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FORMULATION AND IN-VITRO ASSESSMENT OF FUROSEMIDE-LOADED ALGINATE MICROSPHERES VIA IONIC CROSS-LINKING METHOD

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ABSTRACT: Acute or chronic renal failure is a frequent indication for loop diuretics, such as frusemide. At low dosages, it is also used to treat persistent hypertension. It demonstrates properties of pH-independent solubility. Frusemide is primarily metabolized in the kidney and has a half-life of 1.5 hours. Ionotropically gelling sodium alginate in a calcium chloride solution produced the microbeads. The microbeads were then maintained using two different acrylic polymers, Eudragit NE30D and Eudragit S100. The impact of various polymers on the drug's release profile is discussed in this paper. The capacity of the created microbeads to maintain drug release was the main criterion for evaluation. Different formulations were produced using Eudragit S100 (F3, F4) and Eudragit NE30D (F1, F2) at concentrations of 2% and 4%w/w. Various characterization studies were carried out on the final formulations, such as general appearance, particle size determination, rheological studies, moisture content, study of loose surface crystals, drug content and percentage of drug encapsulation efficiency, and in vitro drug release study. The process produced micron-sized alginate spheres with good encapsulation efficiency. In all formulations, only 72% to 90% of the cumulative drug release was visible after 9 hours, suggesting that the drug release was sustained and followed Higuchi's diffusion model. In terms of drug release prolongation and encapsulation efficiency, the Eudragit NE30D 4%w/w formulation F2 fared better than the others.

KEYWORDS: Micropellets, Eudragit, Frusemide, and Ionotropic gelation

I. INTRODUCTION

Micro pellets are solid particles of various shapes (spherical to oval and spheroid) of size ideally less than 125 micron that can be suspended in a suitable aqueous vehicle and injected by 17-20 Gz needle. Each particle is basically a matrix of drug dispersed in a polymer from which drug is released by a mixed order process. Calcium induced ionotropic gelation of sodium alginate was used as a method for preparation of micro pellets. In this method sodium alginate is used as a natural primary polymer and calcium chloride is used as cross linking agent. Droplets of sodium alginate- drug mixture or dispersion immediately forms a cured gel matrix (Ca -Alg) in the presence of calcium ions. The Ca-Alg matrix is used not only as a vehicle for drug delivery, but also as a material in biomedical engineering1, 2. The micropellets thus produced were further made sustained by using different polymers namely Eudragit NE30D (a synthetic water insoluble aqueous polymeric dispersion) and Eudragit S100. As reported in different recent studies, these aqueous polymeric dispersions have played a great role in replacing organic solvents in the coating of solid dosage forms with water soluble polymers. These polymeric dispersions form a homogenous film on drying and provide a diffusion controlled release of the drug from the polymer matrix. The effect of these polymers of varying solubility and other physicochemical properties, on the release profile of the drug has been studied and reported in

this present study by using a technique free from any organic solvent with an objective to encapsulate drugs of varying solubility within water insoluble acrylic polymers in an absolute aqueous environment.

Frusemide3 (C12H11ClN2O5S) is 4-chloro-N-furfuryl-5-sulphamoylanthranilic acid with Molecular Weight 330.74 and categorized as a potent high ceiling loop diuretic agent commonly indicated for acute or chronic renal failure. In low dose it is also used for the treatment of chronic hypertension. Its oral dose recommended in oedema, 20 to 40 mg daily; in oliguria, 250 mg 4 to 6 times daily; by intramuscular or slow intravenous injection. It shows a prompt onset of action and produces a peak diuresis far greater than that observed with other diuretic agents. The drug is practically insoluble in water and has a biological half life of 2 hr in patients with renal insufficiency. It has oral availability 60% and t½ is 1.5 hours. For Frusemide, metabolism occurs predominantly in the kidney. Hence it was selected as the model drug for the present investigations to prepare controlled or sustained release formulations.

II. MATERIALS AND METHODS

Frusemide was received as a gift sample from Diamond Drugs Pvt. Ltd, Howrah, West Bengal. Sodium alginate and Calcium chloride dehydrate (extra pure) were obtained from Loba Chemie, Mumbai. Eudragit (NE30D and S100) (Rhomb Pharma).All other chemicals of Analytical Grade were purchased from local supplier as required.

PREPARATION OF FRUSEMIDE LOADED MICROPELLETS

The micropellets of the drug were prepared by the ionotropic gelation technique. The mucilage of sodium alginate (4%w/v) was prepared by dispersing the sodium alginate in de-ionised water under continuous stirring for 30 minutes. The weighed amount of the drug (40%w/w) was thoroughly mixed with sodium alginate dispersion by using mechanical stirrer maintaining the speed at 500-600 rpm. To this dispersion the desired polymer was mixed in suitable proportions and the entire mixture was stirred for 45 min. The resulted bubble free, homogeneous dispersion was extruded in to 100ml of calcium chloride (4% w/v) solution through hypodermic syringe with flat tip needle (20G) and stirred at 100rpm using magnetic stirrer. The formed micro beads were allowed to cure for 1 hour in the calcium chloride solution to complete the gelation reaction before being filtered and washed thoroughly with distilled water. They are then left overnight at room temperature and then placed in an oven at 50°C for 3 hours for further drying4, 5 .

CHARACTERIZATION OF MICROPELLETS

General appearance Shape, color, odor, stickiness were measured by visualization and touching the beads6 .

Particle Size Determination Particle size distribution of the microspheres was determined by optical microscopy6 using calibrated ocular eyepiece. Fifty microspheres were evaluated and the experiment was performed. Geometric mean diameter was then calculated using the equation: Xg= 10 X [(ni X log Xi) / N] ----- (Equation 1) Where Xg is geometric mean diameter, ni is no of particles in the range, Xi is the mid point of range, N is total no of particles analyzed.

Scanning Electron Microscopy (SEM) Morphological examination of the surface and internal structure of the dried beads was performed by using a scanning electron microscope (SEM). For examination of the internal structure of the beads, they were cut in half with a steel blade. Micropellets before dissolution were only subjected to SEM

study since, after dissolution the pellets become swollen palpable mass. Photographs were taken within a range of 50 - 500 magnifications

Rheological Study The dried beads were allowed to fall freely through a funnel fixed at 1 cm on a horizontal surface and the angle of response (θ) was measured: Where, θ = tan-1 h/r ---- (Equation 2)

Moisture Content Determination It was done by using I.R moisture balance. The beads were placed at 80oC for 10 minutes in I.R moisture balance and % moisture content was calculated.

Loose Surface Crystal (LSC) Study About 100 mg of beads were accurately weighed and suspended in 100 ml phosphate buffer pH 6.8. They were shaken vigorously for 5 min. the leached out solution is analyzed spectrophotometrically at 277.5 nm.7

Drug Content and Drug Entrapment Efficiency About 50 mg of beads were taken and dissolved in 50ml USP phosphate buffer pH 7.4 for overnight in a 100 ml volume flask. Accurately 1 ml was withdrawn and diluted up to 10ml and analyzed in UV max 277.5nm to calculate drug content8 . Drug Entrapment Efficiency (%DEE) 9 was calculated by using the following formula:

$$\%DEE = \frac{\text{Actual drug content} \times 100}{\text{Practical content}} \text{-------- (Equation 3)}$$

In -Vitro Drug Release The in vitro release of the drug from alginate beads was studied in Phosphate buffer of pH 6.8 (900mL) by using USP basket type dissolution rate test apparatus. At regular interval of 1hour, 5ml of the solution was withdrawn and the same amount of fresh medium was replaced to maintain the sink condition. After filtration through 0.45µ filter paper, the absorbance was measured spectrophotometrically at 277.5 nm and the percent drug release was calculated. Release drug data model fitting The suitability of several equation that are reported in the literature to identify the mechanisms for the release of drug was tested with respect to the release data up to the first 50% drug release. The data were evaluated according to the following equations:

Zero order model[10]

$$M_t = M_0 + K_0 t$$

Higuchi model[11]

$$M_t = M_0 + K_H t^{0.5}$$

Korsmeyer-Peppas model[12]

$$M_t = M_0 + K_K t^n$$

Where Mt is the amount of drug dissolved in time t. M0 is the initial amount of the drug. K0 is the Zero order release constant, KH is the Higuchi rate constant, KK is a release constant and n is the release exponent that characterizes the mechanism of drug release.

III. RESULTS AND DISCUSSION

The micropellets were prepared by Ionotropic-Gelation method using different polymers did show significant results during their evaluation. The size of the micropellets found to be in the range of 590 µm to 790 µm and it was observed that with increase in concentration of the Eudragit (copolymers) particle size of the micropellets significantly increased. The average particle size was highest for formulation F2 (i.e. with Eudragit NE30D 4%w/w). The particle size distribution was uniform and narrow. The scanning electron micrograph (Figure 3-5) shows the pellets being spheroid in shape. Surface depression was noticed at the point of contact on the drying paper. On comparison of the pellets prepared from the polymers in highest concentration more roughness was observed with Eudragit NE30D. Eudragit S100 produced particle with smoother surfaced as compared to others. It can be concluded that the roughness is due to the density of the matrix which in turn justifies its sustained release. The micrograph of the blank pellets (Figure 3) act as a control and suggests that increase in total weight of the pellets

makes it more spherical. The rheological parameter like angle of repose of all the pellets (Table 2) confirms better flow properties. Low moisture content in all the micropellets indicates the effectiveness of the optimized drying condition. Low moisture level ensures better stability of the drug in the micropellets.

Loose surface crystal (LSC) study was an important parameter giving an indication of the amount of drug on the surface of the micropellets without proper entrapment. It also confirms net drug loss during processing is minimal. With the increase in the copolymer concentration % LSC decreased significantly owing to high entrapment of drug in the dense network of polymers.

High entrapment efficiency of drug with Eudragit NE30D (Table 2) compared to S100 polymer was observed which confirms it being more rigid among the others.

The in vitro release data of all the formulations were fitted in Zero order, Higuchi matrix and Peppas model and the rate constants (K), correlation coefficient (R2) and n value were compared to know the mechanisms of drug release from the micropellets. Comparing the R2 value of both the kinetic models (Table 3), it is evident that all the batches predominantly showed zero-order release. The formulations of Eudragit NE30D sustained the release of the drug and % cumulative drug release after 9 hours was found to be only 72.39% for formulation F2, which is least among all formulations, while for the formulation S100, the % cumulative drug release varied depending on its concentrations, which is not having any significant variations after 9 hours (Figure 1-2). Predominantly, the drug gets released by passive diffusion through water filled pores. The loose surface crystal study revealed the fact on drug release that due to drug on the surface of the micropellets the in vitro release profile obtained indicated initial fast release followed by a sustained pattern i.e. a biphasic pattern. Batches of Eudragit NE30D micropellets showed more prolonged action as evident from its t50 values when compared with others. Increase in the polymer concentration increased the crosslink density thereby creating barrier for drug diffusion, hence more prolongation.

Table-1: General appearance study of all micropellet formulations

Parameters	FC	F1	F2	F3	F4
Eudragit Composition (% w/w)	—	NE30D-2%	NE30D-4%	S100-2%	S100-4%
Shape	Spherical	More spherical	Spherical in shape with several edges	Spherical	Spherical in shape with irregular edges
Size by visualization	Small	Larger than control	Largest in size	Larger than control	Larger than F2
Colour	Creamish white	More whitish than control	Nearly white beads	White beads	White beads
Stickiness	None	None	None	None	None
Odour	No	No	No	No	No
Surface texture	Smooth	Less than control	Less than F1	Less than control	Less than F3

FC (Control), F1and F2 formulations containing Eudragit NE30D 2%w/w & 4%w/w respectively and F3, F4 formulations containing Eudragit S100 2%w/w & 4%w/w respectively

TABLE-2: Various Characterization Parameters for Micropellets Containing Frusemide and Release Retarded with Eudragit Ne30D and Eudragit S100

Formulation Code	Mean Diameter (μm ± S.D.)	Angle of repose θ ± S.D.)	Moisture content (% ± S.D.)	Drug Entrapment Efficiency (% ± S.D.)	LSC with respect to Entrapped Drug (%)
FC	593.4 ± 0.16	16.36 ±1.16	6.92 ± 0.15	89.34 ± 0.56	3.189
F1	757.93 ± 0.41	15.08 ± 1.13	3.04 ± 0.26	91.23 ± 0.68	2.369
F2	789.57 ± 0.32	14.06 ± 1.17	3.01 ± 0.05	95.74 ± 0.65	2.005
F3	674.37 ± 0.65	22.03 ± 1.78	3.01 ± 0.16	86.09 ± 0.85	4. 801
F4	756.52 ± 0.34	21.93 ± 1.89	1.91 ± 0.41	83.58 ± 0.91	3.041

Results shown are the mean ± SD. n = 6
FC (Control), F1and F2 formulations containing Eudragit NE30D 2%w/w & 4%w/w respectively and F3, F4 formulations containing Eudragit S100 2%w/w & 4%w/w respectively

TABLE-3: Dissolution kinetics and the model fittings (R & K values) for all the formulations.

Formulation Code	t50 (min)	Zero order		Higuchi Square Root		Korsmeyer-Peppas		
		K_0	R^1	K_H	R^1	K_K	R^1	n value
FC	159	12.975	0.9573	29.820	0.878	5.255	0.924	1.135
F1	287	9.348	0.963	23.909	0.757	22.6416	0.948	0.490
F2	347	8.925	0.874	21.251	0.719	14.161	0.939	0.7647
F3	178	11.633	0.968	29.890	0.872	5.558	0.943	1.3369
F4	297	9.126	0.957	30.862	0.884	5.348	0.957	0.8531

Results shown are the mean ± SD. n = 3 (K_0-Zero Order Rate Constant, K_H - Rate Constant Higuchi Model, K_K - Rate Constant Peppas Model, R2 – Correlation coefficient.)
FC (Control), F1and F2 formulations containing Eudragit NE30D 2%w/w & 4%w/w respectively and F3, F4 formulations containing Eudragit S100 2%w/w & 4%w/w respectively

Figure.1: In Vitro drug release profile of Eudragit NE30D retarded Microbeads (FC-control and F1, F2 formulations containing Eudragit NE30D 2%w/w & 4%w/w respectively)

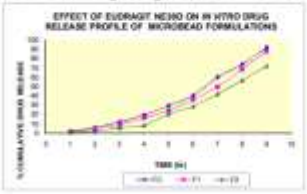


Figure.3: Scanning Electron Micrograph of blank calcium-alginate micropellets.

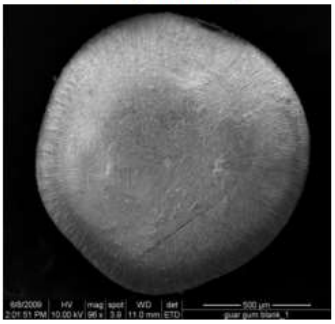


Figure.4: Scanning Electron Micrograph of Frusemide loaded alginate micropellets with Eudragit NE30D (4%w/w) Formulation F2

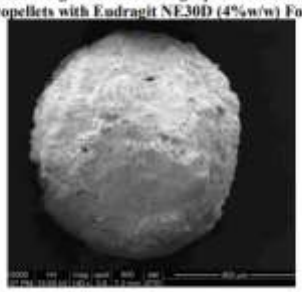
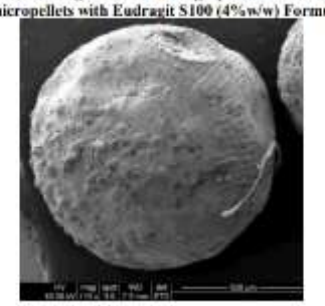


Figure.5: - Scanning Electron Micrograph of Frusemide loaded alginate micropellets with Eudragit S100 (4%w/w) Formulation F4



IV. CONCLUSION

In conclusion, the formulation and in-vitro assessment of furosemide-loaded alginate microspheres prepared via the ionic cross-linking method have demonstrated significant potential in controlled drug delivery applications. The study successfully encapsulated furosemide within alginate microspheres, achieving a controlled and sustained release profile. The in-vitro evaluation indicated that the microspheres possess desirable physicochemical properties, including uniform size distribution and high encapsulation efficiency. The release kinetics of furosemide from the alginate microspheres showed a prolonged release, which is beneficial for enhancing therapeutic efficacy and improving patient compliance. These findings support the viability of this formulation approach for furosemide and potentially other therapeutic agents, highlighting its promise in the development of advanced drug delivery systems.

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