Available online on 15.12.2018 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Formulation and evaluation of polymeric nanoparticles of an antihypetensive drug labetalol

Surendranath Betala[1]*, M. Mohan Varma[2], K. Abbulu[3]

[1] Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andrapradesh. India

[2] Sri Vishnu College of Pharmacy, Bhimavaram, Andrapradesh. India

[3] CMR College of Pharmacy, Hyderabad, Telangana, India

ABSTRACT

Labetalol is an adrenergic receptor blocking agent used in the treatment of hypertension and characterized by high solubility and high permeability which corresponds to BCS class I drug. Plasma half life ranges from 6 & bioavailability is 25%. Ethyl cellulose was used as a rate controlling polymer. Effects of addition of ethyl cellulose on *in vitro* dissolution were studied. Nanoparticles were formulated using different polymer ratios. *In vitro* drug release was carried out by using USP Type II at 50 rpm in 900 ml of acidic dissolution medium (p^H 1.2) for 2 hours, followed by 900 ml alkaline dissolution medium (p^H7.4) for 12 hours. Mean dissolution time is used to characterize drug release rate from a dosage form. Several kinetic models were applied to the dissolution profiles to determine the drug release kinetics. Excipients are selected by FTIR studies. Finally the nanoparticles were evaluated for various characteristics like encapsulation efficiency, percentage yield, partial size and the *In vitro* release for 12 hrs. The nanoparticles were found to be discrete, spherical, and free-flowing. The nanoparticles were uniform in size, and the microencapsulation efficiency was in the range of 52.5-81.7%. The surface morphology of prepared Labetalol nanoparticles was observed under scanning electron Microscopy. Nanoparticles had good spherical geometry. The stability study was performed at 40ºC ± 2ºC and 75 ± 5% RH for 6 months.

Keywords: Nanoparticles; Labetalol, Hypertension, Ethyl Cellulose, Dissolution, entrapment efficiency.

Article Info: Received 21 Oct 2018; Review Completed 03 Dec 2018; Accepted 05 Dec 2018; Available online 15 Dec 2018

Cite this article as:

Betala S, Varma MM, Abbulu K, Formulation and evaluation of polymeric nanoparticles of an antihypetensive drug labetalol, Journal of Drug Delivery and Therapeutics. 2018; 8(6-s):187-191

DOI: http://dx.doi.org/10.22270/jddt.v8i6-s.2109

***Address for Correspondence:**

Surendranath Betala, Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andrapradesh. India

INTRODUCTION

Nanotechnology has been introduced into several aspects of the food science, including apsulations and delivery systems, which has versatile advantages such as incorporation of the bioactive compounds into the food matrices with high physicochemical stability and minimal impact on the properties of the product, as well as protection of the encapsulated bioactive compounds from the interaction with other food components, and maximize the uptake of the encapsulated compounds upon intake and their transport to the sites of action. Nanoparticles are one of the different types of nano-sized carriers generally have size below than 1000 nm that being developed for drug delivery applications. Nanoparticles classified as non-degradable and biodegradable. In recent years, there has been considerable attention to developing biodegradable nanoparticles due to their higher encapsulation efficiency, controlled release and less toxic properties. Numerous processes have been extensively described in past years for the preparation of nanoparticles, including emulsion/solvent evaporation, nanoprecipitation, emulsion and miniemulsion polymerization, salting out, using supercritical fluid technology, electrohydrodynamic atomization, and the generation of nanoparticles using the nano-emulsion template. The nanoprecipitation technique (or solvent displacement method) is a modified solvent evaporation method for nanoparticle production. In this method the water-miscible solvents like acetone or methanol along with the water immiscible organic solvent like chloroform or dichloromethane were used as an oil phase. Rapid dispersion of the polymers in the presence of water can cause precipitation of polymers. This technique has many advantages like rapid and easy to perform where the entire procedure could carry out in only one step. Different methodologies have been reported in the literature to increase the gastric retention of drugs, like intra-gastric floating systems, hydro dynamically balanced systems, extendable or expandable, micro porous compartment system, microballons, bio-adhesive systems,

high-density systems, and super porous biodegradable hydro gel systems. 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. 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. Gastro retention 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 formulate gastro retentive nanoparticles of Labetalol to deliver the drug at a controlled rate to its absorption site so that its oral bioavailability can be enhanced. Mucoadhesive polymers, such as bovine serum albumin, chitosan, and gelatin, were selected to prepare gastroretentive nanoparticles as they intensify the contact between dosage form and the site of absorption, thereby reducing the luminal diffusion pathway of the drug (bioadhesion) and lead to significant improvements in oral drug delivery. These mucoadhesive polymeric nanoparticles in the stomach will offer various advantages such as (i) Longer residence time of the dosage form on mucosal tissues in the stomach. This will improve absorption of the drug and increase the drug bioavailability. (ii) Higher drug concentration at the site of adhesion absorption, which will create a driving force for the paracellular passive uptake. (iii) Immediate absorption from the bioadhesive drug delivery system without previous dilution and possible degradation in the luminal fluids.

MATERIALS AND METHODS

Materials

Labetalol sample obtained from Yarrow chem. Products Mumbai. Polymers Ethyl cellulose from Ozone International Mumbai. Gelatin and Chitosan from S.D.Fine chemicals, Munbai and HPMC K 100M from Loba chem.Pvt Ltd, Mumbai.

Equipments

UV-Visible spectrophotometer - Lab India Model-UV3092, Mumbai, Mechanical stirrer - Kshitij Innovations, Ambala Cantt, Sonicator - Loba Life, Mumbai, Dissolution test apparatus - Lab India- DS 8000, Mumbai, Analytical balance - Shimadzu, Japan, Hot air oven - Fortune, Mumbai, Stability Chamber - Remi Elektro Technik Limited, Vasai.

Drug Profile

Labetalol

Chemical Name: 2-hydroxy-5-[1-hydroxy-2-[(4-phenyl-2-butanyl) amino]ethyl]benzamide

Systematic (IUPAC) Formula: $C_{19}H_{24}N_2O_3$

Mol. mass: 328.41 g/mol

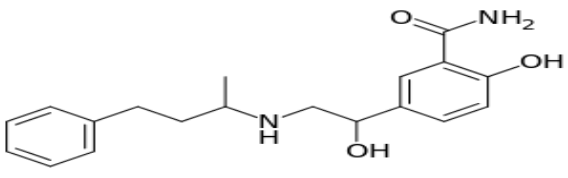

Figure 1: Structure of Labetalol structure

Solubility: sparingly soluble in water and in ethanol (95%), insoluble in chloroform and ether.

Description:

Labetalol is a white crystalline powder. It is an adrenergic receptor blocker drug characterized by high solubility and high permeability which corresponda to BCS class 1 drug. It works by blocking both alpha and beta receptors in the body which lowers blood pressure.

Protein binding: Plasma protein binding is 50%

Metabolism: Primarily hepatic, undergoes significant first pass metabolism

Half-life: 6 hours

Preparation of Nanoparticles

Nanoparticles were prepared according to the nanoprecipitation method with slight modification. Briefly, 200 mg of polymer (HPMC, Ethyl cellulose, chitosan, and gelatin) was dissolved in 25 ml of acetone separately. The Labetalol 100 mg was dissolved in 2 ml of dimethylsulfoxide. Both solutions were mixed and then 50 ml of water was added and stirred for a half hour. Acetone was eliminated by evaporation under reduced pressure using rotary flash evaporator and the final volume of the suspension was adjusted to 10 ml. Then this suspension was centrifuged at 15000 rpm at 4oC for half an hour. The supernatant was discarded and precipitate was washed 3 times with distilled water. The nanoparticles thus obtained were dried overnight in oven at 60ºC and stored in a desiccator. The prepared formulations were characterized for loading efficiency, entrapment efficiency, particle size, particle size distribution, polydispersity index, zeta potential and drug excipient compatibility studies.

Drug-Excipient Compatibility Studies

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

Characterization of Labetalol Loaded Nanoparticles

Loading Efficiency

Drug content in the preparation was determined by extracting the drug from the nanoparticles with 0.1 M hydrochloric acid. In this method, the nanoparticles (50 mg) were stirred in 50 ml of 0.1 M hydrochloric acid until dissolved; it was filtered through a Millipore filter and the drug content was determined, after suitable dilution, at 254 nm by UV spectrophotometry. The loading efficiency (L) of the nanoparticles was calculated according to Equation 1

$$L (\%) = (Qn / Wn) \times 100 \quad .....(1)$$

Where Wn is the weight of the nanoparticles and Qn is the amount of drug present in the nanoparticles.

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. 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.

Then percentage entrapment of a drug was calculated according to Equation 2

$$\% \text{ Drug Entrapment} = (W\text{-}w/W) \times 100 \quad \text{.....(2)}$$

Particle Size, Particle Size Distribution, and Zeta Potential

The particle size and particle size distribution of the formulation was determined by photo correlation spectroscopy with a zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. Every sample was diluted with distilled water. The surface charge (Zeta potential) was determined by measuring the electrophoretic mobility of the nanoparticles using a Malvern zeta sizer (Malvern Instruments, UK). Samples were prepared by diluting with distilled water.

Polydispersity Index

Polydispersity index is a parameter to define the particle size distribution of nanoparticles obtained from photon correlation spectroscopic analysis. It is a dimensionless number extrapolated from the autocorrelation function and ranges from a value of 0.01 for mono dispersed particles and up to values of 0.5-0.7. Samples with very broad size distribution have polydispersity index values > 0.7.

Percentage yield:

The prepared microspheres of all batches were accurately weighed. The measured weight of prepared microspheres was divided by total amount of all the excipients and drug used in preparation of the microspheres, which give the total percentage yield of microspheres.

It was calculated by using following equation;

$$(\%) = \frac{\text{Actual weight of product}}{\text{Total weight of excipients and drug}}$$

***In vitro* dissolution studies**

The dissolution profiles of Labetalol Microspheres were investigated with a dissolution apparatus (LAB INDIA DS-8000), according to type II paddle method, with the rotation speed of paddle was set on 50 and the bath temperature was kept at 37.0 ± 0.5°C. Equivalent 100 percent of drug preparation was filled into the empty capsule. The capsule was put into the vessel containing 900ml of PH 1.2 HCl buffer for 2 hours, followed by alkaline dissolution medium pH 7.4 phosphate buffer for 10 hours. To avoid the float of capsule sinkers were used. At specific intervals, 5ml of aliquot of dissolution medium was sampled, filtered the sample by the U.V-Visible spectrophotometer. Absorbance of the sample solution compared with the standard solution having a known concentration of Labetalol.

RESULTS AND DISCUSSION

The method of nanoprecipitation was used so as to avoid both chlorinated solvents and surfactants to prevent their toxic effect on the body. All the determinations were done in triplicate.

Drug-loading and entrapment efficiency

Although drug loading expresses the percent weight of active ingredient encapsulated to the weight of nanoparticles, 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. 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. The effect of polymer on drug loading efficiency and entrapment efficiency are given in Table.

Table 1: Drug loading and Entrapment efficiency

S.No.	Formulation code	Drug : Polymer	Loading efficiency ± SD	Entrapment efficiency ± SD
1	F 1	1:2	24.23 ± 0.3	45.6 ± 0.6
2	F 2	1:2	29.62 ± 0.2	64.3 ± 0.6
3	F 3	1:2	38.72 ± 05	81.4 ± 0.8
4	F 4	1:2	32.41 ± 0.2	83.47 ± 1.0

* = Average of three determinations

Particle Size Distribution and Polydispersity Index

The particle size and particle size distribution are critical factors in the performance of nanoparticles, as batches with wide particle size distribution show significant variations in drug loading, drug release, bioavailability, and efficacy. Particle size and particle size distribution can be determined using light scattering techniques and by scanning or transmission electron microscopy. Formulation of nanoparticles with a narrow size distribution will be a challenge if emulsion cannot be produced with a narrow droplet size distribution. As nanoparticles are internalized into cells by endocytosis, an increase in particle size will decrease uptake and potentially, affect bioavailability of the drug. The extent of endocytosis is dependent on the type of the target cell.

Table 2: Drug polymer ratio, mean particle size, particle size distribution, poly dispersity index (PDI) and zeta potential.

F. Code	Polymer	Mean Particle Size (nm) ± SD	Size Distribution	PDI ± SD	Zeta Potential (mV) ± SD
F 1	Ethyl cellulose	140.5 ± 1	09.1% (15-30 nm) 90.9 % (200-400 nm)	1.2 ±0.10	25.7± 1.2
F 2	Chitosan	320..6 ± 2	08.8% (48-90 nm) 91.2% (200-525 nm)	1.6 ±0.12	21.1±1.5
F 3	Gelatin	640..3 ± 4	12.2% (70-160 nm) 80.8% (480-1200 nm)	1.7±0.13	12.2±1.5
F 4	HPMC	142.05 ± 2	11.1% (15-30 nm) 89.9 % (200-400 nm)	1.2 ±0.15	25.5±1.2

* = Average of three determination

From the above data it is clear that nanoparticles prepared by using chitosan and HPMC exhibited reduction in mean nanoparticulate diameter and narrower granulometric distribution. But the nanoparticles prepared using gelatin as a polymer resulted in nanoparticulate population of large particles. The higher particle size and polydispersity index may be because of absence of emulsifier as the use of emulsifier decreases the surface tension between organic phase acetone 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 as reported by previous researchers. Therefore results which were obtained in this study may be improved by using increased drug:polymer ratio, using different formulation strategy such as desolvation (for gelatin and albumin) or counter ion induced aggregation (for chitosan and sodium alginate), employing cross linking agent followed by neutralizing residual cross linking agent with cysteine and high speed stirring.

Zeta Potential

The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersions. In general, particle aggregation is less likely to occur for charged particles (i.e. high zeta potential) due to electric repulsion. Generally, Zeta potential values above 30 mV (positive or negative values) lead to more stable nanocapsule suspensions because repulsion between the particles prevented their aggregation. A decrease in zeta potential, i.e. electrostatic repulsion, was considered as the cause for the aggregation process. The charge on the surface of the nanospheres will influence their distribution in the body and the extent of uptake into the cells. Because cell membranes are negatively charged, there is greater electrostatic affinity for positively charged nanoparticles. Therefore, the surface of cationic or neutral nanoparticles may be modified to confer a positive charge to enhance efficacy. The zeta potential values which were in the range of –24.3 - +25.9 mV, indicates that the colloidal suspension may not be stable and may lead to aggregation. Zeta potential values can be altered by modifying the major components such as surfactants, polymer, and surface composition of the nanoparticles, the presence or the absence of adsorbed compounds, composition of the dispersing phase, mainly the ionic strength, and the pH.

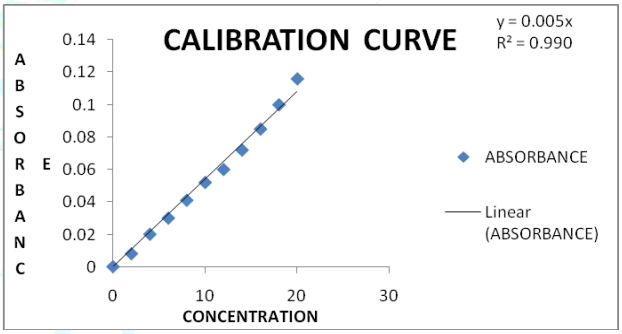

Figure 2: Calibration curve of Labetalol in pH 7.4 Phosphate buffer

Comparative Study

Table 3: comparative study of % CDR

TIME (hrs)	%CDR F 1	%CDR F 2	%CDR F 3	%CDR F 4
0	0	0	0	0
1	18.05	21.66	25.27	32.49
2	21.66	25.27	32.49	36.10
3	30.32	35.37	44.76	51.98
4	31.76	38.98	49.09	54.15
5	34.65	43.32	53.42	59.2
6	36.82	46.2	54.87	63.53
7	40.43	49.09	57.76	66.42
8	43.32	52.7	61.37	70.03
9	47.65	56.31	65.7	72.2
10	51.26	59.2	69.31	75.81
11	54.87	62.81	72.92	80.14
12	60.64	66.42	75.81	83.03

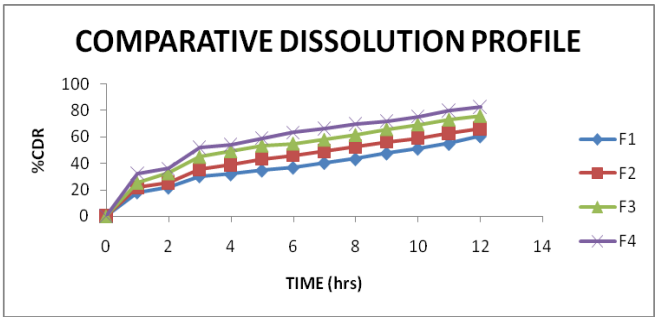

Figure 3: Comparative Dissolution Profile graph for Formulation F1-F4

Inference: Microspheres showed highest release within 12 hours and Formulation F4 showed better sustained release than the remaining formulations.

CONCLUSION

Among different nanoparticulate formulations prepared by nanoprecipitation method formulation F4, with HPMC in 1:2 drug: polymer ratio, showed satisfactory results; i.e. mean particle size of 142 nm (majority of the particles were in the range of 150-500 nm), polydispersity index of 1.2, zeta potential of 25.5 and loading efficiency of 32.41 %, and entrapment efficiency of 83.47 % and in vitro drug release as pes specifications.

REFERENCES

1. Peltonen L., Koistinen P., Karjalainen M.,Hakkinen A., Hirvonen J., The effect ofcosolvents on the formulation of nanoparticles from low molecular weight poly(l)lactide, AAPS PharmSciTech, 2002; 3:1-7.
2. Cui F., Oian F., Yin C., Preparation and characterization of mucoadhesive polymercoated nanopaticles, Int. J. Pharm., 2006; 316:154-161.13.
3. Saparia B., Murthy R.S.R., Solanki A.,Preparation and evaluation of chloroquine phosphate microspheres using cross-linked gelatin for long term drug delivery, Indian J. Pharm. Sci., 2002; 64:48-52.
4. Haznedar S., Dortunc B., Preparation and evaluation of Eudragit microspheres containing acetazolamide, Int. J. Pharm.,2004; 269:131-140.15.
5. Higuchi T., Mechanism of sustained actionmedication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices, J.Pharm. Sci., 1963; 52:1145-1149.
6. Ramteke S., Maheshwari R.B.V., Jain N. K., Clarithromycin based oral sustained release nanoparticulate drug delivery system, Indian J. Pharm. Sci., 2006; 68:479-484.
7. Shashikanth D. Barhate, Yogesh S. Rupnar, Rupesh M. Sonvane et al. Formulation and evaluation of floating nanoparticles of ketorolac trometamol, IJPRD. 2009; 1(9):1-8
8. Atul D karande, Pramod G. Yeole. Comparative assessment of different dissolution apparatus for floating drug delivery systems. Dissolution technologies. 2006: 20-26
9. Ram C. Dhakar, Maurya SD, Saluja V. From formulation variables to drug entrapment efficiency of nanoparticles: A technical review. *Journal of Drug Delivery and Therapeutics,* 2012; 2(6):128-133. https://doi.org/10.22270/jddt.v2i6.160
10. Sachin B Somwanshi, Ramdas T Dolas, Vikrant K Nikam., Floating multiparticulate oral sustained release drug delivery system. J. Chem. Pharm. Res., 2011; 3(1):536-547.
11. Ramesh C Nagarwal, Devendra N Ridhurkar, J K Pandit. In-vitro release kinetics and bioavailability of gastroretentivecinnarizineHCl tablet. AAPS PharmaSci Tech. 2010; 11(1):294-303.
12. Barratt G. Characterization of colloidal drug carrier systems with zeta potential measurements. *Pharma Tech Eur* 1999; 11:25–32.
13. Deshpande AA, Rhodes TN and Shah H. Controlled release drug delivery systems for prolonged gastric residence an overview. *Drug dev Ind pharm* 1996; 22:531-539.
14. Garg R and Gupta GD (2008). Progress in controlled gastroretentive delivery systems. *Trop J Pharm Res* 7: 1055-1066.
15. Gupta SN and Aggarwal N. A gastro-retentive floating delivery system for 5-Fluorouracil. *Asian Journal of Pharmaceutical Sciences* 2007; 2:143-149.
16. Hejazi R and Amiji M. Chitosan-based gastrointestinal delivery systems. *J Control Release* 2003; 89:151-165.
17. Hwang SJ, Park H and Park K. Gastric retentive drug delivery systems. *Crit Rev Ther Drug* 1998; 15:243-248.
18. Lueben HL, Lehr CM, Rentel CO, Noach ABJ, Boer AGD, Verhoef JC and Junginger HE. Bioadhesive polymers for the peroral delivery of peptide drugs. *J Control Release* 1994; 29:329-338
19. M, Indumathy R, Sreeram KJ and Balachandran U. Synthesis of iron oxide nanoparticles of narrow size distribution on polysaccharide templates. *Bull Mater Sci* 2008; 31:93-96.
20. Park K and Robinson JR. Bioadhesive polymers as platforms for oral-controlled drug delivery: method to study bioadhesion. *Int J Pharm* 1984; 19:107-127.
21. Patel JK and Patel MM. Stomach Specific Anti-Helicobacter Pylori Therapy: Preparation and Evaluation of Amoxicillin – Loaded Chitosan Muco adhesive Nanoparticles. *Curr Drug Deliv* 2007; 4:41-50.
22. Pignatello R, Ricupero N, Bucolo, Maugeri F, Maltese A and Puglisi G. Preparation and characterization of eudragit retard nanasuspensions for the ocular delivery of cloricromene. *AAPS Pharm SciTech* 2006; 24(7):E27.