CHAPTER 13

APPENDIX

PUBLISHED PAPERS

1) A STUDY ON THE EFFECT OF DIFFERENT ACRYLIC POLYMERS ON FRUSEMIDE LOADED CALCIUM ALGINATE MICROPELLETS PREPARED BY IONOTROPIC GELATION TECHNIQUE

Authors- GHOSH AMITAVA *, .NATH L.K[1], AND ROY PARTHA

Journal- International Journal of Pharmacy, (www. priory.com), April, 2007

2) A STUDY ON THE EFFECT OF DIFFERENT POLYMERS ON FRUSEMIDE LOADED CALCIUM ALGINATE MICROPELLETS PREPARED BY IONOTROPIC GELATION TECHNIQUE.

Authors- GHOSH AMITAVA *, .NATH L.K[1], DEY B.K.AND ROY PARTHA

Journal – Indian Journal of Pharmaceutical Education and Research, India, 41(4), Oct-Dec, 2007

3) DEVELOPMENT, EVALUATION AND METHOD SELECTION FOR THE PREPARATION OF LAMIVUDINE MICROSPHERES

Authors- AMITAVA GHOSH*, U.K. NAYAK AND PARTHA ROY

Journal- International Journal of Pharmacy, (www. priory.com) June, 2007

PAPERS IN CONFERENCE

1) EVALUATION OF ANTIMICROBIAL ACTIVITY OF FRUSEMIDE

Authors- AMITAVA GHOSH*, K. SENGUPTA AND A. CHATTERJEE

Oral presentation in Indian Pharmaceutical Congress (IPC), Bombay, 2006

PAPERS COMMUNICATED

1) **MATHEMATICAL MODELLING AND STATISTICAL OPTIMIZATION OF IN-VITRO RELEASE BEHAVIOUR OF FRUSEMIDE LOADED CALCIUM ALGINATE MICROPARTICLES**

Authors- GHOSH AMITAVA*, NATH L.K. AND ROY PARTHA.,

Journal- Journal of Americal Association of Pharmaceutical Sciences, USA.

2) **DEVELOPMENT AND OPTIMIZATION OF CALCIUM ALGINATE MICROPARTICLES COINTAINING FRUSEMIDE BY IONOTROPIC GELATION TECHNIQUE**

Authors- GHOSH AMITAVA*, SA. BISWANATH[1]. NATH L.K[2]. AND ROY PARTHA.

Journal- Indian Drugs, India

3) ***IN VIVO* EVALUATION AND ESTABLISHMENT OF *IN VIVO- IN VITRO* CORRELATION OF LAMIVUDINE LOADED MICROSPHERES AFTER ORAL ADMINISTRATION IN RABBITS.**

Authors- GHOSH AMITAVA*, NAG TANUSREE, NAYAK UDAYA KUMAR, ROUT PRASHANT AND ROY PARTHA.

Journal- International Journal of Pharmaceutical Medicine, New Zealand.

4) **MICROSPHERES: A PROMISING APPROACH IN NOVEL DRUG DELIVERY.**

Authors- AMITAVA GHOSH*, SIMLI SARKAR, SOMNATH BHADHURI, , TANUSREE NAG AND PARTHA ROY.

Journal- International Journal of Pharmacy, (www. priory.com)

5) **BIOREMEDIATION OF HEAVY METALS IN LEAF EXTRACT OF NEEM BY CHELATION- A PROSPECTIVE METHOD FOR PHARMACEUTICAL INDUSTRY.**

Authors- P.ROY*, **A.GHOSH,** P.CHAKRABORTI, S.SARKAR, S.BHADURY& T.NAG.

Journal – Indian Journal of Pharmaceutical Education and Research, India

6) **PHARMACOLOGICAL ASSESSMENT AND TOXICOLOGICAL EVALUATION OF CHYAWANPRASH AWALEHA.**

Authors- T.NAG*, **A.GHOSH,** S.SARKAR, S.BHADHURI AND P.ROY

Journal- European Journal of Clinical Nutrition.

7) ***IN-VITRO*** **EVALUATIONS OF SOLVENT DEPOSITED DOMPERIDONE PREPARATIONS FOR SOLUBILITY IMPROVEMENT.**

Authors- DR. B.B. BHOWMIK1*, DR. F.V. MANVI2, DR. R.C. DOIZAD2, A. CHATTERJEE1, **A. GHOSH**[1], P. ROY1, DR. A. MUKHERJEE.

Journal- The Pharma Review, India

8) **DEVELOPMENT AND EVALUATION OF PROPRANOLOL HYDROCHLORIDE TRANSDERMAL PATCHES BY USING HYDROPHILIC AND HYDROPHOBIC POLYMER**

Authors- DEY B.K*, NATH L.K, MOHANTI B[1], **GHOSH A,** BHOWMIK B.B AND ROY P.

Journal – Indian Journal of Pharmaceutical Education and Research, India

9) **DEVELOPMENT AND EVALUATION OF VERAPAMIL HYDROCHLORIDE TRANSDERMAL PATCHES BY USING HYDROPHILIC AND HYDROPHOBIC POLYMER**

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Journal – Indian Journal of Pharmaceutical Sciences

A STUDY ON THE EFFECT OF DIFFERENT ACRYLIC POLYMERS ON FRUSEMIDE LOADED CALCIUM ALGINATE MICROPELLETS PREPARED BY IONOTROPIC GELATION TECHNIQUE

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ABSTRACT Page 1 of 15

The objective of this study is to encapsulate drugs in different acrylic polymers of varying solubility in an absolute aqueous environment. The micropellets were prepared using ionotropic gelation technique, where gelation of anionic sodium alginate, the primary polymer, was achieved with oppositely charged counterion to form microparticles which were further made sustained by using different acrylic polymers namely Acrycoat E30D (poly [ethyl acrylate methyl methacrylate]), Acrycoat L30D (poly [ethyl acrylate methyl methacrylate]), Acrycoat S100 (poly [ethyl acrylate methyl methacrylate]). The effect of these polymers on the release profile of the drug has been reported in this paper. Frusemide, a potent diuretic, was selected as the model drug for the experiments.

Nine set of formulations were prepared using Acrycoat E30D (E1, E2, E4); Acrycoat L30D (L1, L2, L4) and Acrycoat S100 (S1, S2, S4) at concentration (1%, 2%, 4%w/w). The final formulations were subjected to several characterization studies. All the batches sustained the release of the drug for more than 8 hours. Among all acrylic colloidal polymer dispersion, Acrycoat E30D showed high encapsulation efficiencies and maximum prolongation of drug release.

KEYWORDS- Micropellets, Ionotropic gelation, Frusemide, Acrylic polymers

INTRODUCTION

One of the common methods of controlling the rate of drug release is microencapsulation. The encapsulation techniques (e.g., solvent evaporation or coacervation–phase separation) normally involve water insoluble polymers as carriers which require large quantity of organic solvents for their solubilization. (Bodmeier R, et al 1991)(Baken JA. 1987) As a result the processes become vulnerable to safety hazards, toxicity and increases the cost of production making the techniques non reproducible, economically and ecologically at an industrial scale. These

concerns demand a technique free from any organic solvent. Thus, the objective of this study is to encapsulate drugs of varying solubility within water insoluble acrylic polymers in an absolute aqueous environment.

Recently, aqueous polymeric dispersions have played a great role in replacing organic solvents in the coating of solid dosage forms with water soluble polymers.(Lehmann K. 1989)(Steuernagel CR. 1989)(Bodmeier R.et al1993) These polymeric dispersions forms a homogenous film(JamesW.McGinity.1997) on drying and provides a diffusion controlled release of the drug from the polymer matrix.

The micropellets were prepared using ionotropic gelation technique (Lim F. et al 1980) (Segi N. et al 1989)(Bodmeier R.et al 1989) where gelation of anionic polysaccharide sodium alginate, the primary polymer of natural origin, was achieved with oppositely charged calcium ions, acting as counterion,(Lim LY.et al 1997) to form instantaneous microparticles. The micropellets thus produced were further made sustained by using different polymers namely Acrycoat E30D (poly [ethyl acrylate methylmethacrylate]) – a synthetic water insoluble aqueous polymeric dispersion, Acrycoat L30D (methacrylic acid ethylacrylate), Acrycoat S100 (poly [ethyl acrylate methyl methacrylate]). 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 paper.

Acrycoat E30D, which contains 28.7% solids, is one of the first aqueous polymeric dispersions; it was marked initially in Europe and later I the United States for pharmaceutical applications in the name of Eudragit NE30D. It is prepared by emulsion polymerization and consists of neutral copolymers of ethyl acrylate methyl methacrylate esters that are insoluble over the entire physiological pH range. It is thus suitable for the development of pH independent modified-release oral dosage forms, provided that the solubility of the drug is pH independent. Acrycoat L30D, a 30% aqueous dispersion of copolymer of poly (methacrylic acid ethylacrylate) esters. Copolymers of methyl methacrylic acid and ethyl acrylate as ester components with methacrylic acid are used as enteric coatings, because they contain carboxylic groups that are transformed to carboxylate groups in the pH range of 5-7 by salt formation with alkali and amines. In pure water and diluted acids they form water insoluble films resistant to gastric juices. They are popularly applied in formulating preparations which shows pH dependant drug release. Acrycoat S100, a free flowing powder containing 95% w/w solid polymer is sparingly soluble in water but soluble in alcohol and acetone with 3% v/v water. Being copolymers of Methacrylic acid, they are widely used as slow release enteric coating in tablet and capsule manufacturing industry. They are insoluble in gastric fluid but freely soluble in intestinal fluid of pH 7 and above. They are popularly applied in formulating sustained release pH dependant formulations.

Frusemide (Abdurrahman MA. et al 1992) was selected as the model drug for the experiments. It is a potent high ceiling loop diuretic agent commonly indicated for the treatment of edema of hepatic, cardiac and pulmonary systems during acute or chronic renal failure. In low dose it is a drug of choice for the treatment of chronic hypertension. (Gilbert HM. 1975) It shows a prompt onset of action and produces a peak diuresis far greater than that observed with other diuretic agents. This intense diuresis from a conventional tablet provokes major side effects like electrolytic imbalance manifested in the form of tiredness, dehydration and muscular cramps. (The Extra Pharmacopoeia. 2006) The drug is practically insoluble in water and has a biological half life of 2 hr in patients with renal insufficiency. The aim of the experiment is to produce sustained release micropellets of Frusemide, that can be tabletted or capsulated, exhibiting the same diuretic effect as that of a conventional tablet, but eliminating the toxicity, patient discomfort and non compliances.

MATERIALS AND METHODS

Frusemide was received as a gift sample from Aventis Pharma Ltd., Ankleshwar. Sodium alginate (viscosity of 2% aqueous solution at 25°C was 3500cps) was obtained from Loba Chemie, Mumbai. Calcium chloride dihydrate (A.R. Grade, E.Merck, Germany); Acrycoat E30D, aqueous dispersion (solid content-28.7%w/w) Acrycoat L30D (solid content-30 %w/w) (poly [ethyl acrylate methyl methacrylate]), Acrycoat S100 (solid content-95%w/w) (Methacrylic acid copolymer Type B) ;(Corel Pharmaceuticals, Ahmedabad). All other chemicals were purchased from local supplier in A.R. and L.R. Grade as required.

Preparations of micropellets

The drug (30%w/w) was dispersed uniformly in aqueous mucilage of sodium alginate (2%w/v) 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 30 min. The pellets were formed by dropping the bubble free dispersions through a glass syringe into a gently agitated calcium chloride (5% w/v) solution 100 ml. The gelled pellets were cured for 30 min before being filtered and washed thoroughly with distilled water. They are then oven dried for 6 hr at 60°C. The #22 I.P. standard sieve size fractions were used for further studies.

Process variables and Process optimization

The following process variables were investigated (concentration of sodium alginate; concentration of calcium chloride; curing time; height of dropping; variation of drug loading; stirring speed and stirring time) and the different batches thus produced were analyzed for size, shape, ease of preparation, drug content and drug release. On the basis of the result obtained the process parameters were optimized as follows:-

Sodium alginate concentration – 2%w/v

Calcium chloride concentration – 5%w/v

Drug load – 30%w/w

Curing time – 30 min

Height of dropping – 2 cm from the level of CaCl2 solution

Stirring time and speed – 30 min & 500 rpm

Drying condition – oven drying for 6hr at 60°C

Different batches of micropellets were then prepared by using the optimized process variables and the only variation followed was use of different polymers. Nine set of formulations were prepared using Acrycoat E30D (E1, E2, E4); Acrycoat L30D (L1, L2, L4) and Acrycoat S100 (S1, S2, S4) at concentration (1%, 2%, 4%w/w). The final formulations were subjected to several characterization studies.

Characterization of micropellets

Particle size determination

Particle size analysis (Indian Pharmacopoeia. 1996) of the micropellets was done by sieving method using Indian Standard Sieves # 16, #22 and #30. Average particle size was calculated using the formula: - davg = ? dn / ? n, where n=frequency weight, d= mean diameter. (Table-1)

Scanning electron microscopy

Morphological characterization of the micropellets was done by taking scanning electron micrograph in (JEOL JSM Model 5200, Japan). Cross sectional view were obtained by cutting the micropellets with a razor blade. The samples were coated to 200A° thickness with gold-palladium using (Pelco model 3 sputter coater) prior to microscopy. A working distance of 20nm, a tilt of 0° and accelerating voltage of 15kv were the operating parameters. 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. (Fig 1-5)

Rheological study (Indian Pharmacopoeia. 1996)

To determine the rheological properties of the micropellets, the angle of repose of all the samples were measured using funnel method. Bulk density was determined by taking known quantity of micropellets in 100ml measuring cylinder and tapping it 3 times from a height of 1 inch at 2 seconds interval. The bulk density was calculated by dividing sample weight by final bulk volume. (Table-1)

Determination of Moisture content (Indian Pharmacopoeia. 1996)

The formulations were subjected to moisture content study, by placing the micropellets at 60° C for 10 minutes in an IR moisture balance. (Table-1)

Loose surface crystal study (LSC) (Abu IK. et al 1996)

This study was conducted to estimate the amount of drug present on the surface of the micropellets which may show immediate release in the dissolution media. 100mg of micropellets (# 22 sizes) were suspended in 100ml of phosphate buffer (pH 6.8), simulating the dissolution media. The samples were shaken vigorously for 15 min in a mechanical shaker. The amount of drug leached out from the surface was analyzed spectrophotometrically at 277.5nm. Percentage of drug released with respect to entrapped drug in the sample was recorded. (Table-1)

Determination of Drug entrapment efficiency

About 100mg of micropellets (# 22 sizes) were accurately weighed and dissolved in 25ml of Phosphate buffer (pH 7.4) for overnight and an aliquot from the filtrate was analyzed spectrophotometrically, after suitable dilution, using SHIMADZU UV-VIS, at 277.5nm. Reliability of the method was judged by conducting recovery analysis using known amount of drug with or without polymer. Recovery averaged 100±0.89%. Drug content of every batch was determined for every size range of micropellets and the mean± S.D.was calculated. Drug Entrapment Efficiency (DEE) was calculated according to the formula % DEE= (Actual drug content/ Theoretical drug content) x 100. (Table-2)

Disintegration studies (Bodmeier R. et al 1989)

Disintegration studies were performed in 0.1N HCl and simulated intestinal fluid (USP XXI) in a rotating bottle apparatus. 5 pellets per vial were kept in 50 ml medium at 37° C and the vials were rotated at 25 rpm. The measured disintegration time was the time taken by the pellets to disintegrate into crystals, the polysaccharide being soluble and the drug insoluble in the disintegrating fluid. (Table-2)

In vitro dissolution study

The USP rotating – paddle Dissolution Rate apparatus (Veego, Mumbai) was used to study drug release from the micropellets. The dissolution parameters [100mg pellets ; 37± 2°C ; 50 rpm ; 500ml of USP Phosphate buffer (pH 6.8); n=3; coefficient of variation< 0.05] were maintained for all the nine formulations. 2ml of aliquot were withdrawn at specified intervals and after suitable dilution assayed by SHIMADZU UV-VIS PharmSpec 1700 spectrophotometer at 277.5nm. The data for percent drug release was fitted for zero order and Higuchi matrix equation. The polysaccharide did not interfere with the assay as confirmed from conducting a dissolution study of blank alginate beads. (Table-2)

Determination of stability of the micropellets

The formulations showing the best performance, with respect to *in vitro* release, from each set of formulations were stored at 4°C, room temperature and 45°C for a month. Every week samples were withdrawn and were assayed spectrophotometrically at 277.5 nm using Phosphate buffer (pH 6.8) as blank. (Table- 3)

RESULTS AND DISCUSSION

The micropellets were prepared in an environment free from organic solvents by dropping a mixture of colloidal copolymer dispersion, the dispersed drug Frusemide, and mucilage of sodium alginate in calcium chloride solution, which acted as a counterion. The droplets instantaneously formed gelled spherical beads due to cross linking of calcium ion with the sodium ion which remained ionized in the solution. Smaller particle can be prepared by adjusting the height of the syringe from the level of counterion solution, compression force on the plunger of the syringe. The gelled particles were cured to get sufficiently hardened and then filtered and dried. The colloidal polymer particles fused into the polymer matrix during drying with the drug being dispersed in the latex. The *micropellets thus formed using three different polymers did show significant results on evaluation.*

The size of the micropellets ranged between 540 µm to 800 µm and increased significantly with the concentration of the copolymers. The average particle size was on the highest side with Acrycoat E30D polymers followed by S100. The particle size distribution was uniform and narrow. It can be estimated that with further increment in the copolymer concentration the particles would change from micro to granular level.

The scanning electron micrograph (Figure 6-8) shows the pellets being discoid in shape. Surface depression was noticed at the point of contact on the drying paper. On comparison of the pellets prepared from three polymers in highest concentration, it was evident from the photograph that more roughness with Acrycoat E30D copolymers was

achieved than that of the other two. Acrycoat L30D giving the most smooth surfaced particle. It can be concluded that the roughness is due to the density of the matrix which in turn justifies its sustained release. The dense network of drug-polymer-copolymer increases the tortuisity, as evident from Figure-9, thus delaying the release of the drug and retarding the penetration of water required to make the pellets swell for disintegration. The micrograph of the blank pellets (Figure-5) act as a control and suggests that increase in total weight of the pellets makes it more spherical.

The rheological parameters like angle of repose and bulk density of all the pellets (Table-1) confirms better flow and packing properties. Thus, the micropellets if tabletted or encapsulated, requires less amount of lubricants and ensures low production cost leading to its feasibility for large scale production.

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. With the increase in the copolymer concentration % LSC decreased significantly owing to high entrapment of drug in the dense network of polymers.

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.

Significantly high entrapment efficiency of drug with Acrycoat L30D (Table-2) over other polymers confirms it being more rigid among the three.

As described during the discussion on the photomicrograph, formulation E4 showed highest disintegration time which may be due to its stronger latex network structure. The micropellets being less porous among the three, delays the penetration of water needed for swelling and eventual disintegration. No disintegration was observed in 0.1N HCl, even when the samples were kept for overnight in the medium confirming with the fact that all the polymers investigated are insoluble in gastric pH and over and above pH 5.5. The ionic character of the polysaccharide alginate also resulted in pH dependant disintegration of the micropellets. Acrycoat E30D consists of neutral copolymers of ethyl acrylate methyl methacrylate esters that are insoluble over the entire physiological pH range. It is thus suitable for the development of pH independent modified-release oral dosage forms, provided that the solubility of the drug is pH independent. The other two acrylic polymers show pH dependent release.

The in vitro release data of all the formulations were fitted in Zero order and Higuchi matrix model and the rate constants and correlation coefficient were compared to get a trend in the release pattern of the drug from the formulations. From Table -2, comparing the R2 value of both the kinetic models, it is evident that all the batches predominantly showed zero-order release. The formulations of E30D sustained the release of the drug for more than

8 hours while for the formulations of L30D and S100, the release varied depending on their concentrations, within a range of 5-8 hours (Figure 1-3) as compared with the conventional tablet dosage form (Figure-4). Predominantly, the drug gets released by passive diffusion through water filled pores. The loose drug on the surface of the micropellets, on release, exposes the pores or micro channels, through which diffusion of the drug present in the inner matrix occurs. Due to loose drug present on the surface of the micropellets (LSC) the *in vitro* release profile obtained indicated a biphasic pattern i.e. initial fast release followed by a sustained pattern. Batches of Acrycoat E30D micropellets showed more prolonged action as evident from its t50 values when compared with other two acrylic polymers. Increase in the polymer concentration increased the crosslink density thereby creating barrier for drug diffusion, hence more prolongation.

When studied for stability at 4°C, room temperature and 45°C for a month, the drug was found to be stable at 4°C and room temperature and all the formulations showed gradual degradation at high temperatures.

CONCLUSION

Sustained release micropellets containing water insoluble drug were successfully prepared employing ionotropic gelation technique, entirely avoiding the use of organic solvents. Apart from the natural water soluble polymer, namely, sodium alginate, the use of copolymer further prolongs the release of the drug. Acrylic based colloidal polymer dispersions (Acrycoat E30D) showed good encapsulation efficiencies and maximum prolongation of drug release. Hence, further studies can be extended taking Acrycoat E30D as the release controlling copolymer. Considering the end product, the micropellets could be administered as prepared or could be compressed into tablet or filled in capsule shell. The entire process is feasible in an industrial scale and demands pilot study.

ACKNOWLEDGEMENTS

Authors wish to thank Aventis Pharmaceuticals (Ankleshwar, India) for providing gift sample of Frusemide and Corel Pharmaceuticals (Ahmedabad, India) for providing gift sample of Acrycoat E30D, Acrycoat L30D, and Acrycoat S100. We are also thankful to the staff of University Science Instrumentation centre (USIC), Jadavpur University, Kolkata for their relentless cooperation in SEM study.

TABLE- 1

COMPARATIVE STUDY OF VARIOUS PHYSICAL PARAMETERS FOR ALGINATE MICROPELLETS CONTAINING FRUSEMIDE AND RELEASE RETARDED WITH ACRYCOAT E30D, L30D AND S100 RESPECTIVELY

Formulation Code	Acrycoat Composition (% w/w)	Moisture content (% ± S.D.)	LSC with respect to Entrapped Drug (%)	Mean Diameter (µm ± S.D.)	Angle of repose ? ± S.D.)	Bulk density (g/cc ±S.D.)
E1	E30D-1%	1.48 ± 0.48	3.549	608.16 ± 0.59	18.32 ± 0.79	0.658 ± 0.68
E2	E30D-2%	1.44 ± 0.56	2.369	760.89 ± 0.51	20.56 ± 1.03	0.674 ± 1.52
E4	E30D-4%	2.23 ± 0.68	1.567	782.78 ± 0.36	21.24 ± 1.97	0.682 ± 1.96
L1	L30D-1%	2.83 ± 0.67	4.318	547.29 ± 0.54	17.53 ± 1.12	0.617 ± 0.84
L2	L30D-2%	2.14 ± 0.46	3.878	613.58 ± 0.72	20.78 ± 1.48	0.646 ± 1.48
L4	L30D-4%	1.67 ± 0.86	2.972	704.26 ± 0.22	22.19 ± 2.07	0.677 ± 2.36
S1	S100-1%	2.21 ± 0.41	3.943	594.57 ± 0.43	18.24 ± 1.31	0.651 ± 1.19
S2	S100-2%	1.91 ± 0.26	3.018	678.32 ± 0.56	21.33 ± 1.78	0.673 ± 1.76
S4	S100-4%	1.76 ± 0.61	2.454	767.25 ± 0.31	22.53 ± 2.19	0.680 ± 2.48

* Results shown are the mean ± SD. n = 6 for mean diameter and n = 3 for moisture content, angle of repose, bulk density and LSC study.

TABLE- 2

COMPARATIVE STUDY OF VARIOUS PHARMACEUTICAL FACTORS FOR ALGINATE MICROPELLETS CONTAINING FRUSEMIDE AND RELEASE RETARDED WITH ACRYCOAT E30D, L30D AND S100 RESPECTIVELY

Formulation Code	Acrycoat Composition (% w/w)	Drug Entrapment Efficiency (% ± S.D.)	Disintegration Time (min)	t50 (min)	Zero order K0	Zero order R2	Higuchi SQRT KH	Higuchi SQRT R2
E1	E30D-1%	94.48 ± 0.48	42	272	9.3247	0.9477	25.479	0.7939
E2	E30D-2%	93.44 ± 0.56	64	290	9.0388	0.9463	23.909	0.7857
E4	E30D-4%	91.23 ± 0.68	97	341	7.9428	0.8407	20.151	0.6492
L1	L30D-1%	99.22 ± 0.43	31	92	18.622	0.9519	44.784	0.9558
L2	L30D-2%	98.56 ± 0.43	43	153	14.435	0.9776	35.857	0.8773
L4	L30D-4%	97.68 ± 0.43	64	267	10.727	0.9123	27.225	0.7444
S1	S100-1%	91.22 ± 0.34	38	119	17.496	0.9844	38.485	0.9329
S2	S100- 2%	86.09 ± 0.85	51	181	13.333	0.9438	30.280	0.8428
S4	S100-4%	83.58 ± 0.91	79	278	10.061	0.9467	23.862	0.7944

* K0 , KH – Release Rate Constants for Zero Order and Higuchi release Kinetic Model respectively

* R2 – Correlation coefficient.

* Results shown are the mean ± SD. n = 3 for disintegration study, entrapment efficiency and dissolution study.

TABLE- 3

ACCELERATED STABILITY STUDIES OF FRUSEMIDE MICROPELLETS PREPARED WITH DIFFERENT POLYMERS

TIME	S4			L4			E4		
(WEEK)	4°C	RT	45°C	4°C	RT	45°C	4°C	RT	45°C
0	100	100	100	100	100	100	100	100	100
1	98.36	98.23	87.39	99.16	97.98	87.47	99.28	98.52	89.28
2	97.38	96.29	82.54	96.79	96.61	85.18	97.57	96.93	88.42
3	94.85	94.36	77.33	94.82	94.21	83.84	96.62	95.89	86.74
4	90.52	91.72	75.94	91.39	92.22	80.71	95.09	95.37	85.33

RT--- ROOM TEMPERATURE

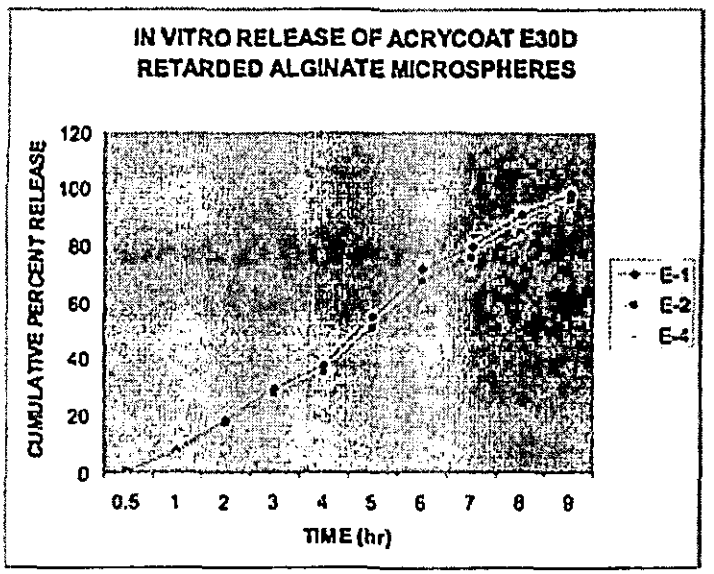

Figure 1

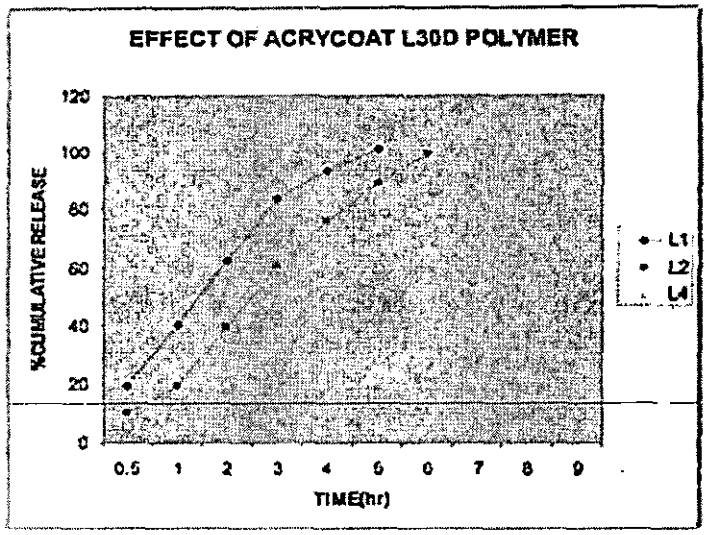

Figure 2

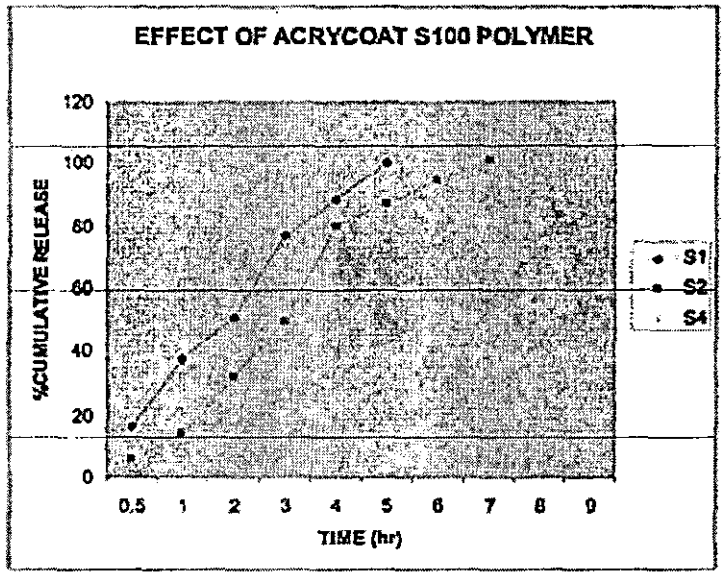

Figure 3

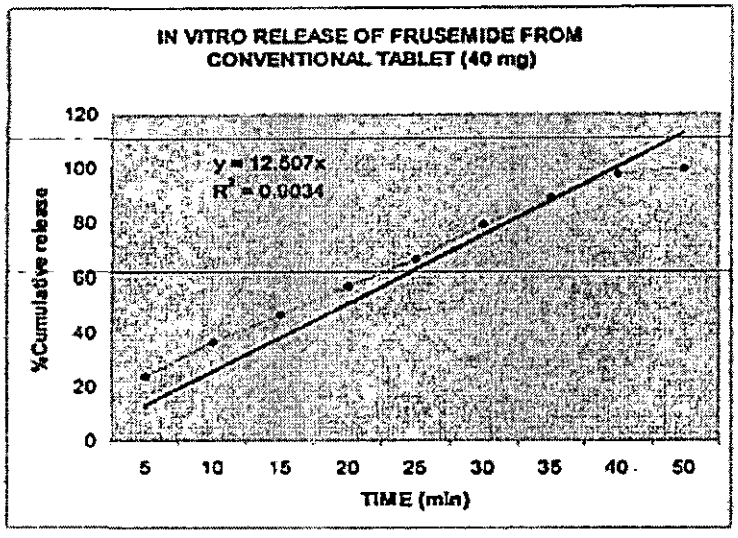

FIGURE 4

Figure 1- SEM Photograph of blank alginate micropellets (50X)

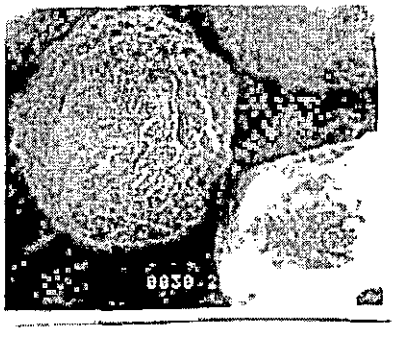

Figure 6- SEM Photograph of Frusemide loaded alginate micropellets with Acrycoat E30D (4%w/w) (50X) Formulation # E4

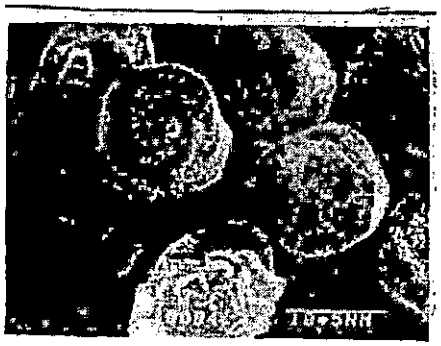

Figure 7 - SEM Photograph of Frusemide loaded alginate micropellets with Acrycoat L30D (4%w/w) (50X) Formulation # L4

Figure 8 - SEM Photograph of Frusemide loaded alginate micropellets with Acrycoat S100 (4%w/w) (50X) Formulation # S4

Figure 9- SEM Photograph of Frusemide loaded alginate micropellets with Acrycoat E30D (4%w/w) (350X) Formulation # E4

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First Published April 2007

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A study on the effect of different polymers on frusemide loaded calcium alginate micropellets prepared by ionotropic gelation technique

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Abstract-

The objective of this study is to encapsulate drugs in polymers of varying solubility in an absolute aqueous environment. The micropellets were prepared using ionotropic gelation technique, where gelation of anionic sodium alginate, the primary polymer , was achieved with oppositely charged counterion to form microparticles which were further made sustained by using different polymers namely Methocel K- 15M (Hydroxy propyl methyl cellulose), Surelease (Ethyl Cellulose) and Acrycoat E30D (poly [ethyl acrylate methyl methacrylate]). The effect of these polymers on the release profile of the drug has been reported in this paper. Frusemide, a potent diuretic, was selected as the model drug for the experiments. Nine set of formulations were prepared using Acrycoat E30D (E1, E2, E4), Methocel K-15M (H1, H2, H4) and Surelease (S1, S2, S4) at concentration (1%, 2%, 4%w/w). The final formulations were subjected to several characterization studies. Batches with Acrycoat E30D and Surelease shown zero-order release whereas batches with Methocel K-15M followed Higuchi model. All the batches sustained the release of the drug for more than 8 hours. Both water soluble and insoluble copolymers were tested and among them acrylic colloidal polymer dispersion (Acrycoat E30D) showed high encapsulation efficiencies and maximum prolongation of drug release.

Keywords- Micropellets, ionotropic gelation, Frusemide, acrylic polymers

INTRODUCTION

One of the common methods of controlling the rate of drug release is microencapsulation. The encapsulation techniques (e.g., solvent evaporation or coacervation–phase separation) normally involves water insoluble polymers as carriers which require large quantity of organic solvents for their solubilization.[1,2] As a result the processes become vulnerable to safety hazards, toxicity and increases the cost of production making the techniques non reproducible, economically and ecologically at an industrial scale. These concerns demand a technique free from any organic solvent. Thus, the objective of this study is to encapsulate drugs of varying solubility within water insoluble polymers in an absolute aqueous environment.

Indian Journal of Pharmaceutical Education & Research
Received on xxxxxxx ; Modified on xxxxxxxxxx
Accepted on xxxxxxxxxx

Recently, aqueous polymeric dispersions have played a great role in replacing organic solvents in the coating of solid dosage forms with water soluble polymers.[3-5] These polymeric dispersions forms a homogenous film[6] on drying and provides a diffusion controlled release of the drug from the polymer matrix.

The micropellets were prepared using ionotropic gelation technique[7-9] where gelation of anionic polysaccharide sodium alginate, the primary polymer of natural origin, was achieved with oppositely charged calcium ions (counterion)[10] to form instantaneous microparticles. The micropellets thus produced were further made sustained by using different polymers namely Methocel K- 15M (Hydroxy propyl methyl cellulose -HPMC) - a water soluble polymer; Surelease (Ethyl Cellulose) – a semi synthetic water insoluble aqueous colloidal polymer; and Acrycoat E30D (poly [ethyl acrylate methylmethacrylate]) – a synthetic water

insoluble aqueous polymeric dispersion. 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 paper.

Frusemide[11] was selected as the model drug for the experiments. It is a potent high ceiling loop diuretic agent commonly indicated for the treatment of edema of hepatic, cardiac and pulmonary systems during acute or chronic renal failure. In low dose it is a drug of choice for the treatment of chronic hypertension.[12] It shows a prompt onset of action and produces a peak diuresis far greater than that observed with other diuretic agents.[12] This intense diuresis from a conventional tablet provokes major side effects like electrolytic imbalance manifested in the form of tiredness, dehydration and muscular cramps.[13] The drug is practically insoluble in water and has a biological half life of 2 hr in patients with renal insufficiency. [11] The aim of the experiment is to produce sustained release micropellets of Frusemide, that can be tabletted or capsulated, exhibiting the same diuretic effect as that of a conventional tablet, but eliminating the toxicity, patient discomfort and non compliances.

MATERIALS AND METHODS

Frusemide was received as a gift sample from Aventis Pharma Ltd., Ankleshwar. Sodium alginate (viscosity of 2% aqueous solution at 25°C was 3500cps) was obtained from Loba Chemie, Mumbai. Calcium chloride dihydrate (A.R. Grade, E.Merck, Germany); Acrycoat E30D, aqueous dispersion (solid content-28.7%w/w) (Corel pharmaceuticals, Ahmedabad); Surelease (Ethyl Cellulose) (Colorcon Ltd.) Hydroxy propyl methyl cellulose (HPMC) (Methocel K- 15M) (Dow Chemical Co.). All other chemicals were purchased from local supplier in A.R. and L.R. Grade as required.

Preparations of micropellets

The drug (30%w/w) was dispersed uniformly in aqueous mucilage of sodium alginate (2%w/v) 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 30 min. The pellets were formed by dropping the bubble free dispersions through a glass syringe into a gently agitated calcium chloride (5% w/v) solution 100 ml. The gelled pellets were cured for 30 min before being filtered and washed thoroughly with distilled water. They are then oven dried for 6 hr at 60°C. The #22 I.P. standard sieve size fractions were used for further studies.

Process variables and Process optimization

The following process variables were investigated (concentration of sodium alginate; concentration of calcium chloride; curing time; height of dropping; variation of drug loading; stirring speed and stirring time) and the different batches thus produced were analyzed for size, shape, ease of preparation, drug content and drug release. On the basis of the result obtained the process parameters were optimized as follows:-

Sodium alginate concentration – 2%w/v
Calcium chloride concentration – 5%w/v
Drug load – 30%w/w
Curing time – 30 min
Height of dropping – 2 cm from the level of $CaCl_2$ solution
Stirring time and speed – 30 min & 500 rpm
Drying condition – oven drying for 6hr at 60°C

Different batches of micropellets were then prepared by using the optimized process variables and the only variation followed was use of different polymers. Nine set of formulations were prepared using Acrycoat E30D (E1, E2, E4); Methocel K-15M (H1, H2, H4) and Surelease (S1, S2, S4) at concentration (1%, 2%, 4%w/w). The final formulations were subjected to several characterization studies.

Characterization of micropellets

Particle size determination

Particle size analysis[14] of the micropellets was done by sieving method using Indian Standard Sieves # 16, #22 and #30. Average particle size was calculated using the formula: - $d_{avg} = \sum dn / \sum n$, where n=frequency weight, d= mean diameter. (Table-1)

Scanning electron microscopy

Morphological characterization of the micropellets was done by taking scanning electron micrograph in (JEOL JSM Model 5200, Japan). Cross sectional view were obtained by cutting the micropellets with a razor blade. The samples were coated to 200A° thickness with gold-palladium using (Pelco model 3 sputter coater) prior to microscopy. A working distance of 20nm, a tilt of 0° and accelerating voltage of 15kv were the operating parameters. 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. (Fig 1-5)

Micromeretic study[14]

To determine the rheological properties of the micropellets, the angle of repose of all the samples were measured using funnel method. Bulk density was determined by taking known quantity of micropellets in 100ml measuring cylinder and tapping it 3 times from a height of 1 inch at 2 seconds interval. The bulk density was calculated by dividing sample weight by final bulk volume. (Table-1)

Determination of Moisture content[14]

The formulations were subjected to moisture content study, by placing the micropellets at 60° C for 10 minutes in an IR moisture balance. (Table-1)

Surface Accumulation study (SA)[15]

This study was conducted to estimate the amount of drug present on the surface of the micropellets which may show immediate release in the dissolution media. 100mg of micropellets (# 22 sizes) were suspended in 100ml of phosphate buffer (pH 6.8), simulating the dissolution media. The samples were shaken vigorously for 15 min in a mechanical shaker. The amount of drug leached out from the surface was analyzed spectrophotometrically at 277.5nm. Percentage of drug released with respect to entrapped drug in the sample was recorded. (Table-1)

Determination of Drug Entrapment Efficiency

About 100mg of micropellets (# 22 sizes) were accurately weighed and dissolved in 25ml of Phosphate buffer (pH 7.4) for overnight and an aliquot from the filtrate was analyzed spectrophotometrically, after suitable dilution, using SHIMADZU UV-VIS, at 277.5nm. Reliability of the method was judged by conducting recovery analysis using known amount of drug with or without polymer. Recovery averaged 100±0.89%. Drug content of every batch was determined for every size range of micropellets and the mean± S.D.was calculated. Drug Entrapment Efficiency (DEE) was calculated according to the formula % DEE= (Actual drug content/ Theoretical drug content) x 100. (Table-2)

Disintegration studies[9]

Disintegration studies were performed in 0.1N HCl and simulated intestinal fluid (USP XXI) in a rotating bottle apparatus. 5 pellets per vial were kept in 50 ml medium at 37° C and the vials were rotated at 25 rpm. The measured disintegration time was the time taken by the pellets to disintegrate into crystals, the polysaccharide being soluble and the drug insoluble in the disintegrating fluid. (Table-2)

***In vitro* dissolution study**

The USP rotating – paddle Dissolution Rate apparatus (Veego, Mumbai) was used to study drug release from the micropellets. The dissolution parameters [100mg pellets ; 37± 2°C ; 50 rpm ; 500ml of USP Phosphate buffer (pH 6.8); n=3; coefficient of variation< 0.05] were maintained for all the nine formulations. 2ml of aliquot were withdrawn at specified intervals and after suitable dilution assayed by SHIMADZU UV-VIS PharmSpec 1700 spectrophotometer at 277.5nm. The data for percent drug release was fitted for zero order and Higuchi matrix equation. The polysaccharide did not interfere with the assay as confirmed from conducting a dissolution study of blank alginate beads. (Table-2)

Determination of stability of the micropellets

The formulations showing the best performance, with respect to *in vitro* release, from each polymer composition were stored at 4°C, room temperature and 45°C for a month. Every week samples were withdrawn and were assayed spectrophotometrically at 277.5 nm using Phosphate buffer (pH 6.8) as blank. (Table- 3)

Study on Drug – Polymer interaction using Infra Red Spectroscopy[14]

The disc method was employed to study the possible interactions between the drug and the selected polymer Acrycoat E30D and sodium alginate. Pure drug, blank alginate pellets and drug loaded pellets with Acrycoat E30D pellets were separately analysed. KBr (IR Grade) discs in a proportion of 1: 100 :: Sample : KBr, were prepared from the samples and are analyzed in FTIR spectrophotometer (SHIMADZU FTIR - 8400S, Japan) over a range of 400- 4000 cm^{-1}. Transmittance (T) spectra were recorded and displayed in an overlay mode in Figure 10.

RESULTS AND DISCUSSION

The micropellets were prepared in an environment free from organic solvents by dropping a mixture of colloidal copolymer dispersion, the dispersed drug Frusemide, and mucilage of sodium alginate in calcium chloride solution, which acted as a counterion. The droplets instantaneously formed gelled spherical beads

due to cross linking of calcium ion with the sodium ion which remained ionized in the solution. Smaller particle can be prepared by adjusting the height of the syringe from the level of counterion solution, compression force on the plunger of the syringe. The gelled particles were cured to get sufficiently hardened and then filtered and dried. The colloidal polymer particles fused into the polymer matrix during drying with the drug being dispersed in the latex. The micropellets thus formed using three different polymers did show significant results on evaluation.

The size of the micropellets ranged between 600 μm to 800 μm and increased significantly with the concentration of the copolymers. The average particle size was on the highest side with Acrycoat polymers followed by Surelease. The particle size distribution was uniform and narrow. It can be estimated that with further increment in the copolymer concentration the particles would change from micro to granular level.

The scanning electron micrograph (Figure 6-8) shows the pellets being discoid in shape. Surface depression was noticed at the point of contact on the drying paper. On comparison of the pellets prepared from three polymers in highest concentration, it was evident from the photograph that more roughness with Acrycoat copolymers was achieved than that of the other two. It can be concluded that the roughness is due to the density of the matrix which in turn justifies its sustained release. The dense network of drug-polymer-copolymer increases the tortuisity, as evident from Figure-9, thus delaying the release of the drug and retarding the penetration of water required to make the pellets swell for disintegration. The micrograph of the blank pellets (Figure-5) act as a control and suggests that increase in total weight of the pellets makes it more spherical.

The rheological parameters like angle of repose and bulk density of all the pellets (Table-1) confirms better flow and packing properties. Thus, the micropellets if tabletted or encapsulated, requires less amount of lubricants and ensures low production cost leading to its feasibility for large scale production.

Surface Accumulation (SA) study was an important parameter giving an indication of the amount of drug on the surface of the micropellets without proper entrapment. With the increase in the copolymer concentration % SA decreased significantly owing to high entrapment of drug in the dense network of polymers.

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.

Significantly high entrapment efficiency of drug with Acrycoat based formulations (Table-2) over other polymers confirms it being more rigid among the three.

As described during the discussion on the photomicrograph, the Acrycoat based formulations showed highest disintegration time which may be due to its stronger latex network structure. The micropellets being less porous among the three, delays the penetration of water needed for swelling and eventual disintegration. No disintegration was observed in 0.1N HCl, even when the samples were kept for overnight in the medium. The ionic character of the polysaccharide resulted in pH dependant disintegration of the micropellets.

The *in vitro* release data of all the formulations were fitted in Zero order and Higuchi matrix model and the rate constants and correlation coefficient were compared to get a trend in the release pattern of the drug from the formulations. From Table -2 it is evident that the batches with Acrycoat E30D (E1-E4) and Surelease (S1-S4) predominantly shown zero-order release whereas micropellets prepared with Methocel K-15M followed Higuchi model. All the batches sustained the release of the drug for more than 8 hours (Figure 1-3) as compared with the conventional tablet dosage form (Figure-4). Predominantly the drug released by passive diffusion technique. On releasing the drug leaves behind pores or channels, through which diffusion of the drug present in the inner matrix of the micropellets occurred. Due to loose drug present on the surface of the micropellets (Surface Accumulation) the *in vitro* release profile obtained indicated a biphasic pattern i.e. initial fast release followed by a sustained pattern. Batches of Acrycoat micropellets showed more prolonged action as evident from its t_{50} values when compared with other two polymers. Increase in the polymer concentration increased the crosslink density thereby creating barrier for drug diffusion.

When studied for stability at 4°C, room temperature and 45°C for a month, the drug was found to be stable at 4°C and room temperature for all the formulations

Table- 1 -Comparative study of various physical parameters for alginate micropellets containing frusemide and release retarded with acrycoat e30d, hpmc and surelease respectively

Formulation	Composition	Moisture content (% ± S.D.)	LSC with respect to Entrapped Drug (%)	Mean Diameter (μm ± S.D.)	Angle of repose (Θ ± S.D.)	Bulk density (gm/cc ± S.D.)
E1	Acrycoat E30D-1%	1.48 ± 0.48	3.549	608.16 ± 0.59	18.32 ± 0.79	0.658 ± 0.68
E2	Acrycoat E30D-2%	1.44 ± 0.56	2.369	760.89 ± 0.51	20.56 ± 1.03	0.674 ± 1.52
E4	Acrycoat E30D-4%	2.23 ± 0.68	1.567	782.78 ± 0.36	21.24 ± 1.97	0.682 ± 1.96
H1	HPMC – 1%	2.43 ± 0.74	3.728	589.25 ± 0.62	20.68 ± 1.05	0.629 ± 0.89
H2	HPMC – 2%	1.74 ± 0.81	2.988	667.58 ± 0.56	22.01 ± 1.88	0.641 ± 1.59
H4	HPMC – 4%	2.32 ± 0.91	2.142	759.26 ± 0.42	23.09 ± 2.57	0.652 ± 2.49
S1	Surelease- 1%	1.21 ± 0.77	3.589	601.69 ± 0.68	19.26 ± 1.36	0.657 ± 1.23
S2	Surelease- 2%	0.91 ± 0.68	3.008	728.14 ± 0.48	21.28 ± 1.98	0.669 ± 1.87
S4	Surelease- 4%	1.76 ± 0.59	2.459	776.19 ± 0.41	22.58 ± 2.89	0.678 ± 2.88

* n= 3

Table- 2 - Comparative study of various pharmaceutical factors for alginate micropellets containing frusemide and release retarded with Acrycoat E30d, HPMC and Surelease respectively

Formulation	Composition	Drug Entrapment Efficiency (% ± S.D.)	Disintegration Time (min)	t_{50} (min)	Zero order		Higuchi SQRT	
					K_0	R^2	K_H	R^2
E1	Acrycoat E30D-1%	94.48 ± 0.48	42	272	11.628	0.9918	25.479	0.7939
E2	Acrycoat E30D-2%	93.44 ± 0.56	64	290	11.175	0.9928	23.909	0.7857
E4	Acrycoat E30D-4%	91.23 ± 0.68	97	341	11.131	0.9397	20.151	0.6492
H1	HPMC – 1%	82.43 ± 0.74	29	117	12.331	0.7806	29.928	0.9531
H2	HPMC – 2%	83.74 ± 0.81	38	120	11.948	0.7971	29.432	0.9464
H4	HPMC – 4%	89.32 ± 0.91	59	129	11.571	0.8841	30.356	0.9452
S1	Surelease- 1%	77.21 ± 0.77	28	158	11.899	0.8853	26.781	0.9057
S2	Surelease- 2%	80.91 ± 0.68	41	187	10.718	0.9576	25.102	0.9131
S4	Surelease- 4%	84.76 ± 0.59	77	316	08.883	0.9746	21.482	0.8386

* *n= 3*

K_0, K_H – Release Rate Constants for Zero Order and Higuchi release Kinetic Model respectively

R^2 – Correlation coefficient.

Table- 3 - Accelerated stability studies of frusemide micropellets prepared with different polymers

TIME	S4			H4			E4		
(WEEK)	4°C	RT	45°C	4°C	RT	45°C	4°C	RT	45°C
0	100	100	100	100	100	100	100	100	100
1	98.28	97.31	84.39	98.83	97.75	86.41	99.28	98.52	89.28
2	96.93	96.62	81.11	95.97	96.13	85.13	97.57	96.93	88.42
3	93.11	94.26	78.44	94.33	95.07	84.21	96.62	95.89	86.74
4	91.37	92.57	76.41	93.78	93.89	83.37	95.09	95.37	85.33

RT--- ROOM TEMPERATURE

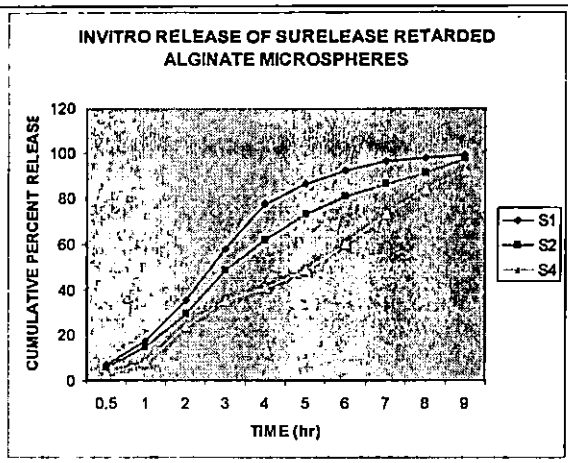

Figure 1

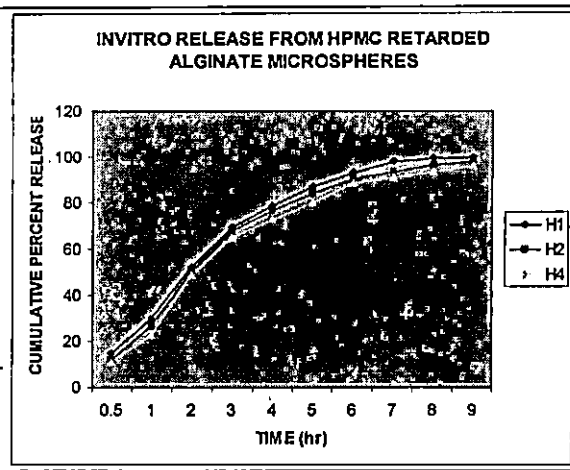

Figure 2

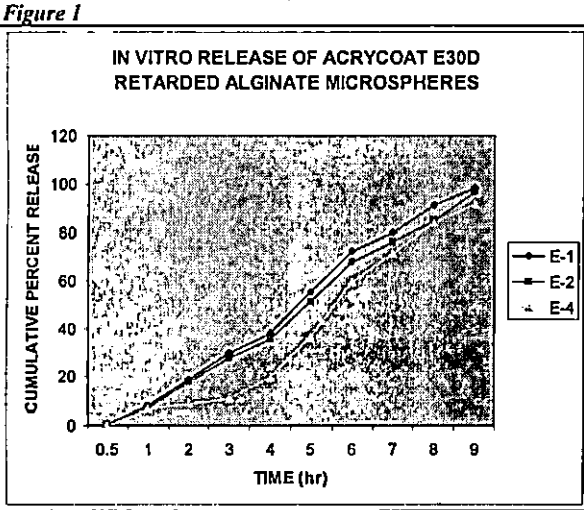

Figure 3

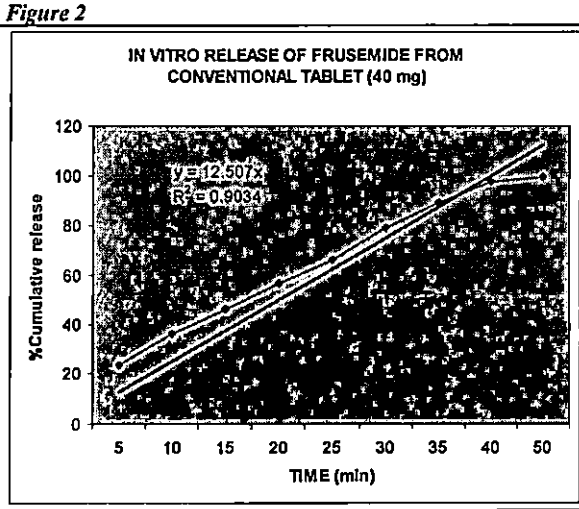

Figure 4

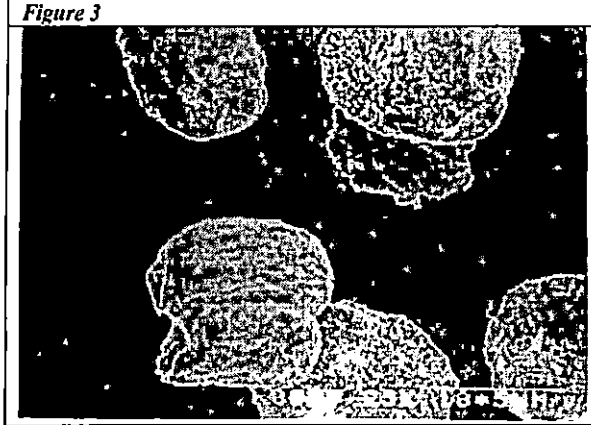

Figure 5- SEM Photograph of blank alginate micropellets (50X)

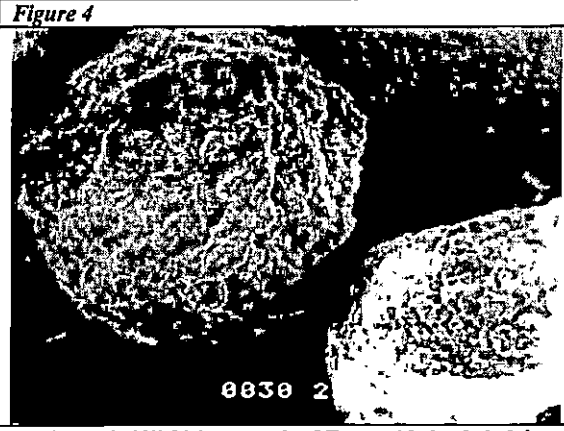

Figure 6- SEM Photograph of Frusemide loaded alginate micropellets with Acrycoat E30D (4%w/w) (50X) Formulation # E4

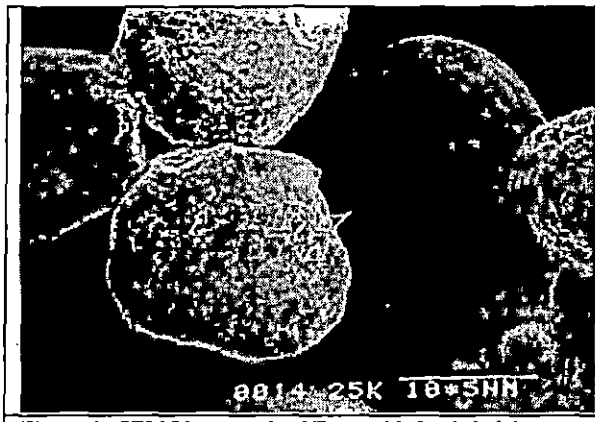

Figure 7 - SEM Photograph of Frusemide loaded alginate micropellets with Surelease (4%w/w) (50X) Formulation # S4

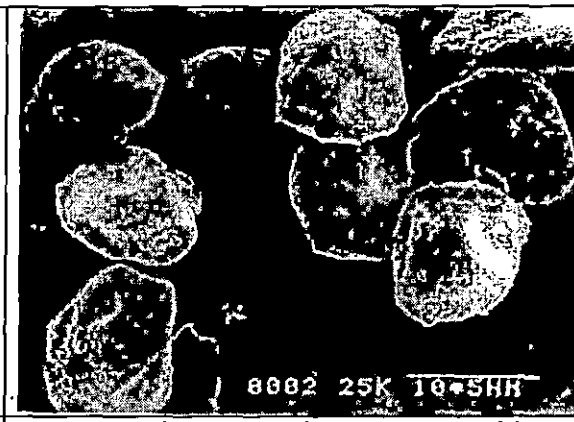

Figure 8 - SEM Photograph of Frusemide loaded alginate micropellets with Methocel K-15M (4%w/w) (50X) Formulation # H4

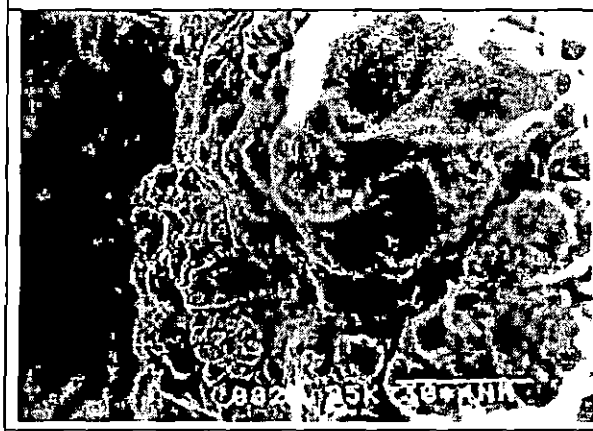

Figure 9- SEM Photograph of Frusemide loaded alginate micropellets with Acrycoat E30D (4%w/w) (350X) Formulation # E4

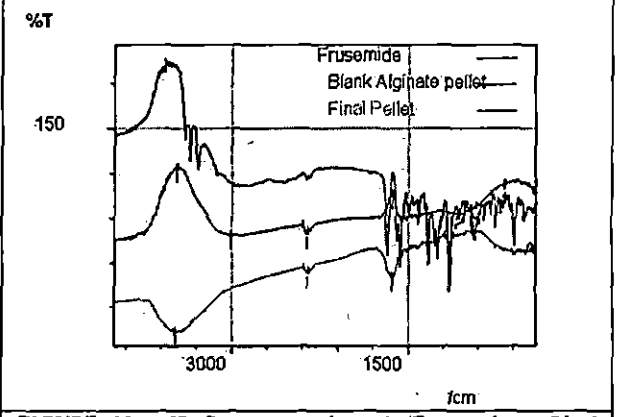

FIGURE 10 - IR Spectra overlapped (Frusemide + Blank Alginate pellets + Final pellets with Acrycoat E30D)

but showed gradual degradation at high temperatures.

From the infrared spectra (Figure 10) it is clearly evident that there were no interactions of the drug. The main peaks in the spectrum of the drug Frusemide like 1143.83 and 1323.21 /cm for S=O bond; 1674.27 /cm for C=O bond; 3487.42 /cm for N-S bond and 582 for C-Cl bond remained undisturbed in the final formulation. This proves the fact that there is no potential incompatibility of the drug with the polymers (alginate and Acrycoat E30D) used in the formulations. Hence, the formula for preparing Frusemide loaded Calcium alginate microspheres can be reproduced in the industrial scale without any apprehension of possible drug- polymer interactions.

In conclusion, sustained release micropellets containing water insoluble drug were successfully prepared employing ionotropic gelation technique entirely avoiding the use of organic solvents. Apart from the natural water soluble polymer, the use of copolymer further prolongs the release of the drug. Both water soluble and water insoluble copolymers were tested and among them acrylic based colloidal polymer dispersions (Acrycoat E30D) showed high encapsulation efficiencies and maximum prolongation of drug release. A further study using different acrylic polymers are on progress for new revelations. Considering the end product, the micropellets could be administered as prepared or could be compressed into tablet or filled in capsule shell. The entire process is feasible in an industrial scale and demands pilot study.

ACKNOWLEDGEMENTS

Authors wish to thank Aventis Pharmaceuticals (Ankleshwar, India) for providing gift sample of Frusemide and Corel Pharmaceuticals (Ahmedabad, India) for providing gift sample of Acrycoat E30D. We are also thankful to the staff of University Science Instrumentation centre (USIC), Jadavpur University,Kolkata for their relentless cooperation in SEM study.

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DEVELOPMENT, EVALUATION AND METHOD SELECTION FOR THE PREPARATION OF LAMIVUDINE MICROSPHERES

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ABSTRACT

The present study concerns the preparation, evaluation and selection of a suitable for the preparation of lamivudine incorporated microspheres composed of ethyl cellulose as release controlling polymeric material. Microspheres were prepared from various methods, namely, modified w/o/o emulsion solvent evaporation method (F1), o/w/o type emulsion solvent evaporation method (F2), thermal change technique (F3), Nobel Quassi emulsion solvent diffusion method (F4), meltable dispersion method (F5) , phase separation coacervation method (non-solvent addition technique) (F6). The prepared microspheres were evaluated for parameters such as, percentage yield, drug entrapment efficiency, particle size determination, drug polymer interaction, stability studies, and *invitro* drug release kinetic study. The drug / polymer ratio (1:2 w/w) and drug load (100 mg), was kept constant throughout the current investigation. The microspheres produced in each batch were smooth and small in size with an average diameter about 27.89 - 41.28 µm. Microspheres exhibited high drug entrapment efficiency with high yield value. FTIR analysis confirmed the absence of drug polymer interactions in all the formulations. The accelerated stability studies were performed following ICH guidelines for 14 Weeks and the results indicated stability of the formulations in varying temperature. Drug release profile of microspheres from F1, F2 and F3 followed zero order kinetics while those from F4, F5 and F6 formulation fit to Higuchi square root model. Among the methods adopted in this study, thermal change method (F3) was most successful in sustaining the release of lamivudine from ethyl cellulose microspheres, a novel trend for effective management of AIDS.

KEYWORDS: Lamivudine, Ethylcellulose, Microspheres, Thermal Change Method.

INTRODUCTION

New drug delivery technologies are revolutionizing the drug discovery, development and creating R&D focused pharmaceutical industries to increase the momentum of global advancements. In this regard novel drug delivery systems (NDDS) have many benefits, which includes improved therapy by increasing the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of adminstration and improved site specific delivery to reduce unwanted adverse effects (Amrita Bajaj et al, 2006). Lamivudine is an active antiretroviral drug belonging to

non-nucleosides reverse transcriptase inhibitor. Lamivudine treatment has gained immense popularity in the AIDS treatment in the present era. Dosage and duration of lamivudine therapy should be individualized according to requirement and response of the patient. The daily recommended dose is 150 mg b.i.d (J.H. Kao et al, 2000) (Indian Pharmacoepia, 1996). The oral administration of lamivudine exhibits side effects in GIT as well as in CNS. Thrombocytopenia, parasthesias, anorexia, nausea, abdominal cramps, depressive disorders, cough and skin rashes etc have also been reported as possible adverse reactions (Caroline M. Perry et al, 1997). Controlled release (CR) preparations helps to achieve maximum therapeutic effect with simultaneous minimization of adverse effects. Microparticulate drug delivery posses many advantages such as high bioavailability, rapid kinetic of absorption as well as avoidance of hepatic first pass effect and improvement of patient compliance (Y.W. Chien, 1992). Absence of sufficient work in the direction of programmed delivery of lamivudine as indicated by literature survey ignited the urge of this research venture, which utilizes six different formulation methods for preparation of lamivudine microspheres and ultimately ascertain the most preferable method for industrial scale-up on the basis of physical characterization and *in vitro* drug release profile.

METHODS AND AIMS

Materials

Lamivudine was received as a gift sample from GlaxoSmithKline Ltd. Mumbai. Ethyl cellulose was obtained from LOBA chemicals, Kolkata, India. All other chemicals and solvents used were of analytical grade and procured from an authorized dealer, USP XXI paddle type dissolution apparatus, FT-IR (Shimadzu IR spectrophotometer, Model 840, Japan) and UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan) were the instruments employed in the current study.

Preparation of microspheres

The Lamivudine loaded Ethyl cellulose microsphere were prepared by modified w/o/o emulsion solvent evaporation method (F1) (Y. Li et al , 2005), o/w/o type emulsion solvent evaporation method (F2) (D. H. Kenneth et al, 2005), thermal change technique (F3) (C. Palanichamy et al, 2006), Nobel Quasi emulsion solvent diffusion method (F4) (Y. Kawashima et al, 1989), meltable dispersion method (F5) (J.B. Schwartz et al 1968), phase separation coacervation method (non-solvent Addition Technique) (F6) (K.N.Shovarani et al, 1994), as described in referred literatures. The ratio of drug and polymer concentration (1:2 w/w) along with drug load (100 mg) was kept constant throughout the study period.

Drug entrapment efficiency (DEE) (M.C. Gohel et al, 2005)

The microspheres were evaluated for percentage yield and percent drug entrapment. The yield was calculated

$$\text{Percentage yield} = \frac{\text{weight of microsphere recovered}}{\text{Weight (drug + polymer)}} \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

Drug loaded microspheres (100 mg) were powdered and suspended in 100 ml methanol: water (1:99 v/v) solvent system. The resultant dispersion was kept for 20 min for complete mixing with continuous agitation and filtered through a 0.45 µm membrane filter. The drug content was determined spectrophotometrically (UV-Visible-1700, Shimadzu, Japan spectrophotometer) at 270 nm (3) using a regression equation derived from the standard graph (r2 = 0.9978). The drug entrapment efficiency (DEE) was calculated by the equation

$$DEE = (Pc / Tc) X 100 \quad \ldots\ldots\ldots\ldots\ldots (2)$$

Pc is practical content, Tc is the theoretical content. All the formulations were analyzed in triplicate (n=3).

Particle size measurement (M.C. Gohel et al, 2005)

The size of the prepared microspheres was measured by the optical microscopy method using a calibrated stage micrometer. Particle size was calculated by using equation

$$Xg = 10 x [(ni x \log Xi) / N] \quad \ldots\ldots\ldots\ldots\ldots (3)$$

Xg is geometric mean diameter, ni is number of particle in range, xi is the mid point of range and N is the total number of particles. All the experimental units were analyzed in triplicate (n=3).

Accelerated stability studies (G.T. Kulkarni et al, 2004) (L. Lachman, et al, 3rd ed., 1991)

Stability studies were performed according to ICH guidelines. The formulations were stored in room temperature at 25 ± 1°, in hot air oven at 37 ± 1°, and at 60± 1° for a period of 14 weeks. The samples were analyzed for drug content every two weeks by spectrophotometer at 270 nm and compatibility of drug with excipients was determined by infrared spectroscopy using a Shimadzu FTIR-840 model IR spectrophotometer.

Fourier Transforms Infrared Radiation measurement (FT-IR) (D.R.Bhumkar et al, 2003)

The FT-IR spectra acquired were taken from dried samples. A FT-IR (Shimadzu IR spectrophotometer, model 840, Japan) was used for the analysis in the frequency range between 4000 and 600 cm-1, an 8 cm-1 resolution and a 0.2 cm-1 rate. The results were the means of 16 determinations. A quantity equivalent to 2 mg of pure drug, empty microspheres of ethyl cellulose and drug loaded microspheres were selected separately.

***In-vitro* drug release**

Invitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using 0.01 M HCl as dissolution medium. Volume of dissolution medium was 900 ml and bath temperature was maintained at 37±1° throughout the study. Paddle speed was adjusted to 50 rpm. At an interval of 1 hr, 5 ml of sample was withdrawn with replacement of 5 ml fresh medium and analyzed for lamivudine content by UV-Visible

spectrophotometer at 270 nm (3). All the experimental units were analyzed in triplicate (n=3).

In vitro drug release kinetics

In order to study the exact mechanism of drug release from the microsphere, drug release data was analyzed according to Zero order (G.M. Khan, 2001), First order (D.M. Morkhade et al, 2006), Higuchi square root (T. Higuchi, 1963), Hixon-Crowell equation (J. Wang et al, 1999) .The criteria for selecting the most appropriate model was chosen on the basis of goodness of fit test.

Statistical Analysis

Statistical data analyses were performed using the ANOVA one way at 5 % level of significance $p < 0.05$ (Bolton S, 1997) and standard error mean.

RESULTS

The Lamivudine loaded ethyl cellulose microspheres were prepared by various methods as mentioned earlier. The microspheres obtained under these conditions were found to be spherical and without aggregation and mean particle size was found in a range of 27.89 to 41.28 µm (Table 1). The particle size distribution of all the formulations is displayed in Figure-2. The percentage yield of all the formulations was found to be satisfactory and each formulation exhibited high drug entrapment efficiency (DEE), as summarized in Table 1. The method F3 showed higher DEE among all the formulations.

The interaction study between the drug (lamivudine) and polymer (ethyl cellulose) in different formulations was evaluated using FTIR spectrophotometer. Four bands present in Lamivudine spectrum at 3445.91, 2930.92, 1736.51 and 1637.7 cm-1, due to the formation of N-H, O-H, C=O, C=N linkage respectively, was also detected and identified in the spectrum of the formulations, confirming no drug-polymer interaction as represented in Figure-3, 4 and 5. The accelerated stability studies were performed according to ICH guidelines for 14 Weeks and the results were found to be stable in varying temperature as shown in Table-2. The results were further verified with one way ANOVA method, the accelerated stability test datas were found significant for F (3.395) at 5 % level of significance ($p< 0.05$).

The *in vitro* drug release profiles for all the batches were tabulated in Table-3 and graphically represented in Figure-1. All the formulations showed constant release profile. To identify the kinetics of drug release from microspheres, release data was analyzed according to different kinetic models. Table-4 indicates that drug release from F1; F2 and F3 formulations obey Zero order kinetics, while the release data of F4, F5 and F6 seems to fit best in Higuchi square root model. The release mechanism was not significantly influenced by formulation variables and was predominately diffusion controlled. Statistical verification with one way ANOVA method attested the fact that the drug release data were found significant for F (27.3731) at 5 % level of significance ($p< 0.05$).

DISCUSSION

Lamivudine loaded Ethyl cellulose microsphere were prepared and evaluated with different reported methods. No significant differences in particle size were found for the microspheres prepared by all the methods. Small variations may be attributed to different conditions (M.C. Gohel et al, 2005), like stirring speed and stirring time or

fluctuations in temperatures. The smaller particle size can be due to application of wide range (5-800) of temperature change with constant stirring at 800 rpm. The reduced particle size helps in achieving our goal by enhancing the controlled delivery of Lamivudine. The method F 3 (C. Palanichamy et al, 2006) showed higher DEE among all the formulations. This can be justified on the basis of minimum process parameters, minimum drug solubility in the external phase and smaller particle size of the thermal change method in comparison with the other methods, leading to minimum drug loss. The minimal use of process parameters during the formulation of Lamivudine microsphere confirms the high yield of F 3.

The FTIR study attests the safety profile of the microspheres due to avoidance of drug polymer interaction. The accelerated stability study indicate the broader horizon of storage conditions complying with the ICH guidelines. The efficiency of release of Lamivudine from ethyl cellulose matrix of prepared microspheres is the key factor in the successful optimization of a method. The present study demonstrated that F3, among all other formulations, have a significantly slower release pattern in terms of their total drug load. The drug release rate was following zero order kinetic which complies with the controlled delivery (G.M. Khan, 2001) of Lamivudine over 10 hours. However the other formulations F1, F2, F4, F5 and F6, had shown insignificant difference in drug entrapment efficiency but found to be significantly (S.E.M < 0.010) distinguished in particle size and percentage of yield. Except F1, F2, F3, the other formulations followed Higuchi square root kinetic model indicating the diffusion controlled drug release, which creates a restriction in optimizing the methods, as zero order model is the most desirable. F1, F2 was rejected on the basis of particle size, yield factor, entrapment efficacy and prolongation of drug release in comparison with F3. The results of the present study suggest that method F3 is the most suited one to develop lamivudine microspheres, keeping in consideration, the zero order release profile, high DEE (99.66 %), small particle size (27.89 µm) and high yield of the microspheres of this method.

In context to the intense world wide research to combat AIDS, it can be envisaged that future workers would indulge in optimization of the various process parameters of the selected method (F3), to promote its commercial scale up, leading to Lamivudine (Caroline M. Perry et al, 1997) loaded ethyl cellulose microspheres for effective management of AIDS.

ACKNOWLEDGMENTS

The authors thank GlaxoSmithKline Pvt. Ltd. Mumbai, India for the gift sample of lamivudine pure drug and Central Drug Research Institute Lucknow, India for providing library facility during the entire work.

Table-1:

Evaluation parameters of various formulations

Formulation code	Yield (%)	Particle size (μm) (X ± S.D)	Drug Entrapment efficiency (X± S.D)
F1	93.00 ± 0.017	36.22 ± 0.015	97.01 ± 0.12
F2	96.00 ± 0.014	29.55 ± 0.021	95.36 ± 0.11
F3	99.10 ± 0.019	27.89 ± 0.026	99.66 ± 0.22
F4	94.56 ± 0.027	28.69 ± 0.019	99.01 ± 0.19
F5	97.55 ±0.026	38.88 ± 0.028	98.45 ± 0.17
F6	98.80 ±0.023	41.28± 0.026	96.21 ± 0.23

All the results are mean ± standard deviation (n=3)

(Standard Error Mean (S.E.M) < 0.01)

F1=W/O/O Emulsion Solvent Evaporation F2= O/W/O Emulsion Solvent Evaporation

F3= Thermal Change Method F4= Quassi emulsion solvent diffusion Method

F5= meltable dispersion Method F6 = phase separation coacervation method (Non-solvent addition method)

Table-2: Stability profile of various formulations in different temperature.

Week	Temp. (° C)	Formulation Code F1	F2	F3	F4	F5	F6
		Percentage of Potency					
Initial	Room Temperature	99.24	99.42	99.56	99.88	101.25	99.23
2	(RT)	99.20	99.01	99.66	99.78	99.44	99.84
4	RT	98.53	98.89	99.05	98.55	98.66	98.39
6	37 ± 1	98.23	98.88	99.00	96.36	99.02	98.81
8	60± 1	99.21	99.0	99.68	99.68	99.12	99.35
10	RT	98.65	98.87	98.97	98.58	98.25	98.01
12	37 ± 1	98.22	98.56	98.91	97.01	98.77	97.55
14	60± 1	98.92	98.97	98.99	98.90	98.88	98.95
	RT	98.56	98.79	98.87	98.53	98.10	98.05
	37 ± 1	98.12	98.50	98.82	97.21	98.62	97.49
	60± 1	98.97	98.83	98.91	98.80	98.68	98.21
	RT	98.62	98.69	98.78	98.41	97.92	97.98
	37 ± 1	98.09	98.32	98.45	97.01	98.36	97.56
	60± 1	98.89	98.45	98.85	98.78	98.59	98.19
	RT	98.58	98.57	98.65	98.35	97.88	97.69
	37 ± 1	97.98	98.29	98.39	97.01	98.26	98.18
	60± 1	98.84	98.39	98.74	98.68	98.47	98.05
	RT	98.52	98.48	98.60	98.31	97.79	97.58
	37 ± 1	97.88	98.19	98.31	96.98	98.18	98.12
	60± 1	98.71	98.31	98.69	98.57	98.41	98.00
	RT	98.48	98.40	98.51	98.29	97.70	97.48
	37 ± 1	97.69	98.08	98.29	96.92	98.10	97.95
	60± 1						

Verifying with one way ANOVA significant at 5 % level of significance (F= 3.395).

Table 3:
***In vitro* drug release profile of formulations**

	Formulation Code					
Time(hr)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
1	19.36	60.22	25.35	23.47	27.62	24.69
2	15.73	70.47	21.30	22.63	30.91	23.34
3	20.03	74.91	19.57	15.59	21.77	19.79
4	17.94	72.09	21.27	21.31	19.66	22.05
5	19.38	74.80	21.28	10.37	18.78	20.83
6	24.02	72.09	18.41	10.89	17.87	23.99
7	22.60	76.29	19.31	15.01	28.61	17.76
8	24.71	72.93	21.06	18.83	28.33	28.47
9	17.38	71.16	21.41	23.12	29.22	32.69

F1=W/O/O Emulsion Solvent Evaporation F2= O/W/O Emulsion Solvent Evaporation

F3= Thermal Change Method F4= Quassi emulsion solvent diffusion Method

F5= meltable dispersion Method F6 = phase separation coacervation method (Non-solvent addition method)

Table-4:
Correlation coefficients according to different kinetic equations

Kinetic Models	F1	F2	F3	F4	F5	F6
					(r)	
Zero	0.9992	0.9952	0.9891	0.9841	0.9968	0.9871
order	0.7397	0.8282	0.8021	0.8724	0.8641	0.8559
First	0.8927	0.9443	0.9803	0.9727	0.9849	0.9853
order	0.9564	0.9931	0.9864	0.9932	0.9985	0.9877
Hixon-crowell model						
Higuchi square root						

Table values represents correlation coefficient (**r**) for linearity according to different kinetic equations used for describing the drug release from various formulations

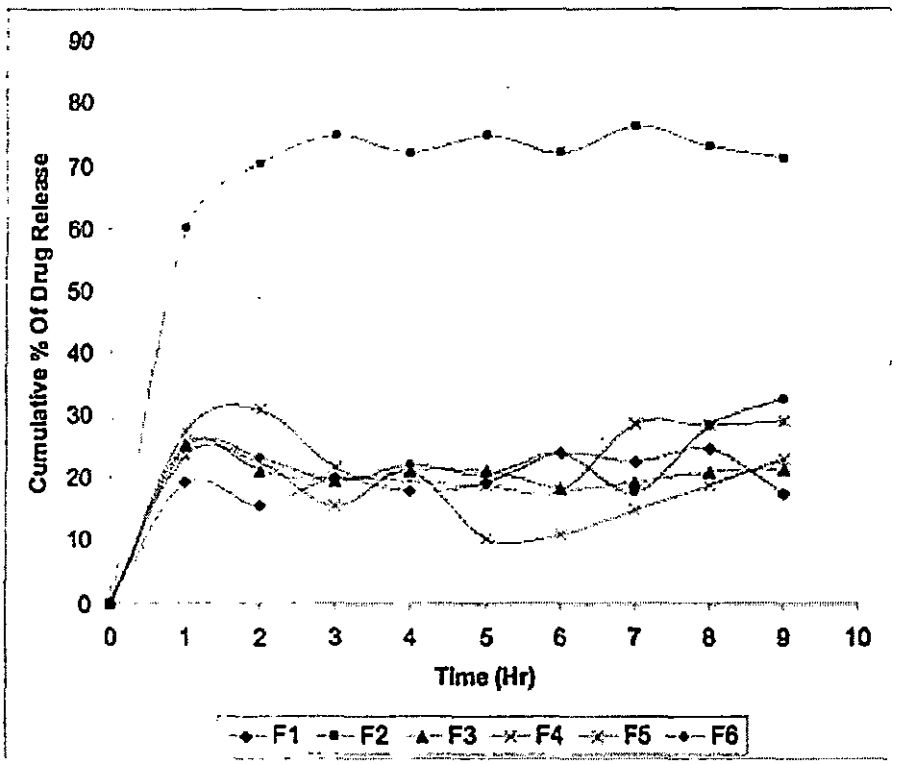

Figure-1: *In vitro* drug release profile of microspheres

F1=W/O/O Emulsion Solvent Evaporation F2= O/W/O Emulsion Solvent Evaporation

F3= Thermal Change Method F4= Quassi emulsion solvent diffusion Method

F5= meltable dispersion Method F6 = phase separation coacervation method

(Non-solvent addition method)

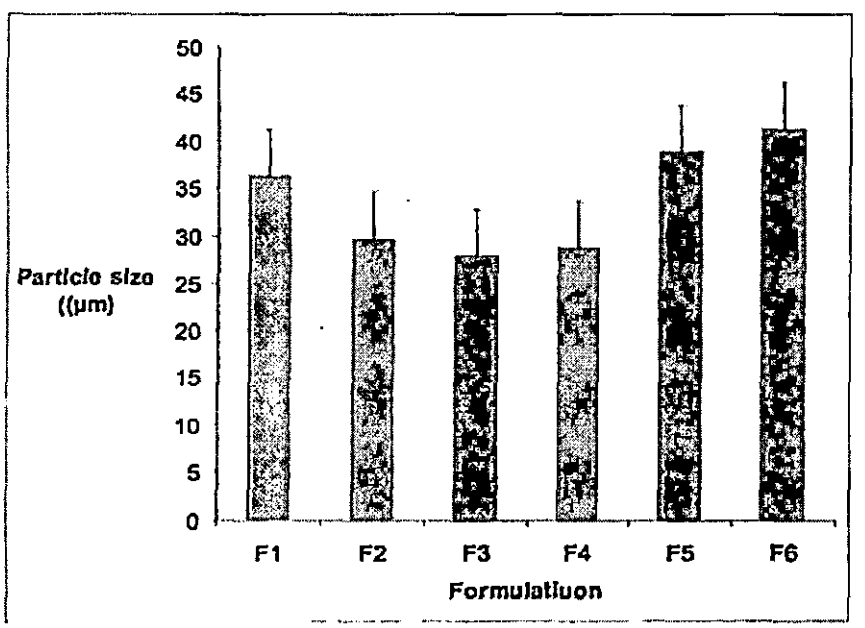

Figure-2: Particle size distribution of various formulations

http://www.priory.com/pharmacy/Lamivudine.htm

F1=W/O/O Emulsion Solvent Evaporation F2= O/W/O Emulsion Solvent Evaporation

F3= Thermal Change Method F4= Quasi emulsion solvent diffusion Method

F5= meltable dispersion Method F6 = phase separation coacervation method

(Non-solvent addition method)

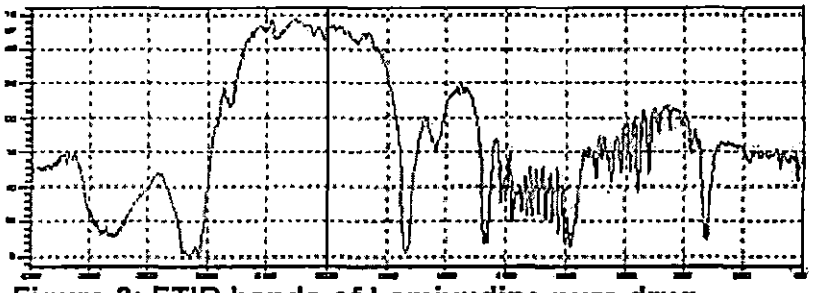

Figure-3: FTIR bands of Lamivudine pure drug

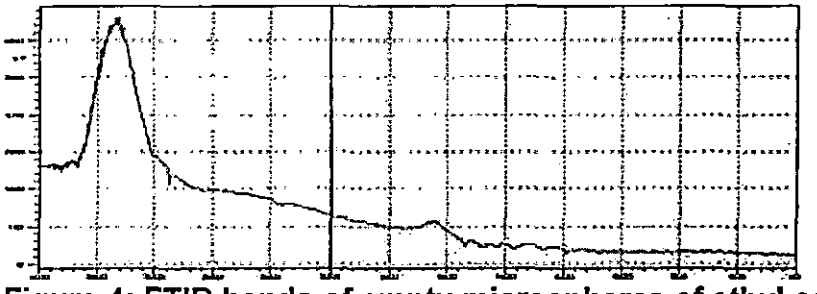

Figure-4: FTIR bands of empty microspheres of ethyl cellulose.

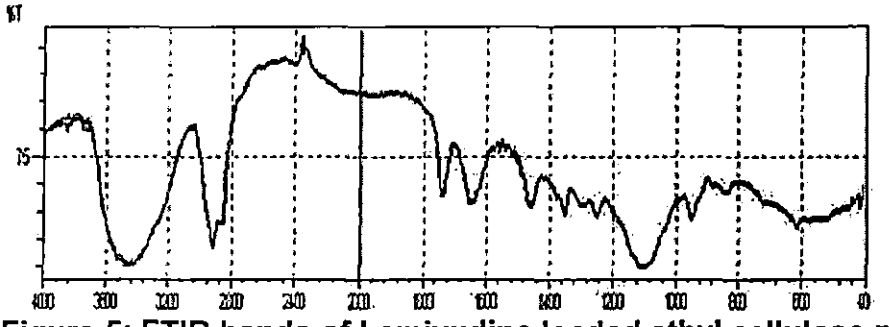

Figure-5: FTIR bands of Lamivudine loaded ethyl cellulose microsphere

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