitro* dissolution study** (9)

Dissolution behavior of the ciprofloxacin nanosuspensions was assessed *in vitro*. To study the dissolution behavior of formulation, 5 mL of nanosuspension was taken and transferred into the open ended test tube tied at one end with 450 nm nanopore membrane filter (Cellulose nitrate, Rankem, Delhi). The test tube was dipped from membrane side in a beaker containing 100 mL 0.1 M hydrochloric acid. The temperature and stirring rate were maintained at 37 ± 2°C and approx. 100 rpm, respectively. Samples were withdrawn periodically and replaced with an equal amount of 0.1 M hydrochloric acid. The samples were analyzed spectrophotometrically at 277 nm wavelength using double beam UV/Visible spectrophotometer (Shimadzu 1700). All measurements were performed in triplicate. The same procedure was followed for *in vitro* drug release study of mucoadhesive nanosuspension.

Physical stability study (8)

Physical stability study of the prepared ciprofloxacin nanosuspension was carried out at 25°C. The changes in appearance, Ostwald ripening and settling behavior were recorded at predetermined time intervals of 24 h, 1 week, and 2 weeks using photo microscope RXLr-3T (Radical, India).

RESULTS AND DISCUSSION

Optimization of process parameters

The optimization of nanosuspension formulations was done on the basis of process parameters like stirring rate, stirring time and increased ultrasound intensity.

Ultrasonic application provided homogeneous and stable nanosuspension system of ciprofloxacin. In the present study we prepared four different formulations of nanosuspension, with different stabilizer concentrations. The pre-dispersion of drug was ensured by high speed homogenization. The energy produced due to conventional bubble collapse might have broken down the drug particle into small microparticles (10). The premixed microsuspension was subjected to ultrasonic homogenization. The ultrasound was applied to the microsuspension at different probe intensities and times to obtain nanosuspension.

Smaller particle sizes of ciprofloxacin were obtained by increasing stirring rate and stirring time, ultrasound intensity and number of cycles. The effect of stirring rate and time on premix suspension of ciprofloxacin was studied by light microscopy. Larger crystals with aggregates were observed when premix suspension of ciprofloxacin was stirred at 15000 rpm for 10 min. On increasing the stirring rate and time (20000 rpm, for 20 min), smaller particles with heterogeneous distribution were

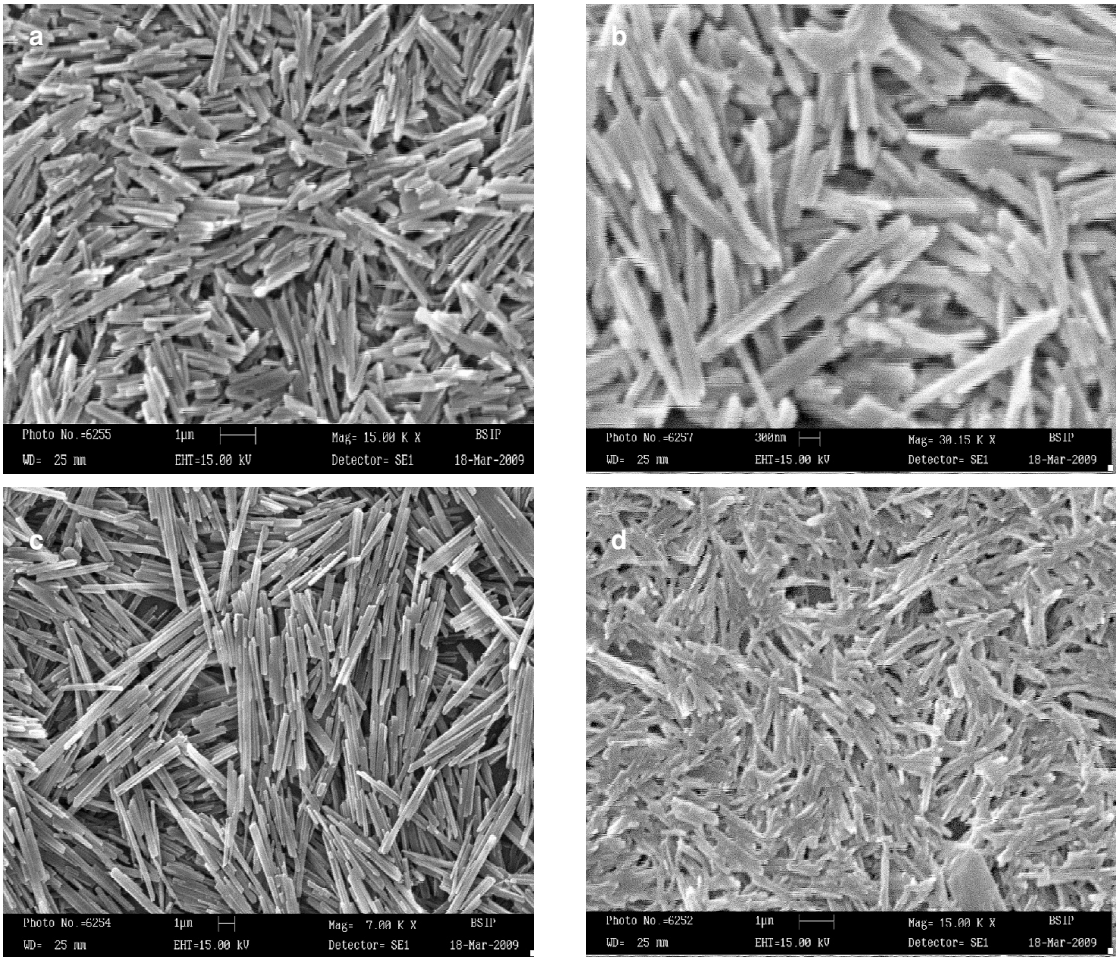

Figure 1. SEM photograph of different nanosuspention formulation prepared at (a) 20% amplitude pressure, (b) 40% amplitude pressure (c) 60% amplitude pressure and (d) 80% amplitude pressure

Table 2. Particle size determination and ζ potential measurements.

Formulation code	F1	F2	F3	F4
Avg. particle size (nm)	540	1844	503	1503
Polydispersity index (PI)	0.823	0.451	0.669	0.045
ζ potential (mV)	–20.8	–4.67	25.8	–18.7

Table 3. Composition of mucoadhesive nanosuspensions.

Formulation code	F3G1	F3G2
Nanosuspension F3 (%w/w)	4	4
Carbopol 934 (%w/w)	0.5	-
Carbopol 971 (%w/w)	-	0.5

Table 4. Mathematical models used to describe the drug release.

Formulation code	Zero order	First order	Peppas equation	Higuchi model
	R			
F3 G1	0.9962	0.9797	0.9901	0.9879
F3 G2	0.9953	0.9867	0.9837	0.9911

obtained. Consequently, on increasing the stirring time up to 30 min at stirring rate of 20000 rpm smaller and uniform microparticles were obtained with in the size range of 1–5 μm. Then, premix microsuspension was subjected to ultrasonic homogenization and the optimization of formulation was further carried out on the basis of ultrasound intensity. Reduction in the size depends on the hardness of the drug and the process parameters. The fineness of the drug nanoparticles depends upon powder density and ultrasound intensity (11). For the preparation of nanosuspension, microsuspension was homogenized for complete one cycle and 20 %, 40 %, 60 %, and 80 % amplitude intensities for 10 min, respectively.

Morphological characterization of nanosuspension formulations prepared at different amplitude intensities and one complete cycle for 10 min was done by scanning electron microscopy. Micrographs clearly showed that production parameter effected the crystal morphology. At low amplitude pressure of ultrasonication, larger rod like crystals with heterogeneous distribution were obtained, while at high amplitude pressure of ultrasonication, smaller, uniform rod shaped nanocrystals were obtained. Similar findings have also been reported for other drugs such as buparvaquone, ascorbyl palmitate and RMKP22 (12–14). The SEM photographs of nanosuspention formulation prepared at different amplitude intensities are presented in Figure 1.

The size of nanosuspension prepared at 80% amplitude pressure for 10 min was significantly smaller than nanosuspension prepared at 20%, 40% and 60% amplitude pressure. This indicated that a higher amplitude pressure could provide more energy to comminute particles leading to smaller size. Therefore, the nanosuspensions were prepared using high speed homogenization at 20000 rpm for 30 min and ultrasonic homogenization at 80% amplitude intensities and for 10 min.

Characterization of nanosuspensions

Four different formulations were prepared on the basis of optimized parameters**.** The photon correlation spectroscopy was used for determination of particle size, polydispersity index (PI). Average particle sizes of formulations F1, F2, F3 and F4 were found to be 380 nm, 1844 nm, 403 nm and 1503 nm, respectively. Polydispersity index of formulations F1, F2, F3 and F4 were found to be 0.823, 0.451, 0.669 and 0.045, respectively.

The different properties of formulations F1, F2, F3 and F4 were investigated by ζ potential measurements. The nanoparticles of formulation F1 had the ζ potential of -20.8 mV, F2 of -4.67 mV, F3 of 25.8 mV and F4 of –18.7 mV respectively. The ζ potential should have at least –30 mV for electrostatic and about ±20 mV for sterically stabilized systems that is necessary to obtain a physically stable suspension. The values of particle size, polydispersity index and ζ potential of different nanosuspension formulations are presented in Table 2.

The mean size of formulation F2 and F4 was found to be significantly higher reaching values higher than 1 μm. Formulation F2 had very low potential at -0.445 mV i.e., particles were practically uncharged. So the formulation F2 and F4 were rejected because of higher particle size and very low ζ potential.

The average size of formulations F1 and F3 was found to be in nano range and also the ζ potential of formulations F1 and F3 was found to be around ±20 mV. This indicated that both the formu-

lations were physically stable, and hence they were subjected to the dissolution studies.

Stability study of nanosuspensions

Physical stability of ciprofloxacin nanosuspensions F1 and F3 was studied at 25 ± 2°C for 2 weeks. Physical instability symptoms like change in appearance, Ostwald ripening and settling behavior were observed at predetermined intervals (1, 7 and 14 days). Ostwald ripening was observed by light microscopy (15). The observations indicated that no crystal growth was noted during the period of study. No changes in appearance and settling behavior were found when observed visually. The results

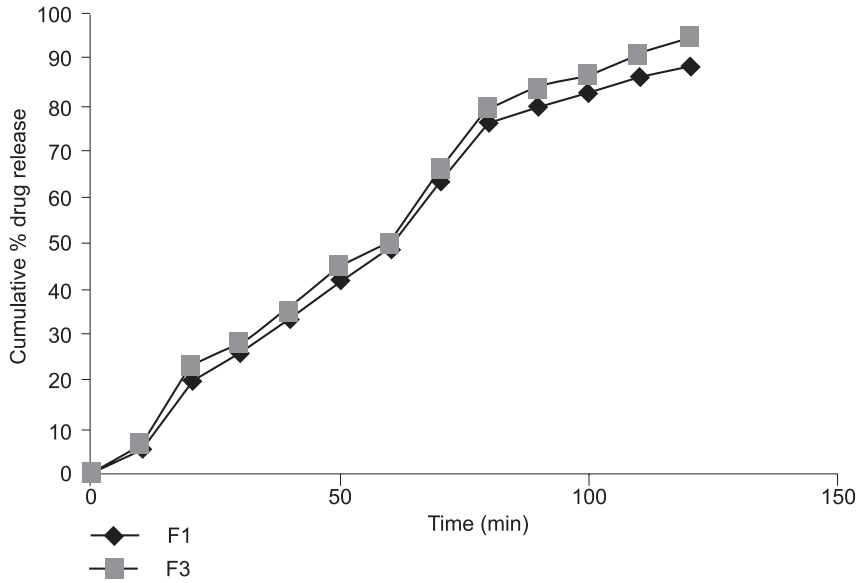

Figure 2. Dissolution profile of nanosuspension F1 and F3

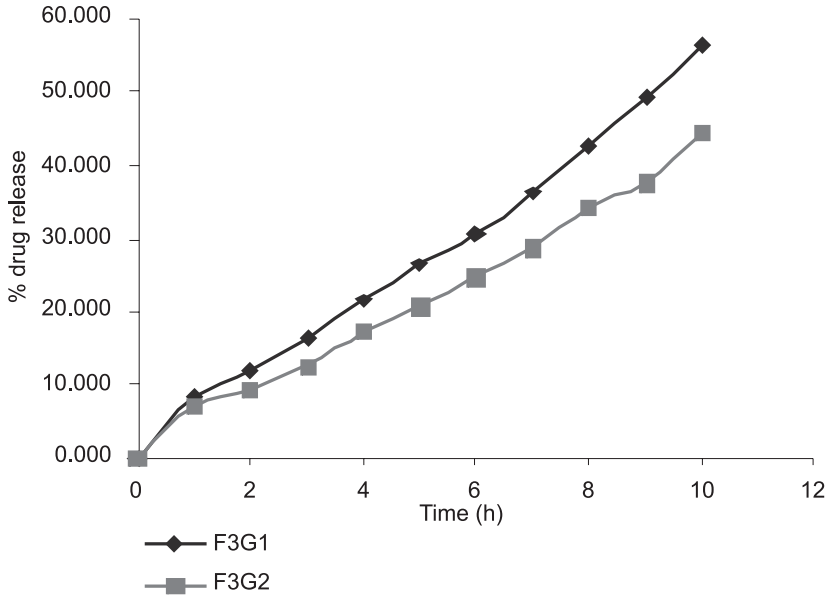

Figure 3. Release profile of mucoadhesive nanosuspension F3G1 and F3G2

indicated that there was no settling and the whole nanosuspension was physically stable.

***In-vitro* dissolution study**

The promising nanosuspension formulation F1 and F3 were subjected to *in vitro* dissolution studies. The results indicated that dissolution of ciprofloxacin increased with the decreasing particle size. In the first and second hour, drug release was found to be 55.5% and 94.5%, respectively, from formulation F3 and 49.5% and 87.4%, respectively, from formulation F1. This could be explained on the basis of Noyes-Whitney equation, which states that the increase of surface area (A) and saturation solubility (Cs) due to the reduction of radius could enhance dissolution velocity of poorly soluble compounds (2). The dissolution profile of formulation F1 and F3 are shown in Figure 2.

From the dissolution study it was clear that F3 had faster dissolution rate as compared to nanosuspension F1. So formulation F3 was selected for incorporation into mucoadhesive polymers and release rate studies.

Preparation of mucoadhesive nanosuspension:

Nanosuspension F3 was incorporated in mucoadhesive polymers carbopol 934, carbopol 971. The composition of mucoadhesive nanosuspensions are given in Table 3.

Release study of nanosuspension with hydrogels

The release behavior of mucoadhesive nanosuspensions was estimated *in vitro* as decribed above. The studies indicated that in 10 h formulations F3G1 and F3G2 could release 56.2% and 44.6% of the drug, respectively. The release profile of formulation F3G1 and F3G2 are shown in Figure 3.

The data obtained from release studies fitted into various kinetics models. The values of regression coefficient indicated that both formulations follow zero order kinetics. The highest value of regression coefficient was found 0.9962 with F3G1. The n values for Peppas model was found to be 0.8391 and 0.8113 for F3G1 and F3G2, respectively, indicating non fickian release. The R values of different kinetic models are presented in Table 4.

CONCLUSION

Ciprofloxacin bearing nanosuspension was successfully formulated. The system exhibited enhanced dissolution rate due to decreased particle size. The ultrasonication method used was shown to be a simple and efficient technique for particle size reduction and the parameters effecting performance of the formulation were optimized. These finding clearly indicate that bioavailability of ciprofloxacin can be maximized by the ultra probe sonication method, which results in a robust formulation. Further, by incorporation the nanosuspension into mucoadhesive hydrogel, the physical stability of the system was enhanced. The incorporation of the nanosuspension into mucoadhesive polymers can increase the gastric residence time of the ciprofloxacin resulting in more efficient system. The enhanced residence time in gastrointestinal tract may decrease the dosing frequency and amount of the drug given. This will further improve the patient compliance.

REFERENCES

1. Jchingunpituk J.: J. Sci. Tech. 4, 139, (2007).
2. Gao L., Eerdenbrugha B.V., Froyen L., Humbeeck J.V., Martensc J.A., Zhang D., Chen M., et al.: Int. J. Pharm. 355, 321, (2008).
3. Patravale V.B., Date A.A., Kulkarni R.M.: J. Pharm. Pharmacol.. 56, 827, (2004).
4. Kesisoglou F., Panmai S., Wu Y.: Adv. Drug Deliv. Rev. 59, 631, (2007).
5. Muller R.H., Jacobs C.: Int. J. Pharm. 237, 151, (2002).
6. Kayser O.: Int. J. Pharm. 214, 83, (2001).
7. Teeranachaideekul V., Junyaprasert V.B., Souto E.B., Muller R.H.: Int. J. Pharm. 354, 227, (2008).
8. Chingunpitak J., Puttipipatkhachorn S., Petmitr C.P., Tozuka P., Yamamoto K.: Drug. Dev. Ind. Pharm. 34, 314, (2008),
9. Zhang X., Tan T., Gao L., Zhao W., Wang P.: Drug. Dev. Ind. Pharm. 33, 569, (2008).
10. Gao L., Zhang D., Chen M., Zheng T., Wang S.: Drug Dev. Ind. Pharm. 33, 332, (2009).
11. Möschwitzer J.M., Georg A.G., Pomper H., Müller R.H.: Eur. J. Pharm. Biopharm. 58, 615, (2004).
12. Muller R.H., Jacobs C.: Int. J. Pharm. 237, 151, (2002).
13. Muller R.H., Peters K.: Int. J. Pharm. 160, 229, (1998).
14. Teeranachaideekul V., Junyaprasert V.B., Souto E.B., Muller R.H.: Int. J. Pharm. 354, 227, (2008).
15. Augustijnsa P., Mootera G.: Eur. J. Pharm. 355, 127, (2008).

Received: 27. 02. 2010