

FORMULATION AND EVALUATION OF LAMIVUDINE LOADED POLYMETHACRYLIC ACID NANOPARTICLES

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ABSTRACT: Nanoparticles represent a promising drug delivery system of controlled and targeted drug release. They are specially designed to release the drug in the vicinity of target tissue. The aim of this study was to prepare and evaluate polymethacrylic acid nanoparticles containing lamivudine in different drug to polymer ratio by nanoprecipitation method. SEM indicated that nanoparticles have a discrete spherical structure without aggregation. The average particle size was found to be $121 \pm 8 - 403 \pm 4$ nm. The particle size of the nanoparticles was gradually increased with increase in the proportion of polymethacrylic acid polymer. The drug content of the nanoparticles was increasing on increasing polymer concentration up to a particular concentration. No appreciable difference was observed in the extent of degradation of product during 60 days in which, nanoparticles were stored at various temperatures. FT-IR studies indicated that there was no chemical interaction between drug and polymer and stability of drug. The *in-vitro* release behaviour from all the drug loaded batches was found to be zero order and provided sustained release over a period of 24 h. The developed formulation overcome and alleviates the drawbacks and limitations of lamivudine sustained release formulations and could possibility be advantageous in terms of increased bioavailability of lamivudine.

KEYWORDS :- nanoparticles, lamivudine , biodegradable, polymethacrylic acid

INTRODUCTION:

During last two decades, considerable attention has been given to the development of novel drug delivery system (NDDS)¹. The rationale for control drug delivery is to alter the pharmacokinetics and pharmacodynamics of drug substance in order to improve the therapeutic efficacy and safety through the use of novel drug delivery system. Besides more traditional matrix or reservoir drug delivery system, colloidal drug delivery system has gained in popularity. The major colloidal drug delivery system includes liposome and polymeric nanoparticles. These systems have been investigated primarily for site specific drug delivery, for controlled drug delivery, and also for the enhancement of dissolution rate/bioavailability of poorly water-soluble drugs. The primary routes of administration under investigation are parenteral route, however, other routes such as the oral, ocular, or topical routes are also being investigated. In era of oral drug delivery system microspheres², microcapsule, nanoparticles, liposomes, and niosomes are better options

to conventional dosage form. Nanoparticles are colloidal polymer particles of a size below $1\mu\text{m}^{3-4}$ and hold promise as drug delivery for parenteral, peroral and ocular administration as well as adjuvant for vaccines. Due to their greater stability and due to their easier manufacturing they offer advantages over other colloidal carriers such as liposomes and cell ghosts. They offer advantages like increased bioavailability, site specific drug delivery, sustained release of drug over longer period of time, retention of dosage form in entire length of gastrointestinal tract and convenient to patient due to reduction in frequent dosing⁵.

Eudragit polymers are series of acrylate and methacrylate polymer available in different ionic forms. Eudragit RS 100 is insoluble in aqueous media but it is permeable and has pH- independent release profile. The permeability of Eudragit RS 100 is due to presence of quaternary ammonium group in their structure⁶. Lamivudine is a potent antiviral agent used in the treatment of AIDS.

Conventional oral formulations lamivudine are administered multiple times a day (150 mg twice daily) because of its moderate half-life (5-7 hours)⁷⁻⁸. Treatment of AIDS using conventional formulations of lamivudine is found to have many drawbacks, such as adverse side effects resulting from accumulation of drug in multidose therapy, poor patient compliance, and high cost. The objective of the present study was to prepare nanoparticles of lamivudine to overcome some of these problems.

MATERIAL AND METHOD:

Lamivudine was a gift sample from Cipla Pvt. Ltd. Mumbai. Eudragit RS 100 was obtained from Rohm Pharma, Germany. Dichloromethane was procured from E. Merck Ltd., Mumbai. All other chemicals used were of analytical grade.

Preparation of nanoparticles

Nanoparticles containing lamivudine were prepared using nanoprecipitation method⁹⁻¹⁰. Drug was dissolved in water, and then cosolvent (acetone) was added into this solution. A cosolvent was needed in order to make the inner phase more homogeneous. Then polymer and 150 mg of propylene glycol were dissolved in chloroform, and this solution was added to the drug solution to form dispersion. The dispersion was added to 10 ml of aqueous ethanol solution (70%). After 5 minutes of mixing, the organic solvents were removed by evaporation at 35° under normal pressure, nanoparticles were separated by using cooling centrifuge (10000 rpm for 20 min), supernatant were removed and nanoparticles washed with water and dried at room temperature in a desiccator. By following the above mentioned procedure five other batches of nanoparticles ratio of 1:1, 1:2, 1:3, 1:4 and 1:5 were prepared and named F₁, F₂, F₃, F₄ and F₅ respectively.

Particle size, surface morphology and zeta potential

The surface morphology (roundness, smoothness, and formation of aggregates) and particle size were studied by scanning electron microscopy (SEM)¹¹⁻¹². Zeta potential of the best formulation (F₄) was determined by zeta potential probe model DT- 300.

Drug content

Drug content was determined by centrifugation method. The redispersed nanoparticles suspension was centrifuged at 15,000 rpm for 40 min at 25° to separate the free drug in the supernatant. Concentration of lamivudine in the supernatant was determined by UV-Vis spectrophotometry at 271 nm after suitable dilution.

Fourier Transform Infra-red Spectroscopy (FT-IR) analysis

The FT-IR spectra of pure lamivudine and Eudragit RS 100 nanoparticles loaded with lamivudine were recorded to check drug polymer interaction and stability of drug.

In vitro release studies

In vitro release studies were carried out by using dialysis tubes with an artificial membrane. The prepared lamivudine nanoparticles and 10 ml of phosphate buffer pH 7.4 was added to the dialysis tube and subjected to

dialysis by immersing the dialysis tube to the receptor compartment containing 250 ml of phosphate buffer pH 6.8. The medium in the receptor was agitated continuously using a magnetic stirrer a temperature was maintained at 37±1°. 5ml of sample of receptor compartment were taken at various intervals of time over a period of 24 h and each time fresh buffer was replaced. The amount of drug released was determined spectrometrically at 271 nm.

Kinetic modeling

In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles were fitted with various kinetic equation like zero order¹³ (cumulative % release vs. time), first order¹⁴ (log % drug remaining vs time), Higuchi's model¹⁵ (cumulative % drug release vs. square root of time). r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots.

Stability study:

The stability study was carried using the batch F₄. The stability of drug loaded nanoparticles was evaluated in terms of its drug content¹⁶. The stability of nanoparticles was evaluated in PBS (pH 6.8). Nanoparticles formulation was incubated at 5-8° and 37 ± 1° for a period of 60 d. After specified time intervals, the suspension was centrifuged at 15,000 rpm for 1 h, supernatant was removed and nanoparticles were dissolved in dichloromethane. After adding of water and separation, the amount of drug was detected by UV-Vis spectrophotometrically method at 271 nm.

RESULT AND DISCUSSION:

Lamivudine nanoparticles with varying proportions of lamivudine and Eudragit RS 100 were prepared by nanoprecipitation method. The scanning electron microphotograph of lamivudine nanoparticles is shown in fig.1. It indicated that lamivudine nanoparticles have a discrete spherical structure without aggregation. The particle size of nanoparticles varied some what among the formulation due to variation in the composition of formulations. Zeta potential of best formulation was determined and it was found +27mV due to quaternary ammonium group of Eudragit. Since there was a decrease of surface potential, it could be concluded that a part of drug was absorbed on the polymeric particles.

The drug content was determined by centrifugation method and it was maximum in formulation FN₄. The nanoparticles exhibited an increase in drug content with an increased in the polymer ratio, up to particular concentration (1:4). A decrease in drug content was observed after that point due to the saturation capacity of polymer. In FT-IR study the characteristic peak due to pure lamivudine has appeared in the spectra of nanoparticles without any markable change in the position. It indicated that there was no chemical interaction between lamivudine and Eudragit RS 100. In stability study there was no remarkable change in the drug content. This indicated that formulation was stable in storage medium condition. The *in vitro* release profile

of all formulation is shown in fig.2. The release of lamivudine mainly depended upon the polymer concentration. The burst release of lamivudine from nanoparticles at initial stage resulted from the dissolution of drug crystals on the surface of nanoparticles. On increasing polymer concentration, the release rate of lamivudine from nanoparticles decreased drastically. The *in vitro* release data was applied to various kinetic models to predict the drug release kinetic mechanism. The release constant was calculated from the slope of appropriate plots, and the regression coefficient (r^2) was determined. It was found that the *in-vitro* drug release of nanoparticles was best explained by zero order kinetics for best formulation F₄ as the plots shows highest

linearity. The correlation coefficient (r^2) was found 0.99 for F₄.

CONCLUSION

The method of preparation of nanoparticles of lamivudine was found to be simple and reproducible. The slow and constant release of lamivudine from nanoparticles maintain constant drug plasma concentration thereby increasing therapeutic efficacy. This study shows that polymethacrylic acid nanoparticles could be a useful carrier for lamivudine. The developed formulation overcome and alleviates the drawbacks and limitations of lamivudine sustained release formulations.

TABLE 1: Formulation and physicochemical characterization of lamivudine nanoparticles

Formulation code	Drug: Polymer ratio	Drug Content* (%)	Particle Size* (nm)
FN ₁	1:1	58.32 ± 0.02	121 ± 8
FN ₂	1:2	64.13 ± 0.08	265 ± 5
FN ₃	1:3	70.83 ± 0.03	287 ± 9
FN ₄	1:4	64.62 ± 0.02	332 ± 5
FN ₅	1:5	59.96 ± 0.04	403 ± 4

* Average of three preparation ± S.D

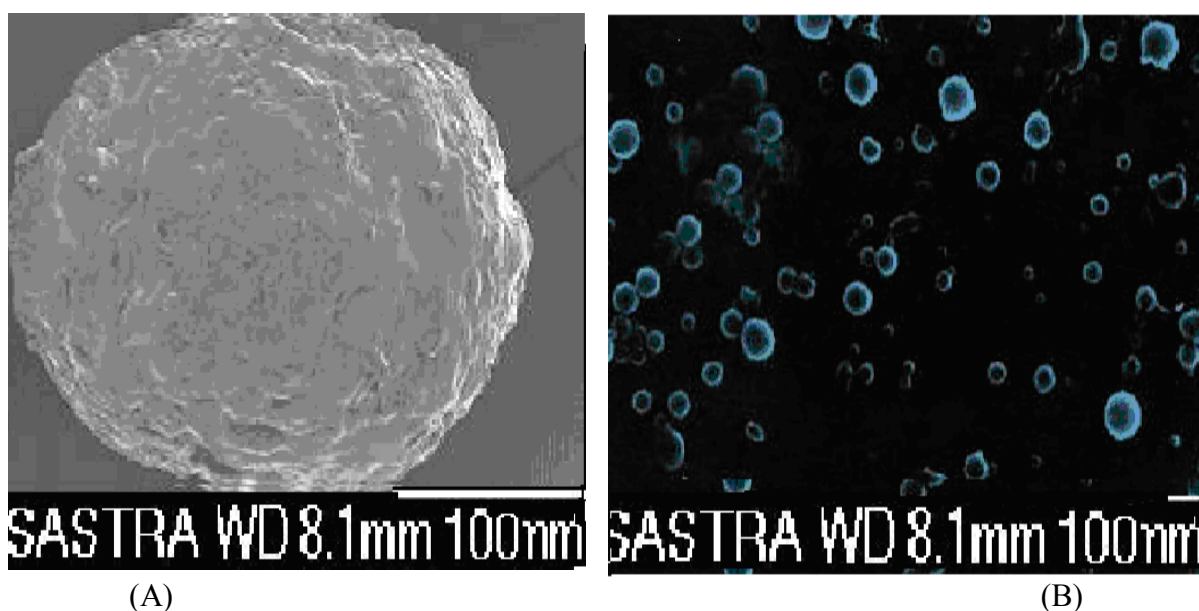


Fig.1 : Scanning electron microphotograph of lamivudine hydrochloride nanoparticles (A) At lower magnification (B) At higher magnification

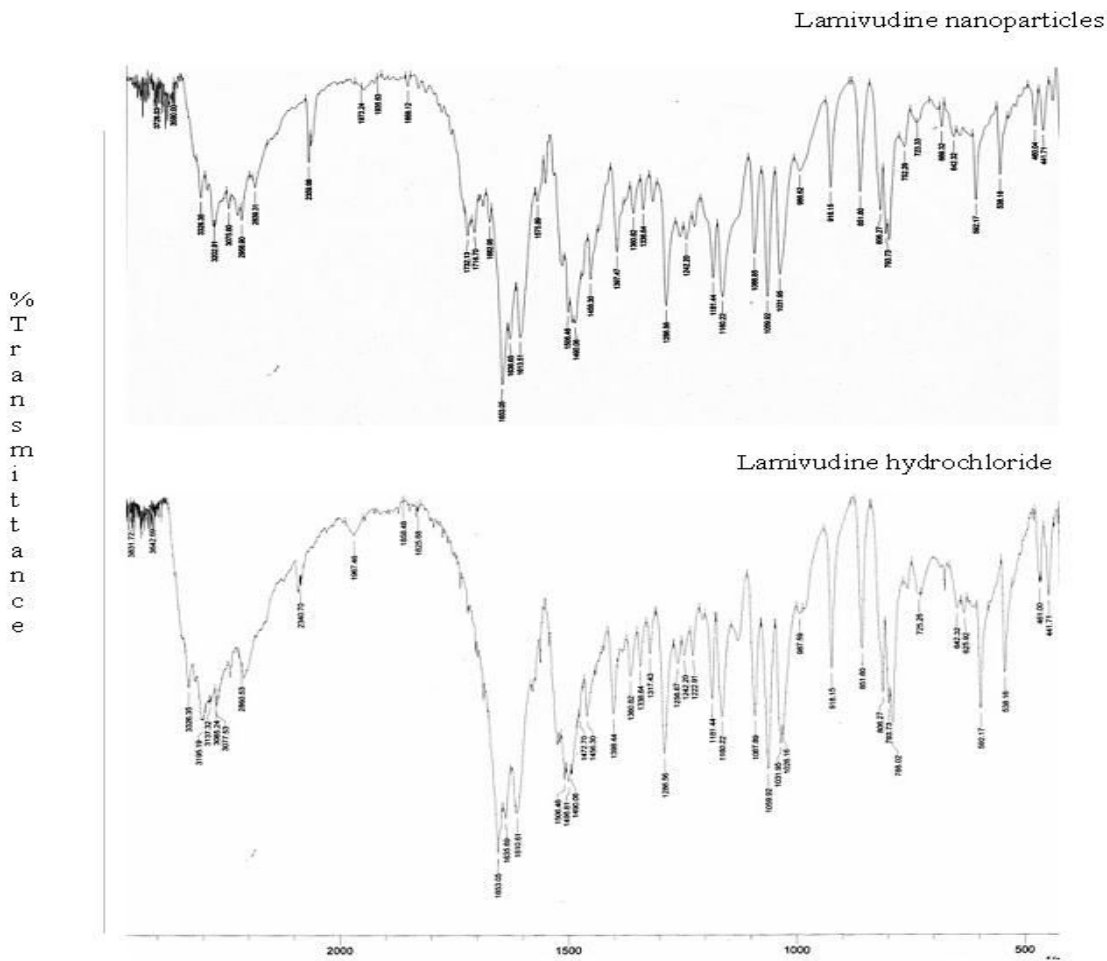


Fig.2 : FT-IR spectra of pure lamivudine and lamivudine loaded nanoparticles formulation (FN₄)

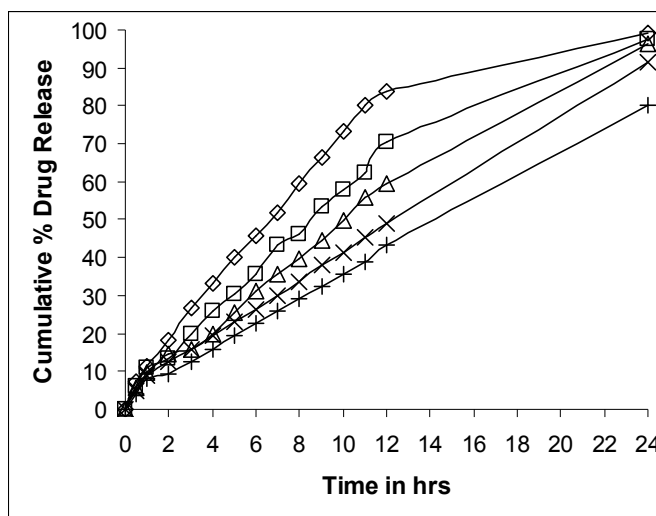


Fig.3 : *In vitro* drug release of lamivudine from polymethacrylic acid nanoparticles formulation FN₁ (-◇-), FN₂ (-□-), FN₃ (-△-), FN₄ (-×-), FN₅ (-+-)

REFERENCES

1. Santhi K., Dhanraj S.A., Nagasamyvenkatesh D., Sangeetha S., Suresh B., Preparation and optimization of sodium alginate nanospheres of methotrexate, **Indian J. Pharm. Sci.**, 2005, 67, 691-696.
2. Tamizharasi S., Rathi J.C., Rathi V., Formulation, characterization and *in vitro* release kinetics of aceclofenac loaded poly(ϵ -caprolactone) microspheres, **Indian Drugs**, 2007, 44, 973-975.
3. Kreuter J., Nanoparticles based drug delivery systems, **J. Control. Rel.**, 1991, 16, 169-176.
4. Bachnav D., Rao M., Madgulkar A., Rao S., Nanotechnology and blood brain barrier, **Indian Drug**, 2007, 44, 245-252.
5. Chen D.B., Yang T. Z., Lu W. L., Zhang Q., *In vitro* and *in vivo* study of two types of long circulating solid lipid nanoparticles containing paclitaxel, **Chem. Pharm. Bull.**, 2001, 49, 1444-1447.
6. Ubrich N., Schmidt C., Bodmeur R., Hoffman M., Maincent P., Oral evaluation in rabbits of cyclosporine loaded Eudragit RS or RL nanoparticles, **Int. J. Pharm.**, 2005, 288,169-175.
7. Punna R.R., Sindhura G., Saha R.N., Design and Study of Lamivudine Oral Controlled Release Tablet, **AAPS PharmSciTech**, 2007, 8, E1-E9.
8. Jain S , Tiwari AK, Sapra B , Jain AK., Formulation & evaluation of Ethosomes for Transdermal Delivery of Lamivudine, **AAPS PharmSciTech**, 2007, 8, E1-E8.
9. Leena Peltonen., The Effect of Cosolvents on the Formulation of Nanoparticles From Low-Molecular-Weight Poly(l)lactide **AAPS PharmSciTech.**, 2002, 3, E1-E7.
10. Barbault S, Gref R., Russo P., Guechot J., Bochot A., Design of poly- ϵ -caprolactone nanospheres coated with bioadhesive hyaluronic acid for ocular delivery, **J. Control. Rel.**, 2002, 83, 365-375.
11. Peltonen L., Koistinen P., Karjalainen M., Hakkinen A., Hirvonen J., The effect of cosolvents on the formulation of nanoparticles from low molecular weight poly(l)lactide, **AAPS PharmSciTech**, 2002, 3, 1-7.
12. Cui F., Oian F., Yin C., Preparation and characterization of mucoadhesive polymer-coated nanoparticles, **Int. J. Pharm.**, 2006, 316, 154-161.
13. 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.
14. Haznedar S., Dortunc B., Preparation and evaluation of Eudragit microspheres containing acetazolamide, **Int. J. Pharm.**, 2004, 269, 131-140.
15. Higuchi T., Mechanism of sustained action medication: theoretical analysis of rate of release of solid drugs dispersed in solid matrices, **J. Pharm. Sci.**, 1963, 52, 1145-1149.
16. 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.
17. Chowdary K.P.R., Rao Y.S., Mucoadhesive microcapsules of glipizide : Characterization, in vitro and in vivo evaluation, **Indian J. Pharm. Sci.**, 2006, 68, 479-484.
18. Chowdary K. P. R., Rao N. K., Malathi K., Ethyl cellulose microspheres of glipizide : Characterization, in vitro and in vivo evaluation, **Indian J. Pharm. Sci.**, 2004, 66, 412-416.
