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FORMULATION AND IN VITRO CHARACTERIZATION OF POLYMETHACRYLIC ACID NANOPARTICLE CONTAINING FRUSEMIDE

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ABSTRACT: The purpose of this research was to prepare Frusemide nanoparticles to reduce dosing frequency. Polymeric nanoparticles have received more attention for preparing sustained dosage forms because of their inertness, solubility in relatively non-toxic solvent. Frusemide is a loop diuretic used in the treatment of congestive heart failure and edema , having short half-life of 1 to1.7 hours.

Frusemide loaded Eudragit RS100 nanoparticles were prepared by nanoprecipitation method for oral delivery. Formulation were prepared in ten different drug to carrier ratio, and characterized for particle size, shape, percentage yield, drug entrapment, stability studies, zeta potential, FT-IR study, in-vitro drug release and release kinetics.

The shape of nanoparticles was found to be spherical by scanning electron microscopy studies, whereas size ranging from 163 nm to 378 nm. FTIR study confirmed that there was no interaction between drug and polymer. Entrapment efficiency was in the range of 14.95±0.06 to69.73±0.03 W/W. No appreciable difference was observed in the extent of degradation of product during 60 days in the nanoparticles, which were stored at various temperatures. Zeta potential of formulation supports the minimum interaction between the particles. The *in-vitro* drug release study revealed that sustained release of some formulation last up to 24 hour. The release followed Higuchi kinetics, which indicates diffusion controlled release pattern of drug.

KEYWORDS: - Nanoparticles, Frusemide, Zeta potential, polymethacrylic acid

INTRODUCTION

Over the past few decades, there has been considerable interest in developing biodegradable nanoparticles (NPs) as effective drug delivery devices. Various polymers have been used in drug delivery research as they can effectively deliver the drug to a target site and thus increase the therapeutic benefit, while minimizing their side effects.The controlled release of pharmacologically active agents to the specific site of action at a therapeutically optimal rate and dose regimen has been a major goal in designing such devices[1]

Furosemide (4-chloro-2- furfurylamino-5-sulphamoyl benzoic acid) is a drug with a diuretic action which acts at the renal level on the ascending limb of the loop of Henle[2].

This drug is used in the treatment of oedema of pulmonary, cardiac or hepatic origin as well as in the treatment of hypertension and in the chronic treatment of cardiac infarction[3]. Frusemide is a loop diuretic used in the treatment of congestive heart failure and edema , having short half-life of 1 to1.7 hours. The present investigation is aimed to formulate the sustained release nanoparticle of furosemide with polymethacrylic acid copolymer.

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 structure[4].

The specific objective of the present study was to prepare frusemide nanoparticles using Eudragit RS100. In present work an attempt made to formulate eudragit RS100 nanoparticles of frusemide to achieve

sustain action, with increasing absorption and thereby to increase its bioavailability.

MATERIAL AND METHODS

Frusemide was obtained as gift sample from Arbro Pharmaceuticals, New Delhi., India. Eudragit RS 100 was obtained from Rohm Pharma, Germany. Dichloromethane was procured from E. Merck Ltd., Mumbai.and All other chemicals and reagents used in the study were of analytical grade.

Formulation of nanoparticles:

Eudagit RS100 nanoparticles containing frusemide were prepared in different drug to carrier ratio F_1 (1:1), F_2 (2:1), F_3 (3:1), F_4 (4:1), F_5 (5:1), F_6 (1:2), F_7 (1:3), F_8 (1:4), F_9 (1:5), F_{10} (1:6) by using nanoprecipitation technique[5-6]. First frusemide was dissolved in 3 ml of 0.1N hydrochoric acid ; then cosolvent (acetone 1 mL) was added into this solution. Then polymer and 150 mg of propylene glycol were dissolved in 4 ml of 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°C 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 desicator.

Nanoparticle characterization:

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)[7-8]. Zeta potential of the best formulation (F8) 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 Frusemide in the supernatant was determined by UV-Vis spectrophotometyrically at 272 nm after suitable dilution.

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

The FT-IR spectra of pure Frusemide and Eudragit RS100 nanoparticles loaded with frusemide 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 frusemide 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 hrs and each time fresh buffer was replaced. The amount of drug released was determined by UV spectrophotometer (Shimadzu UV/VIS) at 272 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 order[9] (cumulative % release vs. time), first order[10](log % drug remaining vs time), Higuchi's model[11] (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 F_8. The stability of drug loaded nanoparticles was evaluated in terms of its drug content[12]. 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 days. 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 272 nm.

RESULT AND DISCUSSION

Frusemide nanoparticles with varying proportions of frusemide and Eudragit RS 100 were prepared by nanoprecipitation method. The scanning electron microphotograph of frusemide nanoparticles is shown in fig.1. It indicated that frusemide 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 formlation FN_8. 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, which is shown in Table.1 In FT-IR study the characteristic peak due to pure frusemide has appeared in the spectra of nanoparticles without any markable change in the position. It indicated that there was no chemical interaction between frusemide and Eudragit RS 100. In stability study there was no remarkable change in the drug content. This indicated that formulation was stable at different storage condition.

The in vitro release profile of all formulation is shown in fig.2. The release of frusemide mainly depended upon the polymer concentration. The burst release of frusemide 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 frusemide 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 FN_8 as the plots shows highest linearity. The correlation coefficient (r^2) was found 0.99 for FN_8.

CONCLUSION

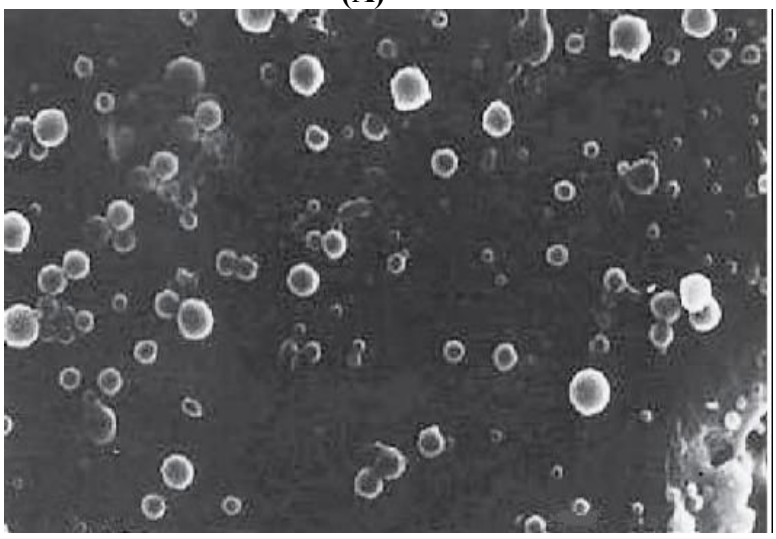
This study confirms that the nanoprecipitation technique is suitable for the preparation of frusemide nanoparticles with high encapsulation efficiency. The method of preparation of nanoparticles of frusemide was found to be simple and reproducible. The slow and constant release of frusemide from nanoparticles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

TABLE 1: Formulation and physicochemical characterization of frusemide nanoparticles

Formulation code	Drug: Polymer ratio	Drug Content*(%)	Particle Size*(nm) Size*(nm)
FN_1	1:1	48.32±0.02	163
FN_2	2:1	39.18±0.03	198
FN_3	3:1	23.39±0.05	207
FN_4	4:1	18.09±0.04	236
FN_5	5:1	14.95±0.06	324
FN_6	1:2	56.85±0.03	175
FN_7	1:3	63.13±0.07	254
FN_8	1:4	69.73±0.03	310
FN_9	1:5	67.62±0.02	346
FN_{10}	1:6	62.98±0.08	378

*** Average of three preparation ± S.D**

(A)



(B)

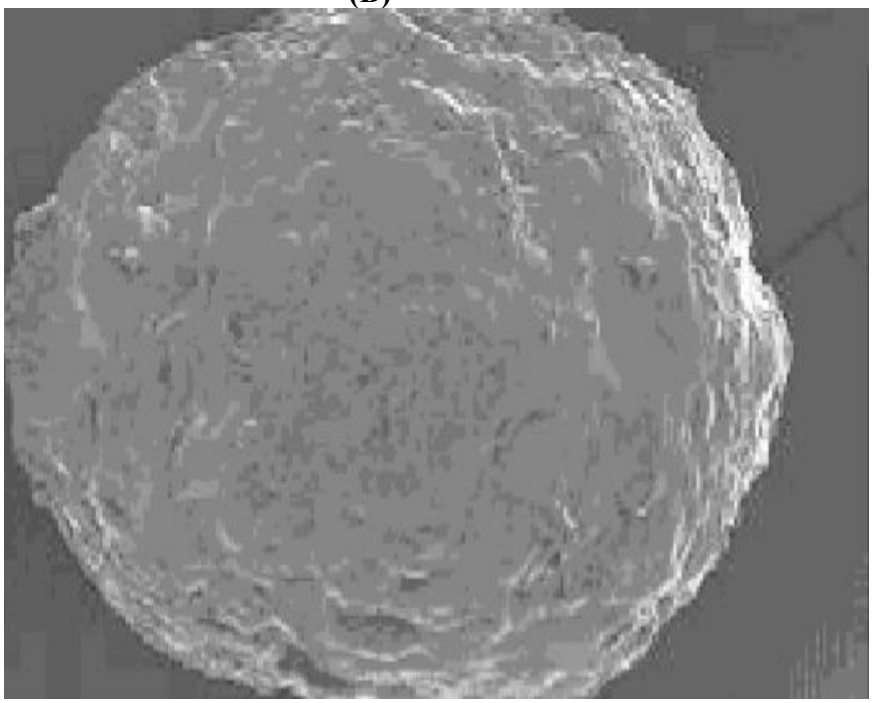


Fig.1: Scanning electron microphotograph of frusemide nanoparticles (A) At lower magnification (B) At higher magnification

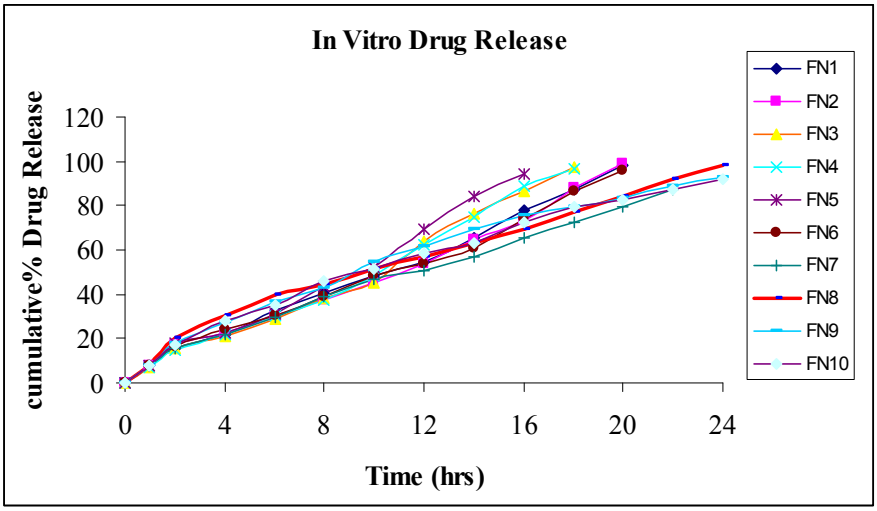


Fig.2: In vitro drug release of prepared formulations.

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. Ebihara et al., Controlled release formulations to increase the bioadhesive properties, Drug Res. 1983; 33: 163.

3.Verhoeven, J. et al., controlled-release formulations, a hydrophilic matrix containing furosemide. Int. J. Pharm. 1988; 45: 65.

4.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.

5 Leena Peltonen., The Effect of Cosolvents on the Formulation of Nanoparticles From Low- Molecular-Weight Poly(l)lactide AAPS PharmSciTech., 2002, 3, E1-E7.

6.Barbault S, Gref R., Russo P., Guechot J., Bochot A., Design of poly-e-caprolactone
Nanospheres coated with bioadhesive hyaluronic acid for ocular delivery, J. Control. Rel., 2002, 83, 365-375.

7. 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.

8.Cui F., Oian F., Yin C., Preparation and characterization of mucoadhesive polymercoated nanopaticles, Int. J. Pharm., 2006, 316, 154-161.

9. 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.

10. Haznedar S., Dortunc B., Preparation and evaluation of Eudragit microspheres containing acetazolamide, Int. J. Pharm.,2004, 269, 131-140.

11. 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.

12. 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.

13. 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.

14. 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.

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