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Research Paper

Development and Evaluation of Furosemide Microspheres Made by Mixed Solvency Concept

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The purpose of this investigation was to prepare and evaluate Floating Microspheres of Furosemide made by Mixed Solvency Concept. Mixed solvency concept was used in this formulation because Furosemide drug is poorly water soluble drug. It enhanced the solubility of Furosemide and also explores possibility of used ethyl acetate: ethanol as a combination of solvents to prepare hollow floating microspheres replaces dichloromethane: ethanol combination. Result of our present study suggest that floating microsphere of Furosemide can be successfully designed and develop by mixed solvency concept which can reduce individual concentration of solubilizer and so reduce their toxicity and it provided environmentally friendly methods. Evaluate all characteristics parameter of microspheres i.e. particle size, encapsulation efficiency, surface morphology and *In-Vitro* Drug Release.

Key words : Floating Microspheres, Furosemide, solubilizer, parameters, evaluation.

INTRODUCTION

Furosemide drug has been classified as a class IV drug as per the biopharmaceutical classification system (BCS) as a result of its low solubility and oral bioavailability; one of the major causes of its low oral bioavailability is its solubility. Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules. For this purpose enhance the aqueous solubility of Furosemide by application of mixed solvency. Mixed solvency concept show all substances have solubilizing power and all substances whether liquids, solids or gases may enhance the solubility of poorly soluble drugs. It is increase the solubility of poorly soluble drugs by the addition of more than one solubilizing agent. Solubilizers like Hydrotropes (Niacinamide, sodium ascorbate, urea, and sodium benzoate), co-solvents (propylene glycol, PEG 200, 300, 400) and water soluble solids (PEG 4000, 6000, Cyclodextrins) in varying concentrations may be used that show additive or synergistic enhancement in solubility. These Solubilizers do not cause any toxicity and are non volatile**.** It reduced the total concentration of individual solubilizer necessary to produce modest increase in solubility by employing combination of agents in lower conc.

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from the point of view of safety of solubilizer.

Furosemide is also categorized a potent loop diuretic. It increases renal excretion of water, sodium, potassium, chloride, calcium, magnesium, hydrogen, ammonium and bicarbonate. It is used in the treatment of edema of hepatic, cardiac, pulmonary and renal failures and in chronic hypertension. It also causes renal venodialation and transiently increases glomerular filtration rate. In this Drug dose related adverse effects have been observed This assists in improving the bioavailability of the drug at the site of absorption.

Gastroretentive drug delivery system is also known as type of controlled drug delivery system. It is also called as floating systems or hydrodynamically controlled systems are low-density systems that have sufficient buoyancy to float over the gastric contents and remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. This results in an increased GRT and a better control of the fluctuations in plasma drug concentration.

The following approaches have been used for the design of floating dosage forms of

and the treatment with conventional tablets produced short period of maximum diuresis, which is inconvenient to the patients. For this purpose development of oral Gastroretentive controlled drug delivery system, it modify the GI transit time which can overcome these problems and release the drug to maintain its plasma concentration for a longer period of time.

The following approaches have been used for the design of floating dosage forms of single and multiple unit systems. In this. research may be formulated multiple-unit single and multiple unit systems. In this research may be formulated multiple-unit dosage form that is hollow microspheres. Hollow Microspheres have high loading capacity and many polymers have been used such as albumin, gelatin, starch, polymethacrylate, polyacrylamine, and poly alkyl cyano acrylate. Microspheres have a characteristic internal hollow structure and show an excellent in vitro floatability. The solvent evaporation technique of microencapsulation is widely applied in pharmaceutical industries to obtain the controlled release of drug. The obtained polymer microspheres with drug trapped inside can degrade and release the encapsulated drug slowly with a specific release profile. Hollow microspheres may be prepare by the emulsion solvent evaporation method using Eudragit as an

entericacrylic polymer with Furosemide as various polymer/drug ratios in a mixture of ethyl acetate and ethanol it replace the dichloromethane: ethanol mixture. This method may be reduces the toxicity of organic solvent.

MATERIALS AND METHODS

Furosemide was a gift sample from IPCA Laboratories Ltd, Ratlam (M.P). All other chemicals used were of analytical grade.

Preliminary solubility studies of furosemide

The solubility determination of furosemide was carried out in mixed blends and different solvent [demineralized water, 0.1N HCl and ethyl acetate: ethanol (70:30)] were carried out at. The excess drug was added gradually to 5 ml of different solutions (of solubilizers) and solvent contained in 10ml glass vials and vials were sealed with rubber closures an aluminum seals. The vials were shaken for 12hr and allowed to equilibrate for 24 hrs. undistributed. The solutions containing excess of drug were centrifuged and filtered through whatmann filters. Aliquots of filtrate were suitably diluted and the dilutions were analyzed on UV-Visible spectrophotometer.

Preparation of Microspheres

Floating microspheres of Furosemide were prepared by using emulsification solvent evaporation technique. Accurately weighed polymer Eudragit RSPO and drug were dissolved in internal phase ethyl acetate: ethanol (70:30). To it, fixed amount of solubilizer Niacinamide was added and shaken with the help of vortex. The internal phase, was then added in a stream, at once to external phase in a 250 ml beaker containing 50 ml demineralized water with 0.8% PVA as stabilizer, and allowed the stirring using a mechanical stirrer. Stirring was continued for 2hrs at room temperature until no detectable smell of ethyl acetate: ethanol remained and microspheres were formed. Demineralized water was added to dilute the contents and the formed microspheres were filtered through Whatmann filter paper. The residue was washed 3 times with 30 ml portions of Demineralized water. The product was first kept at room temperature for 24 hrs and then subjected to drying in oven at 65^{o}C till the solvent was completely removed.

Characterization of Micro -Spheres

Microspheres were characterized for the Drug Encapsulation Efficiency, *Invitro* drug release, Particle Size, Floating Study, Surface morphology, X-Ray Diffraction Studies and Stability Studies

Encapsulation Efficiency

Twenty five mg of drug loaded microspheres were accurately weighed and dissolved in 25 ml of ethyl acetate: ethanol

(70:30) sonicated for 15 min. Then it was analyzed at 341 nm on a double beam UV/Visible spectrophotometer. The percentage encapsulation efficiency was calculated.

$$\text{Encapsulation Efficiency} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \text{ X } 100$$

Particle Size

Particle size was determined by optical microscopy using light optical microscope. An ocular eyepiece calibrated with stage micrometer was used. Microspheres were dispersed in 0.5% Tween 80 and spread on microscopic slide. Using 10X objective. Average size was determined.

Floating Study

Floating efficiency of microspheres was tested using U.S.P. XXIV dissolution test apparatus with paddle to rotate at 50 r.p.m. A quantity of 100 mg of microspheres was spread on the surface of dissolution medium. Medium used for floating was 900ml of 0.1 N HCl with 0.02% v/v Tween 80 (used as dispersing agent). After 2.5hrs, the floating fraction was separated by pippetting and microspheres were collected on Whatmann filter paper and dried at 40°C. The percentage of microspheres found floating was determined by formula.

$$\text{\%floating after 2.5 hrs} = \frac{\text{Amount of microspheres floating after 2.5hrs}}{\text{Weight of microspheres taken initially}} \text{ X } 100$$

***In-Vitro* Drug Release**

Microspheres of Furosemide were tested for their dissolution rate using U.S.P. XXIV dissolution test apparatus with paddle to rotate at 50 r.p.m. Nine hundred ml of 0.1 N HCl was taken as dissolution medium with temperature of 37±0.5°C. For maintaining sink conditions, a solubilizer was needed. Polysorbate 20 was added in dissolution medium to provide sink condition. This shows solubility of Furosemide at different concentrations of Polysorbate 20 in 0.1 N HCl needed for sink condition. At definite time intervals, 5 ml of the samples were withdrawn and were analyzed for drug content. Withdrawn samples were replaced with fresh dissolution media and calculations for the amount of drug were done using respective regression equation. Drug releases rate was calculated.

Surface morphology

Morphology of the microspheres was studied by scanning electron microscopy. Dried samples were mounted on metal stubs with double side tape. Metal stub was examined under SEM at 20kv. It

reveals the presences of spherical and hollow structure of microspheres.

X-Ray Diffraction Studies

The solid drug powder, Eudragit RSPO and microspheres were analyzed forcrystal arrangement and its crystalline nature by the virtue of diffraction patternanalyzed by Powder X-ray diffractometer (Bruker) at power: 4 KW, source: Cu K-aand wavelength: 1.5418 A°.

Stability Studies

Furosemide microspheres were kept at different storage conditions. Test samples were kept at room temperature and at 40°C.The samples were withdrawn at different time intervals and the drug contents were determined. The percent drug remaining is reported in table. The controlled release Furosemide microspheres were found to be stable at the different Storage condition for one month period.

RESULT AND DISCUSSION

Solubility studies

Results of solubility studies of Furosemide revealed that enhancement in solubility in a mixed hydrotropic solution of Niacinamide and Eudragit RSPO both enhance the solubility of Furosemide in internal phase. Therefore, in addition to polymer Eudragit RSPO, Niacinamide was selected as a solubilizer. It is concluded that the solubility of Furosemide increases synergistically by mixed hydrotropy. Ethyl acetate and ethanol in the ratio of 70:30 was used as internal phase. Ethanol has weaker solubilizing power for Furosemide. The addition Niacinamide (a solubilizer) in ethanol showed very good solubility of Furosemide hence this solvent system was employed to solubilizer Furosemide and polymer technique. The solvents have low toxic potential listed under class 3 of ICH Q3.

Evaluation of Microspheres Formulation Batches

The trial batches of Furosemide microspheres were evaluated for particle size, encapsulation efficiency, floating properties. Encapsulation efficiency is calculated 91% to 98% and Floating properties of microspheres was found to be 45% to 69. Mean size range of microspheres is 299 to 145 µm.

Floating microspheres showed sustained release of the drug in acidic environment and the drug release was found to be approximately linear. The in vitro releases of the drug from microspheres were studied at pH 1.2using USP XXIV basket method. The results are given in the fig. From this study, it shows that the release

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Table 1: Evaluation of Optimized Batches of Furosemide Microspheres

Batch code	Obtained Results		
	Mean Size (µm)	% Encapsulation Efficiency	% Floating after 2.5hrs
F1	299.37	91.6	45
F2	200.93	92.1	64
F3	135.95	85.2	67
F4	183.08	95.9	69
F5	131.17	97	71
F6	120.17	98.2	70
F7	123.96	94.7	66
F8	145.20	96	61

Table 2:% Cumulative Release of Furosemide Microspheres Batches

Batch code	Percent cumulative drug release					
	0.5 hr	1hr	1.5hr	2hr	2.5hr	3hr
F1	6.94	35.07	58.92	79.06	97.18	99
F2	2.86	42.73	66.45	75.83	87.64	93.22
F3	2.45	22.36	40.34	63.51	70.21	73.39
F4	5.99	29.38	62.71	70.96	75.25	91.10
F5	10.88	43.12	72.4	81.30	85.32	87.92
F6	12.37	36.89	64.41	82.67	92.4	95.01
F7	6.20	30.80	67.07	76.87	83.49	91.25
F8	8.99	43.27	66.27	84.83	97.40	100.00

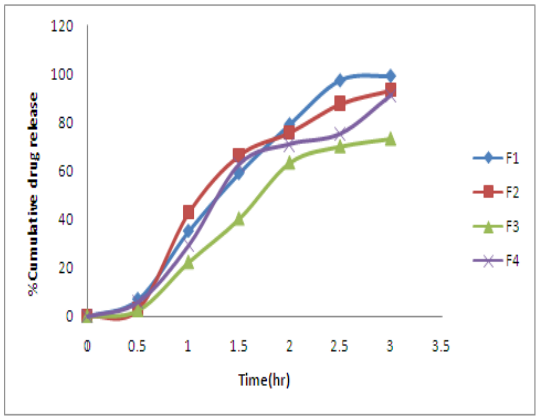

Fig 1: %Cumulative Drug Release v/s Time plot of Furosemide Microspheres (optimized batches F1, F2, F3&F4)

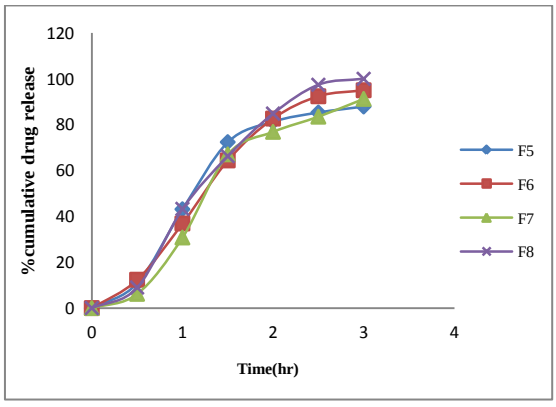

Fig. 2: %Cumulative Drug Release v/s Time plot of Furosemide Microspheres (optimized batches F5, F6, F7& F8)

rates of drug increases with time. The cumulative percent drug release was found to be 95% at 3hrs. Formulation F6 was concluded to be most suitable.

Stability studies

Stability studies performed for a one period of month showed no degradation in formulation F6. %Encapsulation efficiency values showed little changes at the end of month. The Eudragit RSPO, Niacinamide which is the used in formulation F6, was concluded to be most suitable carrier.

Table 3: Stability data of Furosemide Microspheres

S.N.	Storage condition	% Encapsulation efficiency after			
		Initial	7 days	15 days	30 days
1	Room Temp.	100	99.35	99.81	99.47
2	40°C	100	99.75	98.95	99.05

Surface morphology

The SEM of microspheres shows a hollow spherical structure with a smooth surface morphology and exhibited arrange of sizes within each batch. Some of the microspheres showed a dented surface structure but they showed good floating ability on the surface of the medium, indicating intact surface. The outer surface of the microspheres was smooth and dense, while the internal surface was porous. The shell of the microspheres also showed some porous structure.

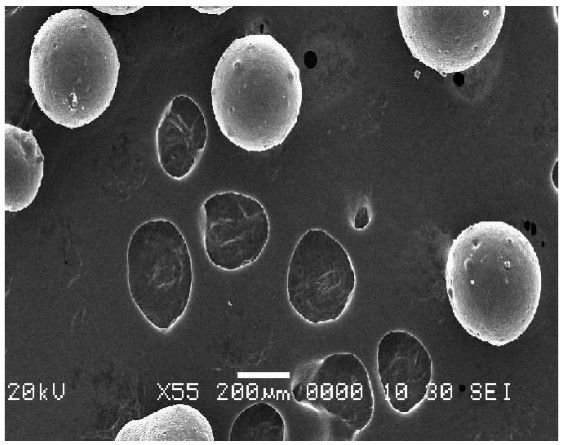

Fig 3: Surface Morphology of Microspheres

It may be caused by the evaporation of solvent entrapped within the shell of microspheres after forming a smooth and dense skin layer.

X-Ray Diffraction Studies

Diffraction spectra of furosemide, Eudragit RSPO, Furosemide microspheres were obtained. The diffraction spectrum of the pure drug, Eudragit RSPO, Furosemide microspheres, indicated the changes produced in drug crystal structure. The X-ray pattern of pure furosemide revealed a drug fingerprint with intense and sharp peaks, indicating its crystalline nature as demonstrated by sharp peaks observed in the range from 0 to 55(degrees 2θ). A reduction in Crystallinity was observed in microspheres spectrum indicating that the Crystallinity of the drug was reduced to greater extent. All the principal peaks from pure furosemide were present in their

Eudragit RSPO and microspheres, although with lower intensity

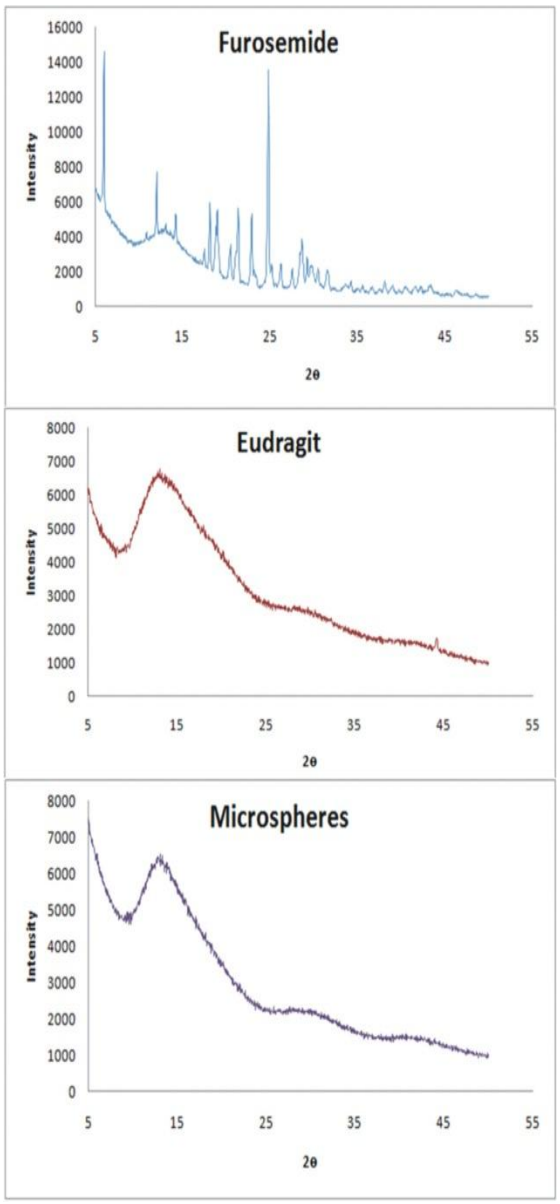

Fig 4: X-ray Diffractograms of pure Furosemide drug, Eudragit RSPO and Microspheres

No new peaks could be observed, suggesting the absence of interaction This is shown in fig.4.

CONCLUSION

It can be concluded that with the proper use of solubilizer (Eudragit RSPO and Niacinamide) with combination, improved the solubility of Furosemide. This concept was provided new, simple, cost effective, safe, accurate, precise and environmentally friendly method for the formulation of poorly water soluble drug. It reduced the total concentration of individual Solubilizers and it enhanced the solubility of Furosemide in ethyl acetate and to make ethyl acetate a strong solvent for emulsification solvent evaporation process by the use of solubilizer and limit the use of toxic organic solvents since ethyl acetate is safer (class 3 solvent) than those generally employed for microsphere production i.e. methylene chloride (class 2 solvent). This technique would be economical, convenient and safe. Microspheres drug delivery system were reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest.

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