www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN(USA): IJPRIF ISSN : 0974-4304
Vol.1, No.2, pp 390-393, April-June 2009

Preparation and Characterisation of PLGA-Nanoparticles containing an Anti-hypertensive agent

N.Jawahar*, T.Eagappanath, Nagasamy Venkatesh, Jubie.S, Samanta M.K,
Department of Pharmaceutics,J.S.S. College of Pharmacy,
Rock lands, Ootacamund- 643001,Tamilnadu, India
E.Mail: jajupharma@yahoo.co.in

Abstract : The objective of this work was to design and characterize Poly (D,L-Lactide-co-Glycolide) (PLGA) nanoparticles of ramipril an anti-hypertensive agent loaded by nanoprecipitation method using tribloere polymeric stabilizer (Pluronic ^{R}F-68). The particles were characterized for drug content, particle size and particle morphology by Transmission electron microscope(TEM). *Invitro* studies were determined by the bulk equilibrium reverse dialysis bag technique. The particle size of the prepared nanoparticles ranged from 20nm to 340nm. nanoparticles of ramipril were obtained with high encapsulation efficiency (68-75%). The drug release from the ramipril nanoparticles was sustained in Batch (F_3) for more than 24hrs with 72% drug release. This study suggest that the feasibility of formulating Ramipril loaded PLGA nanoparticles can be used to improve the therapeutic efficacy of Ramipril in the treatment of hypertensive disorder.
Key words: Nano particles, PLGA, ramipril,drug release

Introduction

It is estimated that up to 40 percent of new chemical entities (NCEs) discovered by the pharmaceutical industry today and many existing drugs are poorly soluble or lipophillic compounds which leads to poor oral bio-availability, high intra and inter-subject variability and lack of dose proportionality [1]. In recent years, technological advancements have brought us many new innovative drug delivery systems. Among the those, polymeric nanoparticulate system from biodegradable and biocompatible polymer are interesting option for controlled drug delivery and drug targeting [2-4].Poly (D,L-Lactide-co-glycolide) (PLGA) has gained attention for the preparation of wide variety of delivery systems containing several drugs [5-6] due to their biodegradable, biocompatible properties [7-8] and low toxicity [9].

Ramipril, potent anti-hypertensive agent has been used in are treatment of hypertensive disorders. It is highly lipophillic (log p (octanol/water, 3.32), poorly water soluble drug with absolute bioavailability of 28-35%. It undergoes significant 'first pass' metabolism. The active metabolite of ramipril is ramiprilate (a dicarboxylic acid). The half life of ramipril and and its metabolite is 2 and 18hrs respectively [10].Based on these, the aim of this work was to produce and characterize ramipril loaded PLGA nanoparticles using pluronic F-68 as stabilizer with a view to improving the dissolution rate of Ramipril that would increase the biological activities.

Experimental

Ramipril was obtained from Ananth Pharmaceuticals (Pondicherry, India) as a gift sample. PLGA (50:50), pluronic ^{R}F-68 and the dialysis bag with a 12,000 molecular weight Cut off were purchased from sigma-alduich chemicals private ltd (Bangalore, India). All other chemicals were analytical grade.

Preparation of nanoparticles

Nanoparticles were prepared by nanoprecipitaion method [11]. PLGA was dissolved in acetone (25ml). The solubility of ramipril in aqueous solution is less than 0.001% but the drug was soluble in polymer/acetone solution. This organic phase was added to 50ml of an aqueous solution containing pluronic F-68. Acetone was eliminated by evaporation under reduced pressure at 40^{o}C. The final volume of suspension was adjusted to 10ml. The final nanosuspension was centrifuged to separate the ramipril loaded polymeric aggregates, which were used for further polymeric aggregates, which were used for further characterisation.

Nanosuspension formula were established (Table 1) with different PLGA and pluronic F-68 concentrations to obtain higher encapsulation efficiency, desired particle size and suitable drug-release studies.

Nanoparticle Characterisation

Particle size determination

To analyse particle size, nanosuspension was diluted with filtered (0.22µm) ultra pure water. Samples were analysed using Master Sizer 2000 (Malvern

instrument, UK) which allows sample measurement in the range of 0.020-2000.00µm.

Polydispersity studies:

Polydispersity was determined according to the equation,

$$\text{Polydispersity} = \frac{D(0.9) - D(0.1)}{D(0.9)}$$

Where, D(0.9) corresponds to particle size immediately above 90% of the sample, D(0.5) corresponds to particle size immediately above 50% of the sample. D(0.1) corresponds to particle size immediately above 10% of the sample.

Table No.1: Formulae of Nanosuspension

For mul atio Code	Amt of Ramipril (mg)	Amt of PLGA (mg)	Amt of Pluronic F-68 (mg)	Amt of Acetone (ml)	Amt of Water (ml)
F_1	5	125	100	25	50
F_2	5	250	100	25	50
F_3	5	125	200	25	50
F_4	5	250	200	25	50
F_5	---	125	100	25	50

Table No. 3 Drug Content of the Formulations

Formulation Code	Drug Content (mg)
F_1	3.12
F_2	4.29
F_3	2.94
F_4	3.92

Table No. 4 Amount of Free Dissolved Drug in the Formulations

Formulation Code	Free dissolved drug (mg)
F_1	0.72
F_2	0.62
F_3	0.76
F_4	0.68

External Morphological StudieS (TEM):

External morphological of nanoparticles was determined using Transmission Electron Microscopy (TEM) with Philips EM-CM 12, 120 kr. Sample were prepared by placing one drop on a copper grid, dried under vaccum pressure before being examined using a TEM without being stained.

Drug Content and Drug Entrapment Efficiency [12,13]:

The total drug amount in the suspension was determined spectrophotometrically at 207nm.Entrapment efficiency of ramipril in the nanoparticles were determined by the following formula,

$$\text{Entrapment Efficiency} = \frac{\text{Wt. of the drug incorporated}}{\text{Wt. of the drug initially taken}} \times 100$$

I*nvitro* release Studies [14,15]:

The dialysis bag diffusion technique was used to study the *invitro* drug release of ramipril nanoparticles. The prepared nanoparticles were placed in the dialysis bag and immersed in to 50ml of PBS (7.4). The entire system was kept at $37 \pm 0.5°C$. With the continous magnetic stirring at 200rpm/min. Samples were withdrawn from the receptor compartment at predetermined intervals and replaced by fresh medium. The amount of drug dissolved was determined with UV-Spectrophotometry at 207nm.

Table No. 5 Entrapment Efficiency of the Formulations

Formulation Code	Entrapment Efficiency (%)
F_1	72.14
F_2	68.28
F_3	77.16
F_4	74.86

Results and Discussion

I.R Spectroscopy

I.R. Study was carried out to confirm the compatibility between the selected polymer PLGA, drug ramipril and nanoparticles. The spectra obtained from the I.R. Studies are from $3600cm^{-1}$ to $450cm^{-1}$. It was confirmed that there are no major shifting as well as no loss of functional peaks between the spectra of drug, polymer and drug loaded nanoparticles ($1652cm^{-1}$, $1701cm^{-1}$, $1743cm^{-1}$, $2866cm^{-1}$, $1323cm^{-1}$).

Particle Size Determination

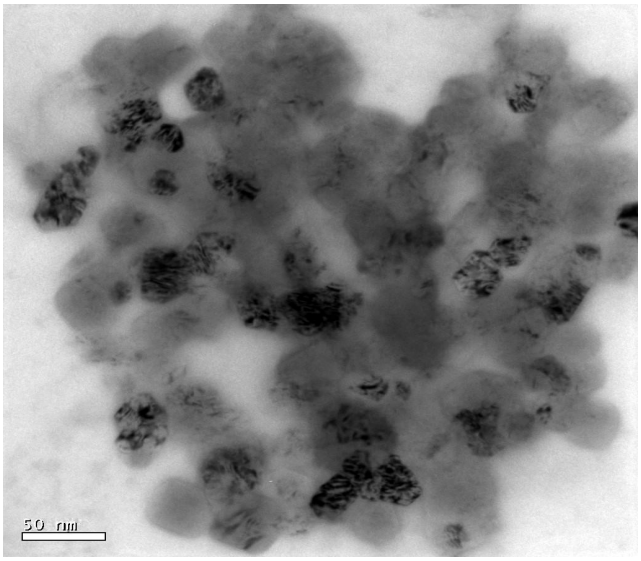
The particle size distribution curves for all the samples are unimodel. The nanoparticles size were 199nm, 340nm, 189nm, and 279nm from F_1, F_2, F_3 and F_4

respectively. The particle size dependant on PLGA concentration. The small particle size of 20nm were found in batch F_3 and the largest particle of 340nm was found in batch F_2. The data suggest that in an increase in polymer concentration increase the particle size. However the polydispersity of the nanoparticles increased with an increase in polymer concentration and particle size of the nanoparticle.

External Morphological Studies

The External Morphological Studies revealed that maximum nanoparticles were nearly spherical or crystal . The nanoparticle size observed by TEM, correlated well with the particle size distribution measured by Mastersizer(Malvern Instrument).

FIG.1.TEM of ramipril nanoparticles

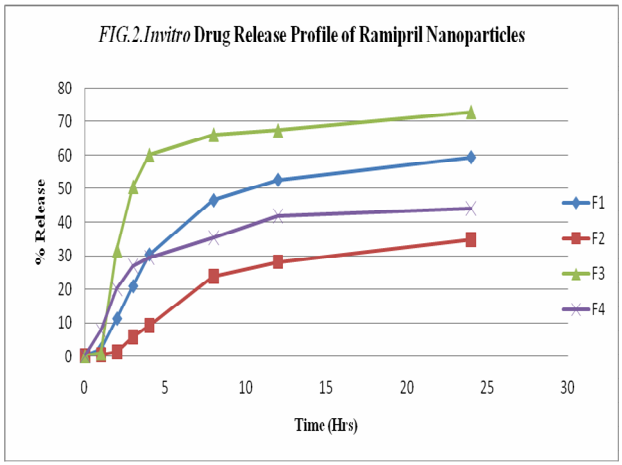


Drug Content & Entrapment Efficiency

The total drug content in nanosuspension were varied from 2.94-4.25 mg (F_1-F_4). The drug content increased with an increase in the concentration of PLGA. The amount of free dissolved drug in nanosuspension ranged from 0.68-0.76 mg. This was due to the limited solubility of Ramipril in aqueous phase. The increase in PLGA concentration increase the encapsulation efficiency.

***Invitro* Release Studies**

The nanosuspension (F_3, F_1) showed burst release followed by sustained release. After 24hrs of dialysis in PBS (pH 7.4) the percentage of release were 73% & 59%. The initial burst effect on the release of Ramipril may be due to the free dissolved drug observed with nanosuspension and higher concentration of Pluronic F-68. Moreover smaller sized nanoparticles prepared with lower amount of PLGA and high concentration Pluronic F-68 exhibited higher drug release. The other batches (F_2, F_4) showed initial release and prolong the effect and increased size of polymeric nanoparticles.



Conclusion

This study confirms that the nanoprecipitation technique is suitable for the preparation of ramipril nanoparticles with high encapsulation efficiency. This formulation approach can be used to improve the therapeutic efficacy of poorly soluble drugs. The change in nanoparticle size and release kinetics were affected by changes in polymer and stabilizer concentration. The sustained release of drug from the ramipril nanoparticle suggest that the frequency of administration, dose and adverse effects of this molecule could be reduced. We can conclude that there is large scope for improving the use of ramipril in hypertensive treatments through nanoparticle as a drug delivery system.

Acknowledgement

We thank Mr. Varadharajan, CECRI Karaikudi, India in carrying out TEM analysis and particle size distribution analysis of Nanoparticles.

References

1. T.R.Kommuru, B.Gurbey, M.A.Khan, I.K.Reddy, Self emulsifying drug delivery systems (SEDDS) of coenzyme Q_{10} : Formulation development and bioavailability assessment, Int.J.Pharm.212 (2001), 233-246.
2. Grof.R, Minamitake.Y: Biodegradable long circulating Nanospheres. Science 263, 1600-1603 (1994).
3. Labhastwar V.Song, C.Levy RJ: Nanoparticle drug delivery systems. Adv.drug delivery.Rev.24, 63-85 (1997).
4. Sinha VR, Bansal K, Kousik R et. al: Polt-E-Caprolactone microspheres and nanospheres an overview. Int.J.Pharm. 278, 1-23 (2004).

5. E.Allemann, R.Gumy, E. Doclker, Drug loaded nanoparticles preparation methods and drug targeting tissues. Eur.J.Pharm. Biopharm. 39 (1993) 173-191.
6. J.Mandit, M.Vert, Les polymers a base diacids lactiques et glycoliques et la deliverance des principles actifs-S.T.P.Pharma sci.3 (1993) 197-212.
7. D.H.Lewis, controlled release of bioactive agents free lactide/glycolide polymers, in: M.Chasis, R.Langer (eds) Biodegradable polymers as drug delivery systems, Marcel dekkar, New york, 1991, 1-41.
8. S.J.Halland, B.J.Tighe, Polymers for degradable devices. The potential of polyesters as controlled release systems, J.control.rel.4 (1986) 155-180.
9. Y.Ogava, Monthly microcapsule- Depot form of LHRH agonist, Leupreclin acetate: Formulation and Pharmcokinetics in animals, Eur.J.Hosp.Pharm, 2(1992) 120-127.
10. Karen tu. Et.al., (2006)
11. Zili Z.Sfars, Fessi. H: Preparation and characterization of poly-e-caprolactone nanoparticles containing griseofulvin. Int. J.Pharm.294.261-267 (2005).
12. Chothy M.Fishbein, J.Danenberg HD et.al; Lipophillic drug loaded nanospheres prepared by Nanoprecipitation technique: effect of formulation variables on size, drug delivery and release kinetics.J.Control.Release 83, 389-400 (2002).
13. Levy M Y, Benita S: Drug release from submicronized O/W emulsion: a new *invitro* kinetics evaluation model. Int.J.Pharm. 66, 29-37 (1990).
14. Verger ML. Fluckiger L, Kim Y et.al.,: Preparation and characterization of nanoparticles containing an anti-hypertensive agent. Eur.J.Pharm. Biopharm 46, 137-143 (1998).
15. Yang SC, LU LF. Cai Y et. al.,: Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J. Control. Release, 59.299-307 (1999).

*****