Formulation and Evaluation of Gastroretentive Drug Delivery System of Acyclovir as Mucoadhesive Nanoparticles

Ankit Anand Kharia[1,2]* and Akhlesh Kumar Singhai[2,3]

[1]*Oriental College of Pharmacy, Department of Pharmaceutics, Thakral Nagar, Raisen Road Bhopal, Madhya Pradesh, 462021, India;

[2]Uttarakhand Technical University, Department of Pharmacy, Chandanwadi, Prem Nagar, Sudhowala, Dehradun, Uttarakhand, 248007, India;

[3]Lakshmi Narain College of Pharmacy, Department of Pharmaceutics, Raisen Road, Bhopal Madhya Pradesh, 462021, India

*Corres.author: ankitanandkharia@yahoo.co.in
Mob. No. 09685360536

Abstract: The objective of this study was formulation and evaluation of mucoadhesive nanoparticles of acyclovir. Nanoparticles were prepared by one step desolvation method by using gelatin as a mucoadhesive polymer. The effects of amount of gelatin and Pluronic F-68 on particle size, polydispersity index, entrapment efficiency, loading efficiency, mucoadhesive strength were studied. Nanoparticulate formulation F4 with 1:2 gelatin and Pluronic F-68 in 1:2 showed satisfactory results i.e. average particle size 207.7 nm, polydispersity index 0.338, entrapment efficiency 54.21%, loading efficiency 43.13% and mucoadhesive strength of 6.012 g. FTIR study concluded that there was no major interaction occurred between the drug and polymers used in the present study.
Keywords: Acyclovir, mucoadhesion, gelatin, nanoparticles, gastroretention.

Introduction

Acyclovir, a cyclic analogue of the natural nucleoside 2-deoxyguanosine, clinically used in the treatment of *herpes simplex, varicella zoster, cytomegalovirus*, and *epstein barr* virus infections.[1] Acyclovir is currently marketed as capsules (200 mg), tablets (200, 400 and 800 mg) and suspension for oral administration, topical ointment and intravenous injection. Oral acyclovir is mostly used as 200 mg tablets, five times a day.[2] Absorption of orally administered acyclovir is slow, variable, and incomplete, with a bioavailability of 15%-30% and the elimination half-life of acyclovir is approximately 3 hours.[3] It has narrow absorption window and is primarily absorbed from stomach and upper part of the small intestine;[4-6] reduced bioavailability of acyclovir may be because of transportation of dosage form from the region of absorption window to site where it is less absorbed. Therefore there was a need to increase the gastroretention time of dosage form so that drug would be available at the site of absorption and results in improved bioavailability.

Several attempts are being made to increase the gastric retention of drugs, like intra-gastric floating systems, hydro dynamically balanced systems, extendable or expandable, microporous compartment system, microballons, bio/muco-adhesive systems, high-density systems, and super porous biodegradable hydro gel systems.[7] After oral administration, such a dosage form would be retained in the stomach for several hours and would release the drug there in a controlled and prolonged manner, so that the drug could be supplied continuously to its absorption sites in the upper gastrointestinal tract.[8] Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility of drugs that are less soluble in a high pH environment. It is also suitable for local drug delivery to the stomach and proximal small intestine.[9] Gastroretention helps to provide better availability of new products with suitable therapeutic activity and substantial benefits for patients.

The aim of the present study was to study the effect of amount of gelatin and Pluronic F-68 on formulation of mucoadhesive gastroretentive nanoparticles of acyclovir. Gelatin was selected as a mucoadhesive polymer to prepare gastroretentive nanoparticles as they strengthen the contact between dosage form and the site of absorption, thereby reducing the luminal diffusion pathway of the drug and lead to significant improvements in oral drug delivery.[10,11] These mucoadhesive polymeric nanoparticles in the stomach will offer diverse advantages such as (a) Longer residence time of the dosage form on gastric mucosa which will improve absorption of the drug and increase the bioavailability. (b) Higher drug concentration at the site of adhesion absorption, which will create a driving force for the paracellular passive uptake. (c) Immediate absorption from the bioadhesive drug delivery system without previous dilution and probable degradation in the luminal fluids.[12]

Materials And Methods

Acyclovir was obtained as a gift sample from M/s Modern Laboratories Pvt. Ltd., Indore, India. Dialysis tubing (cut-off 12 kDa) was purchased from Sigma (USA). Gelatin (type B) and Pluronic F-68 were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All other ingredients used throughout the study were of analytical grade and were used as received.

Preparation of Acyclovir Loaded Nanoparticles

Gelatin nanoparticles were prepared by desolvation method as described by Coester et al. with slight modification. Gelatin was dissolved in 25 ml of distilled water under gentle heating and 200 mg acyclovir was dispersed in 50 ml of distilled water. Drug dispersion was then added to the polymeric solution followed by addition of Pluronic F-68 as a stabilizer under continuous stirring at 1000 rpm by using magnetic stirrer and the pH of solution was adjusted to pH 2 ±0.05 (by 1N HCl or 1N NaOH). Then 50 ml of acetone was added at addition rate of 3 mL/min, after 10 min of acetone addition 0.3 mL of glutaraldehyde was added to the reaction vessel to crosslink the nanoparticles. Finally after stirring for 3hr, the particles were purified by three fold centrifugation (16000 g for 20 min at 4 ºC) and redispersion in 10 ml mixture of acetone: water (3:7). The supernatant was removed and the pellets were resuspended in distilled water and finally, the nanoparticles were freeze-dried using 5% glucose solution as a cryoprotector and powder was stored in vials.[13]

Evaluation of Formulations

Particle Size and Particle Size Distribution

Average particle size and polydispersity index (PI) which is a range of measurement of the particle sizes within measured samples were determined using the zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. For analysis nano-suspensions were diluted five times with filtered (0.45µm) bidistilled water.[14]

Entrapment Efficiency

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV spectrophotometer at 254 nm (UV-1700 Spectrophotometer, Shimadzu Scientific

Instruments, Inc. Maryland, USA). A standard calibration curve of drug was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (W). Effectively, (W-w) will give the amount of drug entrapped in the particles.[15]

Then percentage entrapment of a drug is obtained by using following equation

% Drug Entrapment = (W-w) $\times$ 100 / W

Drug Loading

The acyclovir content in the nanoparticles was determined by pulverizing the acyclovir loaded nanoparticles (10mg) followed by immersing them in 100 ml simulated gastric fluid (SGF, pH 1.2, without enzymes) with agitating at room temperature for 12 h. After filtration through a 0.45µm membrane filter (Millipore), the drug concentration was determined spectrophotometrically at the wavelength of 254 nm (UV-1700 Spectrophotometer, Shimadzu Scientific Instruments, Inc. Maryland, USA). The filtered solution from the empty nanoparticles (without acyclovir) was taken as blank. All samples were analyzed in triplicate and the drug loading (DL) was calculated according to the following equation:[16]

DL (%) = WD/WT $\times$ 100

Where, DL: drug loading; WD: the weight of the drug loaded in the nanoparticles;

WT: the total weight of the nanoparticles.

Table 1: Formulation of mucoadhesive nanoparticles of acyclovir

Code	Amount of gelatin (mg)	Amount of Pluronic F-68 (mg)	Amount of glutaraldehyde (mL)	Acetone Addition rate (mL/min)	pH	Stirring Speed (rpm)	Stirring Time (h)
F1	400	50	0.3	3	2	1000	3
F2	800	250	0.3	3	2	1000	3
F3	200	250	0.3	3	2	1000	3
F4	200	400	0.3	3	2	1000	3
F5	600	400	0.3	3	2	1000	3
F6	600	150	0.3	3	2	1000	3
F7	1200	150	0.3	3	2	1000	3
F8	1000	400	0.3	3	2	1000	3

Drug amount was kept constant at 200 mg

Measurement of Mucoadhesive Strength

The method is based on the measurement of shear stress required to break the adhesive bond between a mucosal membrane and the formulation. The formulation is sandwiched between two mucosal membranes fixed on flexible supports in the assemblies for a sufficient period of time. After the adhesive bond has formed, the force (weight) required to separate the bond was recorded as mucoadhesive strength.

Mucoadhesive properties of acyclovir nanoparticles were evaluated by Texture analyzer (M/s TA. XT. Plus, Stable Microsystem, UK) using porcine gastric mucosa. Stomach of pig was obtained immediately after slaughter at local slaughterhouse. The stomach was washed with fresh water to remove non-digested food from stomach then placed in SGF at 4^{0}C (used within 6 h). The membrane was then attached both on the base of texture analyzer and to the stainless steel probe (using two sided adhesive tape), probe is then fixed to the mobile arm of the texture analyzer. The 10 mg of nanoparticulate formulation was placed on the membrane placed on lower surface moistened with 1 ml of SGF. The mobile arm (with attached membrane) was lowered at a rate of 0.5 mm s^{-1} until contact with the formulation was made. A contact force of 10 g was maintained for 500 s, after which the probe was withdrawn from the membrane. After the adhesive bond has formed, the force (g) required to separate the bond was recorded as mucoadhesive strength.[17]

Drug-Excipient Compatibility Studies

The Drug excipient compatibility was performed by using FT-IR spectrophotometer (Perkin Elmer). The FT-IR spectra of Drug and formulations were analyzed separately and then correlated for incompatibility.

Results And Discussion

The results for evaluation of mucoadhesive nanoparticles for particle size, PDI, entrapment efficiency, loading efficiency and mucoadhesiveness are given in table 2. The size of all nanoparticulate formulations prepared varied between 207.7 and 452.2 nm, PDI between 0.292 to 0.506, entrapment efficiency between 36.29 and 78.11, loading efficiency between 24.45 and 43.13 and mucoadhesive strength between 6.012 to 10.974 g.

Particle Size and Particle Size Distribution

The particle size and size distribution are the most important characteristics of nanoparticles systems. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system. It was found that as the amount of gelatin was increased the particle size of nanoparticles also increases. While increasing the quantity of Pluronic F-68 results in reduction in size of the formulation because it decreases the surface tension between organic and aqueous phase and leads to the formation of smaller solvent droplets, which in turn causes decrease in particle size. It also stabilizes newly generated surfaces and prevents aggregation of the particles which is similar to the previous studies.[18] While high stirring speed and amount of polymer resulted in lowering of PDI. Considering that the polydispersity (PDI) is calculated from the square of the standard deviation/mean diameter, less value of polydispersity index indicates enhanced homogeneity of the nanoparticles. The report for particle size analysis of optimized formulation is shown in figure 1.

Table 2: Evaluation of mucoadhesive nanoparticles of acyclovir

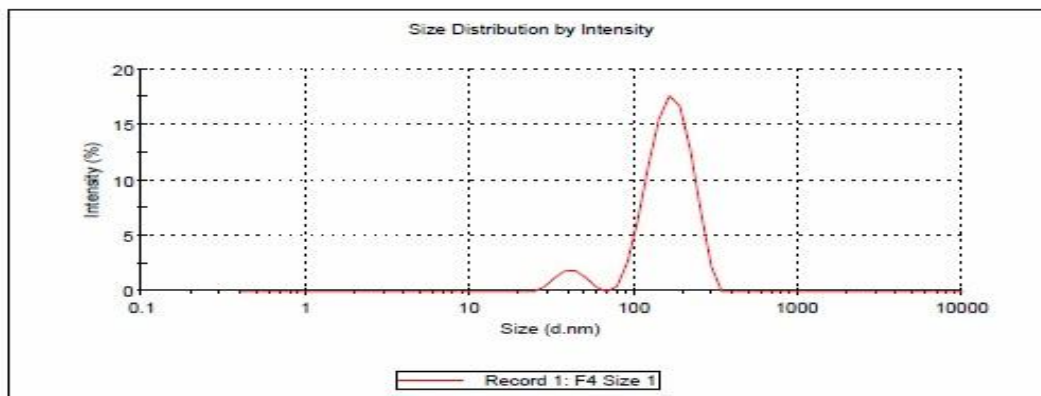| Formulation	Particle size (nm)	PDI	Entrapment efficiency* %	Drug loading* %	Mucoadhesion strength* (g)
F1	219.1	0.506	36.29±2.48	26.45±2.44	7.425±0.157
F2	386.5	0.292	78.11±2.54	33.04±1.14	8.544±0.079
F3	324.1	0.310	59.35±3.17	24.45±2.07	6.045±0.145
F4	207.7	0.338	54.21±3.41	43.13±3.54	6.012±0.725
F5	224.1	0.486	58.99±2.15	39.57±2.78	6.254±0.452
F6	380.6	0.493	65.51±2.49	37.74±3..42	7.142±0.275
F7	452.2	0.312	76.28±3.07	40.57±2.54	10.974±0.145
F8	272.0	0.361	69.14±2.41	26.45±2.97	7.452±0.078

*Mean ± SD (n = 3)

Figure 1: Particle size analysis report for formulation F4.

Entrapment Efficiency

Entrapment efficiency is the ratio of the experimentally determined percentage of drug content compared with actual or theoretical mass, of drug used for the preparation of the nanoparticles. Entrapment efficiency of formulation was in the range of 36.29% to 78.11%. It was observed that when the concentration of Pluronic F-68 was kept constant the entrapment efficiency increases with increase in the polymer concentration, so that sufficient quantity of polymer will be available to entrap the drug present in the solution. When concentration of polymer was kept constant, entrapment efficiency decreases with increase in the concentration of Pluronic F-68. This may be because of solubilization effect of Pluronic F-68 which reduces the entrapment of drug by the polymer during rigidization of nanoparticles by the glutaraldehyde and results in leakage of drug during cross linking.

Drug Loading

Drug loading expresses the percent weight of active ingredient encapsulated to the weight of nanoparticles. The loading efficiency depends on the polymer-drug combination and the method used. Hydrophobic polymers encapsulate larger amounts of hydro phobic drugs, whereas hydrophilic polymers entrap greater amounts of more hydrophilic drugs. Several formulation parameters, such as emulsifier type, weight ratio of polymer to drug, and organic to aqueous phase ratio, will influence the extent of drug loading. Loading efficiency of formulation was in the range of 24.45% to 43.13%. In most of the cases loading efficiency of the formulation was showing positive trend on increasing the amount of gelatin and Pluronic F-68.

Mucoadhesive Strength

Mucoadhesion studies were performed by using texture analyzer and representative graph showing force of detachment is given in figure 2. Point F represents the force at which the breaking of the adhesive bond starts (i.e., the force of detachment). It was found that increase in polymer ratio increases the mucoadhesiveness of the formulation due to bioadhesive nature of the polymer. While increase in concentration of Pluronic F-68 and glutaraldehyde results in lowering of mucoadhesiveness which may be due to surfactant effect of Pluronic F-68 which reduces the adhesive property of the mucoadhesive polymer due to its solubilizing effect.

Drug-Excipient Compatibility Studies

The results of drug-excipient compatibility studies are shown in table no.3. From the IR data it is clear that functionalities of drug have remained unchanged including intensities of the peak. This suggests that during the process of formulation polymer has not reacted with the drug to give rise to reactant products. So it is only physical mixture and there is no interaction between them which is in favor to proceed for formulation.
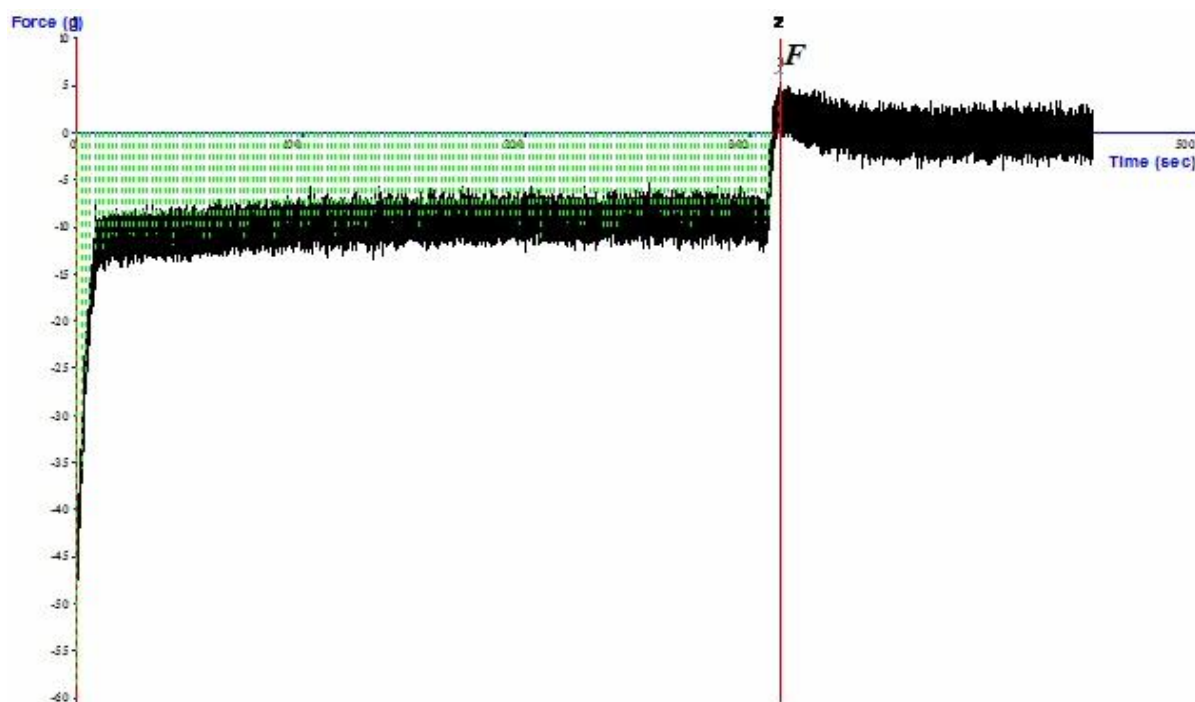

Figure 2: Graph showing force of detachment (point F represents the force of detachment).

Table 3: Major peaks observed in the FT-IR spectrum

S. No.	Bands	Wave number (cm^{-1})	
		Pure drug	F4
1.	-OH	3447.8 and 3337.9	3441.1
2.	N-H bend, assy.	1632.5	1635.4
3.	C-C ring str.	1537 and 1483.7	1532.1 and 1481.1
4.	Aromatic amines, C-N str.	1305.9 and 1392.1	1303.6
5.	Assy. C-O-C str.	1219	1216.5
6.	Sym C-O-C str.	1013	1011.2
7.	N-H wagging	900.5 and 864.2	899.5
8.	Mono-substitution in ring	777.1 and 681.2	769.8 and 670.9

Conclusion

Among different nanoparticulate formulations prepared by one-step desolvation method formulation F4 with gelatin: Pluronic F-68 in the ratio of 1:2 showed satisfactory results i.e. particle size 207.7 nm, polydispersity index 0.338, entrapment efficiency 54.21, loading efficiency 43.13 and mucoadhesive strength of 6.012 g. FTIR study concluded that there was no major interaction occurred between the drug and polymers used in the present study.

References

1. O'Brien JJ, Campoli-Richards DM. Acyclovir: an updated review of its antiviral activity, pharmacokinetics properties and therapeutic efficacy. Drugs 1989;37(3):233-309.

2. Ruhnek-Forsbeck M, Sandstrom E, Andersson B, Eriksson G, Hersle K, Lovhagen GB, Mobacken H, Hillstrom L, Svensson L. Treatment of recurrent genital herpes simplex infection with oral acyclovir. J Antimicrob Chemother 1985;16(5):621-8.

3. Shao Z, Mitra AK. Bile salts-fatty acid mixed micelles as nasal absorption promotors: III. Effect on nasal transport and enzymatic degradation of acyclovir prodrug. Pharm Res 1994;11(2):243-50.

4. Groning R, Berntgen M, Geogarakis M. Acyclovir serum concentrations following per oral administration of magnetic depot tablets and the influence of extra corporal magnet to control gastrointestinal transit. Eur J Pharm Biopharm 1996;46(3):285-91.

5. Meadows KC, Dressman JB. Mechanism of acyclovir uptake in rat jejunum. Pharm Res 1990;7(3):299-303.

6. Park GB, Shao Z, Mitra AK. Acyclovir permeation enhancement across intestinal and nasal mucosae by bile salt–acylcarnitine mixed micelles. Pharm Res 1992;9(10):1262-7.

7. Singh BN, Kim KH. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J Control Release 2000;63(3):235-9.

8. Streubel, A., Siepmann, J., Bodmeier, R., 2006. Gastroretentive drug delivery systems. Expert Opin. Drug Deliv. 3 (2), 217-33.

9. Kharia AA, Hiremath SN, Singhai AK, Omray LK, Jain SK. Design and optimization of floating drug delivery system of acyclovir. Indian J Pharm Sci 2010;72(5):599-606.

10. Lueben HL, Lehr CM, Rentel CO, Noach ABJ, de Boer AG, Verhoef JC, Junginger HE. Bioadhesive polymers for the peroral delivery of peptide drugs. J Control Release 1994;29(3):329-38.

11. Park K, Robinson JR. Bioadhesive polymers as platforms for oral-controlled drug delivery: method to study bioadhesion. Int J Pharm 1984;19(2):107-27.

12. Hejazi R, Amiji M. Chitosan-based gastrointestinal delivery systems. J Control Release 2003;89(2):151-65.

13. Coester CJ, Langer K, van Briesen H, Kreuter J. Gelatin nanoparticles by two step desolvation-a new preparation method, surface modifications and cell uptake. J Microencapsul 2000;17(2):187-93.

14. Elshafeey AH, Kamel AO, Awad GA. Ammonium methacrylate units polymer content and their effect on acyclovir colloidal nanoparticles properties and bioavailability in human volunteers. Colloids Surf B Biointerfaces 2010;75(2):398-404.

15. Das S, Banerjee R, Bellare J. Aspirin loaded albumin nanoparticles by coacervation: implications in drug delivery. Trends Bio Artif Organs 2005;18(2):203-11.

16. Ma N, Xu L, Wang Q, Zhang X, Zhang W, Li Y, Jin L, Li S. Development and evaluation of new sustained-release floating microspheres. Int J Pharm 2008;358(1-2):82-90.

17. Thirawong N, Nunthanid J, Puttipipatkhachorn S, Sriamornsak P. Mucoadhesive properties of various pectins on gastrointestinal mucosa: an in vitro evaluation using texture analyzer. Eur J Pharm Biopharm 2007;67(1):132-40.

18. Schubert MA, Muller-Goymann CC. Solvent injection as a new approach for manufacturing lipid nanoparticles-evaluation of the method and process parameters. Eur J Pharm Biopharm 2003;55(1):125-31.

*****