CHAPTER 6

PROCESS VARIABLES AND OPTIMIZATION

PROCESS VARIABLES AND PROCESS OPTIMIZATION

6.1 Introduction

To initiate a systematic and scientific approach in the designing and evaluation of calcium alginate micropellets of Frusemide, employing ionotropic gelation technique, preformulation studies on the micropellets were conducted before the preparation of final set of formulations. In order to attain this, lot of process variables were investigated with an objective to produce pellets with greater sphericity, desired particle size and optimum rigidity. The following process parameters were investigated to achieve these objectives. The result obtained from these investigations were analyzed and optimized on the basis of best result relevant to our research work.

6.2 Preparation of Frusemide loaded calcium alginate micropellets using ionotropic gelation technique[1,2]

Mucilage of sodium alginate was prepared by soaking properly weighed quantity of alginate salt in 100ml distilled water and stirring at low speed in an electrical stirrer until homogenous mucilage was formed. To this release controlling copolymer, Acrycoat E30D was mixed in suitable proportions (0, 2 and 4 % w/w) and the entire mixture was stirred for 30 min. The water insoluble drug, Frusemide was then mixed in small portions and dispersed uniformly in the polymer mixture for 15 minutes using electrical stirrer maintaining the speed at 500-600 rpm. The bubble free dispersions were pulled in a glass syringe and extruded through a hypodermic needle of size 17G (gauze) into a gently agitated 100ml calcium chloride solution. During extrusion the height of the tip of the needle was maintained above the level of the solution, simply by graduating the beaker in terms of height, prior to the addition of the drug-polymer dispersion. Instantaneous gelation was observed with the formation of wet, heavy calcium alginate micropellets which gradually settles down at the bottom of the beaker. The gelled micropellets were cured or hardened by keeping them in contact with the calcium chloride solution. The white coloured micropellets were then filtered using filter paper and washed thoroughly with distilled water to ensure removal of any excess calcium ion adhered on the surface of the wet micropellets.

The filtered micropellets were kept in a petri dish on a dry filter paper and dried in open air, so as to soak free water from their surface .They are then dried in hot air oven for 6 hr at 60°C until the wet micropellets got fully dried into non adhering micropellets. The #22 I.P. standard sieve size fractions of the pellets were used for further studies.

6.3 The List of Process Variables Investigated for Optimization

To optimize the process variables the following enlisted process parameters were investigated, recorded and optimized.

1. **Bore diameter** of the needle - 17G, 20G, 22G, 24G
2. **Height of dropping** the dispersion in calcium chloride solution – 1cm, 2cm, 3cm, 4cm, 5cm (difference in height between the tip of the needle and upper level of calcium chloride solution)
3. **Drying time and Drying temperature**
   i) In Hot Air Oven -- 60°C for 2,4,6,8 hr; 90°C for 3hr; 120°C for 30 min
   ii) At Room Temperature (23-26°C) for overnight (>12hr)- In Desiccators and Open Air
4. **Concentration** of **sodium alginate** (Primary polymer)
   1%, 2%, 3%, 4%, 5% w/v
5. **Concentration** of **Calcium Chloride** solutions (Counterion)
   0.5%, 1%, 2%, 5%, 10% w/v
6. **Curing** or **Hardening Time** --(Contact time of the pellets with $CaCl_2$ solution)
   5min, 10min, 15min, 20min, 30min
7. **Concentration of Drug loading** - 30%, 45%, 60% w/w

Copolymers used along with **Sodium Alginate:**

8. **Concentration** of **Acrycoat E30D** – 1%, 2%, 4% w/w
9. **Concentration** of **Acrycoat L30D** – 1%, 2%, 4% w/w
10. **Concentration** of **Acrycoat S100** – 1%, 2%, 4% w/w
11. **Concentration** of **Methocel K-15M** (HPMC) – 1%, 2%, 4% w/w
12. **Concentration** of **Surelease** (Ethyl cellulose) – 1%, 2%, 4% w/w

6.3.1 Effect of the Bore Diameter of the needle

Methodology - Sodium alginate, the primary polymer in the formulation is a mucilaginous substance which enhances in viscosity with the increase in concentration. The secondary polymers are all viscosity enhancers themselves by their inherent properties. Keeping this fact in mind the investigator went for optimization of the needle size as the first and foremost step before proceeding further. For this mucilage of sodium alginate of various concentrations (1 – 5% w/v) in water were prepared which were then extruded into calcium alginate solutions using different needles of sizes 17G, 20G, 22G, 24G (G - Gauze). The wet micropellets formed were filtered and dried in oven at 60°C for 6hr. Two things came out from this experimentation, firstly, finding the ease of extrusion from different needles and, secondly, estimating the effect of different needle size on the diameter of the pellets produced.

Observation - On primary investigation it was found that 24G needle (lowest bore diameter) though giving pellets of minimum size yet poses strong resistance during extrusion of mucilage containing 3% w/v and above of sodium alginate. In the next step, along with the mucilage of primary polymer the copolymers were mixed at their highest concentration (4% w/w) and the effect on extrusion was reassessed. The combination of maximum concentrations (4%) of two polymers has been able to be extruded only from the 17G needle.

Inference - Though a compromised decision had to be made with the resultant pellet diameter, but to avoid any problem during the entire study regarding extrusion of colloidal polymer mixture from the needle, 17G needle had been optimized for the entire work.

6.3.2 Effect of Height of dropping the drug-polymer dispersion from the needle in respect to the top level of the counterion solution

Methodology - Height of dropping the colloidal dispersion of drug-polymer combination in calcium chloride solution can be defined as the difference in height between the tip of the needle and upper level of calcium chloride solution. The investigator optimized this variable on the basis of the effect on particle size and shape generated by changing height of dropping. Distance of 1cm, 2cm, 3cm, 4cm and 5cm were tried as different heights.

 # Observations -

1 cm – Droplets got spread on the surface of the counterion solution forming incomplete micropellets.

2 cm & 3 cm – Droplets were more spherical producing regular discoid shaped free flowing micropellets on drying. (Figure 6.1)

4 cm & 5 cm - Formation of long tailed droplets with rounded on one side and having a conical ending at the other. The shape remained so even after drying.

 # Inference - From overall observations it was found that with the increase in height the wet micropellets formed in the calcium chloride solution contains a tail which retained after drying. With low height micropellets were shapeless. Hence for further study, 2 cm height of the tip of the needle from the level of solution was adjusted using graduation on the beaker and was optimized for the entire work.

6.3.3 Effect of Drying time and Drying temperature

 # Methodology - Wet micropellets obtained by ionotropic gelation, using 2% w/v of sodium alginate and 1% w/v of calcium chloride and keeping height of dropping at 2 cm and needle size 17G as constant, the effect of drying temperature and drying time on the shape and size of dry micropellets were investigated. The main objective of this study was to optimize the drying condition which can retain the physicochemical stability of the drug and the polymers, consumes less time and energy and does produce micropellets completely free from any entrapped moisture. To achieve this goal the micropellets were kept in Hot Air Oven at 60°C for 2, 4, 6 and 8 hr. Further three different conditions were studied, namely, 60°C for 6hr; 90°C for 3hr; and 120°C for 30 min, the wet gelled micropellets were also kept at Room Temperature (23°C -26°C) for overnight (> 12hr), in Desiccators and in Open Air.

 # Observations - At 60°C for 2 and 4 hours the pellets obtained, on visual inspection, were found to be soft, palpable lacking firmness in shape showing signs of incomplete drying. Increasing the time of drying to 6 and 8 hours produced free flowing dried micropellets with negligible moisture content as confirmed by using I.R.Moisture balance (Chapter 8). Since there was no significant difference in moisture content with 6 and 8 hours of study, 6 hours of drying time was selected for further process variables.

From the study of three different conditions of drying, namely, 60°C for 6hr; 90°C for 3hr; and 120°C for 30 min, pellets obtained from drying at higher temperatures were found to

char, especially at the point of contact of the micropellets with the filter paper. High temperature conditions does not reduce more amount of moisture compared to 60°C for 6hr. Over and above they have got a potential risk of thermal degradation of the drug. Hence, among the different thermal conditions in hot air oven 60°C for 6hr was selected to be the best condition giving optimal drying. A comparative study was also performed with thermal and non thermal methods. For this, samples obtained from drying at 60°C for 6hr were compared with their moisture content and final shape with the samples kept at room temperature (23°C -26°C) for overnight (> 12hr) in desiccators and also in open air. Shape wise samples kept at room temperature were more spherical but on comparing the moisture content in I.R.balance thermal method was found to score much higher with respect to the non thermal methods.

Inference - Thermal condition 60°C for 6hr was optimized as drying condition for the entire research work and the dried samples obtained were stored in room temperature in a dessicator.

6.3.4 Effect of the variation in concentration of sodium alginate

Methodology - Frusemide loaded micropellets of calcium alginate were prepared by varying the concentration of sodium alginate in order to optimize the concentration which gave desired result. For the study 1.0% - 5.0% w/v of sodium alginate were used keeping the drug load constant at 30%w/w, calcium chloride concentration at 1 % w/v, height of dropping at 2cm, needle size of 17G, drying condition as 60°C for 6hr in hot air oven and stirring time and speed as 30 min and 500 rpm respectively. Curing time in calcium chloride solution was kept at 15 min.

Table- 6.1 Effect of the variation in the concentration of Sodium alginate on the physical characterization of the Calcium alginate micropellets

Formulation Code	Conc. of Sodium alginate (% w/v)	Mean Diameter (μm)	Drug Entrapment Efficiency (%) (DEE)	t_{50} (min)	Disintegration time (min)
SA1	1	502.78	95.71	24	17
SA2	2	588.45	95.87	31	23
SA3	3	621.26	97.66	36	32
SA4	4	844.63	98.27	43	41
SA5	5	888.92	99.07	44	56

Table- 6.2 *In vitro* drug release data of 30% w/w Frusemide loaded pellets prepared by using different concentration of Sodium alginate

TIME (min)	% Cumulative Release				
	SA1	SA2	SA3	SA4	SA5
5	12.36	10.26	8.85	7.45	7.25
10	25.24	21.35	16.42	14.56	14.05
15	32.21	29.88	28.92	21.51	19.97
20	45.67	39.58	36.77	30.04	28.29
30	60.82	48.68	45.22	39.38	37.04
40	80.23	59.34	56.61	48.19	46.11
50	90.42	71.21	69.31	55.52	54.91
60	100.16	83.52	78.49	64.18	63.02
70		91.25	86.28	74.58	72.42
80		95.74	92.39	82.05	80.19
90		100.39	97.46	88.46	87.25
120			100.42	95.28	93.26
150				99.93	97.44

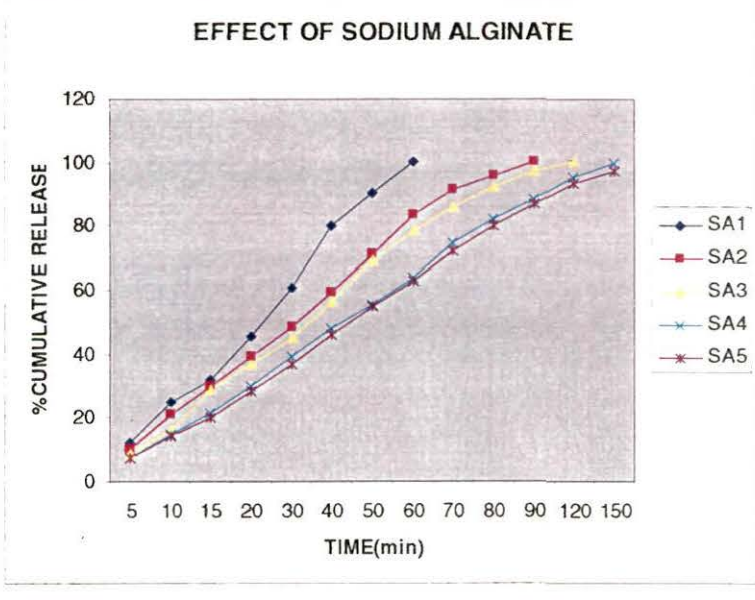


Figure: 6.2 ***In-vitro*** **drug release profile of micropellets prepared with variation in the concentration of sodium alginate**

 # Observations - From the Table 6.1, 6.2 and the Figure 6.2 it is evident that as the concentration of the polymer increases, the release of the drug from the micropellets got decreased. The release retarding effect of 4 and 5 % w/v of sodium alginate (SA4 and SA5) were more or less similar as also their t_{50} were very close. The release of the drug was extended by one hour just by increasing the concentration of sodium alginate from 1 to 3 % w/v (SA1 & SA3). Regarding disintegration time SA5 recorded maximum time for

disintegration which may be attributed to the fact that at higher concentration alginate forms a dense network which slows the penetration of dissolving medium, thus enhancing both disintegration and dissolution time. Considering the works on diffusion behavior of the micropellets by T.Yotsuyanagi[3] *et al;* when gelled micropellets are used as a drug delivery system the diffusion coefficient become smaller as polymer concentration increases. The results obtained comply with the earlier findings. Same reason can be put forward for the results in entrapment efficiency. Particle size varied from 500 – 900 μm well within a range to be termed as micropellets. For obvious reasons the increase in concentration of the polymer increased the total mass, resulting in bigger particle. From the Figure 6.3, the formulations SA2 and SA4 were found to show more spherical particle with smooth surface topography.

 # Inference - For further process optimization studies the investigator selected 2% w/v concentration of sodium alginate as constant. During final set of formulations, the primary polymer sodium alginate was again varied in three different concentrations (1, 2 and 4% w/v) along with the optimized copolymer using 3^2 factorial analyses.

6.3.5 Effect of the variation in concentration of calcium chloride[4]

 # Methodology - Frusemide loaded micropellets of calcium alginate were prepared by varying the concentration of calcium chloride, acting as counter ion to sodium alginate, and to study the effect of this variation in order to optimize the concentration. In the study, 0.5%, 1%, 2%, 5% and 10% w/v of calcium chloride solution were used keeping drug load constant at 30%w/w, sodium alginate concentration at 2% w/v, height of dropping at 2cm, needle size of 17G, drying condition as 60°C for 6hr in hot air oven and stirring time and speed as 30 min and 500 rpm respectively. Curing time in calcium chloride solution was kept at 15 min. The resultant micropellets were physically characterized.

Table 6.3 Effect of the variation in the Concentration of Calcium chloride on the physical characterization of the Calcium alginate micropellets.

Formulation Code	Conc. of $CaCl_2$ (% w/v)	Mean Diameter (μm)	Drug Entrapment Efficiency (%) (DEE)	t_{50} (min)	Disintegration time (min)
C-0.5	0.5	623.42	90.99	22	21
C-1	1.0	588.45	95.87	31	23
C-2	2.0	578.25	96.63	32	25
C-5	5.0	523.95	98.13	33	30
C-10	10.0	521.26	98.61	35	32

Table 6.4 Weight loss and contraction of the alginate micropellets with the variation in concentration of Calcium chloride

Conc. of $CaCl_2$ (% w/v)	Weight of fully cured 10 micropellets (mg)
0.5	160.2
1.0	159.1
2.0	158.6
5.0	153.7
10.0	142.8

Table- 6.5 *In- vitro* drug release data of 30% w/w Frusemide loaded micropellets prepared using different concentration of Calcium chloride solutions

TIME (min)	% Cumulative Release				
	C-0.5	C-1	C-2	C-5*	C-10*
5	17.85	10.26	9.62	5.36	4.26
10	28.32	21.35	20.51	9.85	9.11
15	36.39	29.88	25.38	17.56	16.73
20	47.56	39.58	36.19	25.81	23.98
25	56.49	44.94	43.28	36.22	35.85
30	66.42	48.68	48.08	47.41	45.14
35	72.93	56.32	55.36	53.73	50.21
40	81.26	59.34	58.66	57.46	56.08
45	89.48	67.65	65.28	64.56	63.39
50	96.35	71.21	70.84	69.47	68.29
60	100.23	83.52	82.52	77.85	76.59
70		91.25	88.55	83.92	83.03
80		95.74	94.54	90.69	89.37
90		100.39	100.61	99.43	98.54

'* ' indicates ($p < 0.05$), n=3

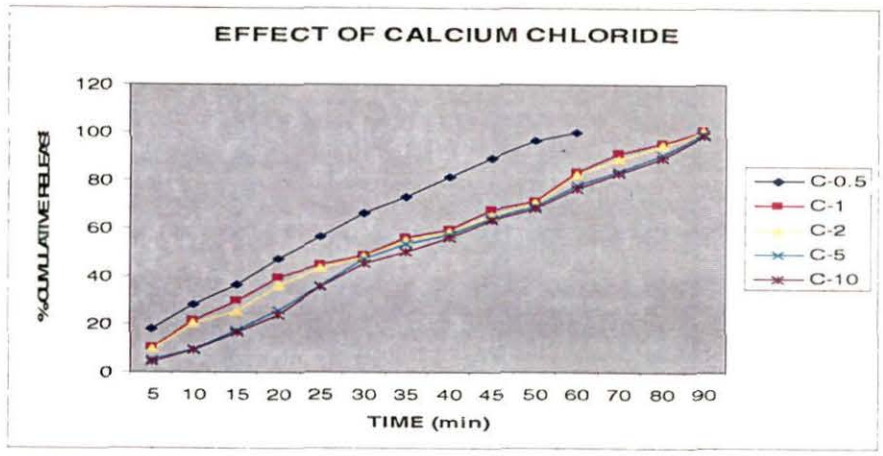


Figure: 6.5 ***In-vitro*** **drug release profile of micropellets with variation in the concentration of calcium chloride**

 # Observations - Morphologically, pellets formed from low concentration of calcium chloride were flaky in nature. With the increase in the concentration of the counterion from 1% w/v and above the pellets got a discoid shape rather than being spherical and were rigid in nature (Figure 6.4). As reported by T.Yotsuyanagi[3] *et al,* with the increase in the concentration of the counterion produces more dehydration of the alginate molecule due to osmotic effect of the chloride salt. This leads to contraction of the wet micropellets and finally leading to the formation of smaller dry pellets. The result obtained as shown in Table 6.3 complies with the reported literature. It is further supported by the weight loss study presented in Table 6.4, where clear losses in weight of the micropellets are evident owing to more dehydration. Regarding drug entrapment, from Table 6.3, it is seen that again higher the concentration of counterion more the value of entrapment efficiency. However, 5% and 10% concentration did not have much difference in this regard. The results give a similar trend for disintegration time and dissolution profile. It can be inferred that as the concentration of sodium alginate was kept constant in all the formulations, the concentration of the associate calcium ion can be expected to remain same which implies that there will not be any appreciable change in the time of disintegration with the variation of calcium chloride concentration. Since the drug release depends on swelling and disintegration, the trend is also followed in the pattern of dissolution (Figure 6.5).

 # Inference - From the data obtained as above, the investigator selected and optimized 5% w/v of calcium chloride solution as the fixed parameter for the final set of formulations since it has no significant difference with that of 10% w/v formulations, considering all the characterization data.

6.3.6 Effect of the Curing time of micropellets in Calcium chloride solution[4]

 # Methodology - Calcium alginate micropellets loaded with Frusemide were prepared by varying the curing or hardening time of the wet micropellets inside calcium chloride solution and to study the effect of this variation in order to optimize the curing time for the final set of formulations. Curing time can be defined as the time of contact of the sodium alginate micropellets with calcium chloride solution. This time was allowed to complete the ionotropic gelation process where sodium ion gets replaced by calcium ion making the resultant micropellets more rigid. This time factor must have some effect on the sphericity, size and release profile of the micropellets .Hence for this study, curing time was varied from 5,10,15,20 and 30 min in 5 % w/v of calcium chloride solution with drug load constant at 30%w/w, sodium alginate concentration at 2% w/v, height of dropping at 2cm, needle size of 17G, drying condition as 60°C for 6hr in hot air oven and stirring time and speed as 30 min and 500 rpm respectively. The resultant micropellets were physically characterized.

Table 6.6 Effect of the variation in the Curing time on the physical characterization of the Frusemide loaded Calcium alginate micropellets.

Formulation Code	Curing Time (min)	Mean Diameter (μm)	Drug Entrapment Efficiency (%) (DEE)	t_{50} (min)	Disintegration time (min)
H-5	5	652.25	95.71	23	20
H-10	10	649.56	97.49	26	22
H-15	15	588.45	95.87	31	23
H-20	20	573.42	98.27	36	29
H-30	30	523.95	99.07	39	30

Table 6.7 Weight loss and contraction of the alginate micropellets with variation in Curing time

Curing Time (min)	Weight of fully cured 10 micropellets (mg)
5	152.2
10	148.3
15	146.6
20	141.5
30	137.9

Table 6.8 *In- vitro* release data of 30% w/w Frusemide loaded micropellets prepared with different Curing time in Calcium chloride solution

TIME (min)	% Cumulative Release				
	H-5	H-10	H-15	H-20	H-30*
5	16.17	10.41	10.26	8.02	7.87
10	26.58	21.27	21.35	15.95	14.17
15	35.28	31.33	29.88	24.13	23.22
20	45.49	42.23	39.58	37.58	35.49
30	58.45	54.72	48.68	44.29	42.58
40	71.46	78.21	59.34	56.98	54.46
50	84.13	94.56	71.21	66.38	63.28
60	95.36	97.65	83.52	75.49	72.49
70	100.31	100.5	91.25	82.19	79.87
80			95.26	91.37	87.04
90			100.39	97.46	95.68

'* ' indicates ($p < 0.05$), n=3

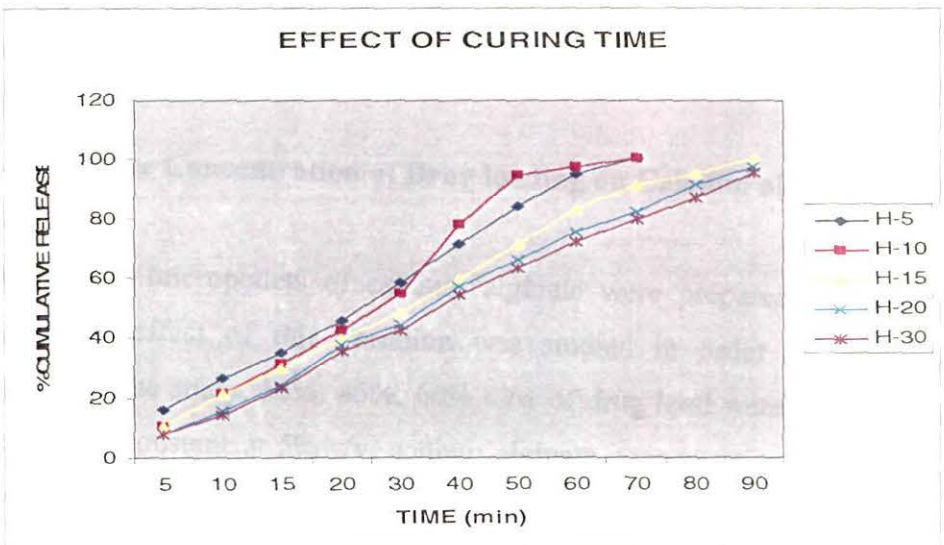


Figure: 6.6 ***In-vitro*** **drug release profile of micropellets with variation in the curing time.**

 # Observations - The curing or the hardening time of wet gelled alginate micropellets in the counterion solution was assumed to have an important role to play in characterization of the dried micropellets. Hence, the investigator carried out a study by varying curing time from 5 to 30 minutes keeping all other parameters constant. Significant effect on the size and morphological properties of the pellets were observed. With low curing time (5, 10 min) the pellets obtained after drying were found to be flaky and of undefined shape (Figure 6.7). With the increase in the curing time over 15 minutes the pellets were found to have a

regular discoid shape. From the micromeretic data (Table 6.6), it has been proved that sizes of the pellets are inversely related with the curing time. From the Table 6.7, this phenomenon can be explained as, with more contact time more influx of calcium ion occurs in the wet micropellets increasing the mass and squeezing out water from the gelled matrix. As a result there is a gross reduction in the particle diameter before and after drying. Though, time of contact has got an effect on size and morphology it has found not to influence much on the drug entrapment efficiency, disintegration time and dissolution pattern of the drug from the dried pellets. It has been reported in literatures[4] that rearrangement occurs in the calcium alginate molecule with prolongation of curing time. As the gelling in the counterion solution was instantaneous, the investigator limited the variation in curing time upto 30 minutes so as to make it a fast, practical and reproducible process industrially.

 # Inference - From all the primary data obtained from the results 30 minutes was optimized by the investigator as the curing time during the preparation of final set of formulations

6.3.7 Effect of the Concentration of Drug loading on Calcium alginate micropellets[5,6]

Frusemide loaded micropellets of calcium alginate were prepared by varying the drug loading and the effect of this variation was studied in order to optimize the drug concentration. In the study, 30%, 45%, 60% w/w of drug load were used keeping calcium chloride solution constant at 5%w/v, sodium alginate concentration at 2% w/v, height of dropping at 2cm, needle size of 17G, drying condition as 60°C for 6hr in hot air oven and stirring time and speed as 30 min and 500 rpm respectively. Curing time in calcium chloride solution was kept at 15 min. The resultant micropellets were physically characterized.

Table 6.9 Effect of the variation in Drug loading on the physical characterization of the Frusemide loaded Calcium alginate micropellets.

Formulation Code	Conc. of Sodium alginate (% w/w)	Mean Diameter (µm)	Drug Entrapment Efficiency (%) (DEE)	t_{50} (min)	Disintegration time (min)
D-30	30	588.45	95.87	31	23
D-45	45	742.31	97.25	55	30
D-60	60	868.52	96.66	59	32

Table 6.10 ***In- vitro*** **drug release data of Frusemide loaded micropellets prepared with different Drug Loading Concentration.**

TIME (min)	% Cumulative Release		
	D-30	**D-45**	**D-60**
5	10.26	7.18	6.29
10	21.35	18.05	13.18
15	29.88	26.95	18.69
20	39.58	37.52	33.56
30	48.68	43.85	41.25
40	59.34	56.81	55.05
50	71.21	68.95	66.41
60	83.52	78.88	75.93
70	91.25	84.56	82.91
80	95.74	89.85	87.57
90	100.39	94.58	93.08
120		97.46	96.44
150		100.42	99.86

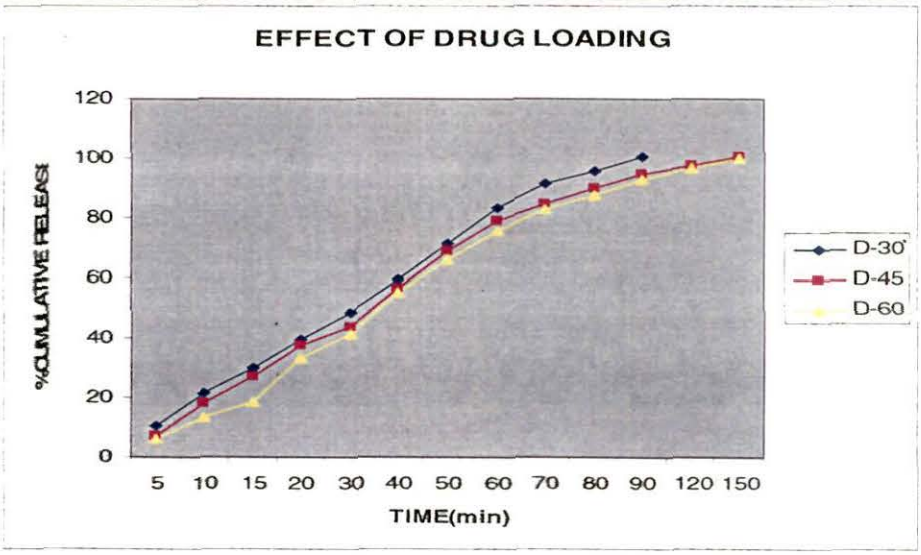


Figure: 6.8 ***In-vitro*** **drug release profiles of micropellets with variation in the drug loading concentration.**

 # Observations - From the Figure 6.9, it appears that with the increase in the drug loading, more spherical, white and smooth micropellets resulted, producing harder and dense matrix. Since the viscosity of drug-polymer dispersion increased with the increase in drug loading, the size of the particle formed after drying also showed similar trend. Drug load did not have much significant effect on the drug entrapment efficiency factor, but it had noticeable effect on the dissolution behavior of the micropellets. The Figure 6.8 displays the fact that as drug load increases, the release of the drug decreases.

 # Inference - At higher drug load concentration, the number of drug particles per unit weight of the calcium alginate micropellets is greater. With higher load the viscosity of the drug-polymer dispersion increased with the formation of hard and dense matrix which hinders penetration of dissolving medium in to the pellets. This delays the time of disintegration and also sustains the release of the drug from the dried drug-polymer matrix. But since the aim of the research work was to formulate a product with two polymers that can sustain the release of the drug more than 8 hours, further experiments were done keeping the drug load concentration minimum at 30% w/w.

6.3.8 Effect of Copolymers along with Sodium Alginate

Sodium alginate was the primary polymer in the formulation of the frusemide loaded calcium alginate micropellets. Different copolymers were tried along with it in order to achieve the following benefits :

1. Better penetration of the dissolution medium in to the micropellets.
2. Improved and controlled swelling.
3. More prolonged and sustained effect on the drug release.

The following copolymers[7,8] were studied in the preformulation stage in order to optimize the best polymer. Ethyl cellulose, hydroxy propyl methyl cellulose, aqueous dispersion of acrylic polymers (Acrycoat E30D, Acrycoat L30D and Acrycoat S100). The common property among all the polymers selected for preformulation study is their solubility in water. As the ionotropic gelation method employed throughout the study is completely done in organic solvent free aqueous environment, the polymers were selected solely on the basis of very high water solubility. Each of the five polymers were varied in concentration (1%, 2%, 4% w/w) and were incorporated in the mucilage of sodium alginate before the addition of the drug in the dispersion, as per the method described in **Section 6.1**. The resultant micropellets were physically characterized. On the basis of the best result obtained, especially in reducing the dissolution time of the drug, the optimization of copolymer was done for the final set of formulations.

6.3.8.1 Effect of Acrycoat E30D on calcium alginate micropellets

Table-6.11 Effect of the variation in the concentration of Acrycoat E30D on the physical characterization of the Calcium alginate micropellets.

Formulation Code	Conc. of Acrycoat E30D (% w/w)	Mean Diameter (μm)	Drug Entrapment Efficiency (%) (DEE)	t_{50} (min)
E1	1	608.16 ± 0.59	94.48 ± 0.48	272
E2	2	760.89 ± 0.51	93.44 ± 0.56	290
E4	4	782.78 ± 0.36	91.23 ± 0.68	341

Table- 6.12 *In- vitro* drug release data of 30% w/w Frusemide loaded micropellets prepared with different concentration of Acrycoat E30D.

Time (hr)	% Cumulative Release		
	E1	E2	E4
0.5	0.23	0.02	0.24
1	7.41	6.98	6.92
2	17.98	17.32	8.77
3	28.76	27.8	10.53
4	36.99	35.7	19.49
5	53.9	51.3	36.34
6	69.96	67.96	59.32
7	77.99	75.88	71.36
8	90.9	85.06	83.83
9	96.59	96.82	91.94

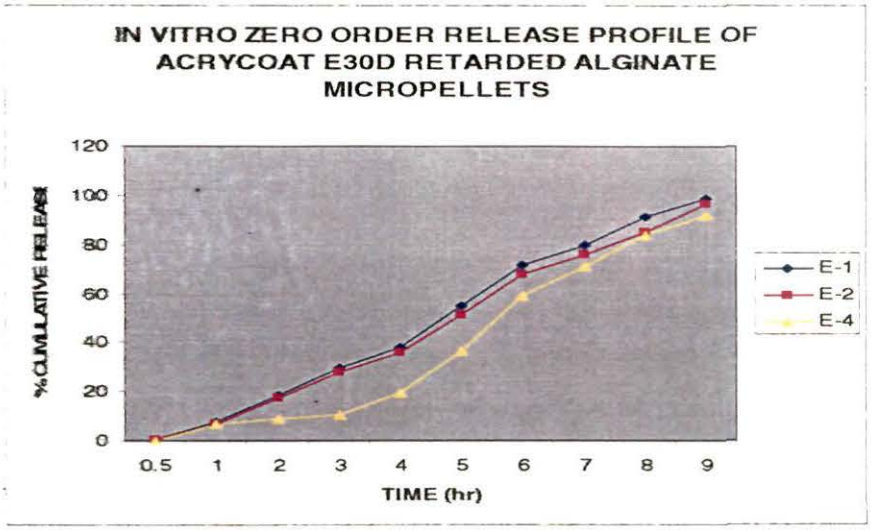


Figure: 6.10 *In-vitro* drug release profiles of micropellets prepared with Acrycoat E30D as copolymer

6.3.8.2 Effect of Acrycoat L30D on calcium alginate micropellets

Table- 6.13 Effect of the variation in the concentration of Acrycoat L30D on the physical characterization of the Calcium alginate micropellets

Formulation Code	Conc. of Acrycoat L30D (% w/w)	Mean Diameter (μm)	Drug Entrapment Efficiency (%) (DEE)	t_{50} (min)
L1	1	547.29 ± 0.54	99.22 ± 0.43	92
L2	2	613.58 ± 0.72	98.56 ± 0.43	153
L4	4	704.26 ± 0.22	97.68 ± 0.43	267

Table- 6.14 *In- vitro* drug release data of 30% w/w Frusemide loaded pellets prepared with different concentration of Acrycoat L30D.

TIME (hr)	% Cumulative Release		
	L1	L2	L4
0.5	19.21	10.11	6.23
1	40.13	19.22	13.46
2	62.36	39.25	19.49
3	83.56	60.41	33.32
4	93.54	76.31	44.15
5	101.02	89.05	60.27
6		99.59	84.39
7			98.78

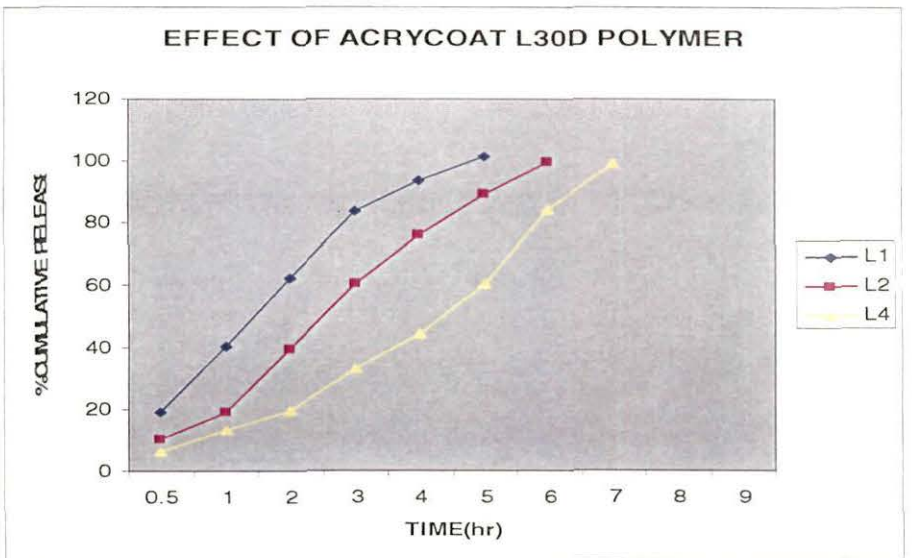


Figure: 6.11 *In-vitro* drug release profiles of micropellets prepared with Acrycoat L30D as copolymer

6.3.8.3 Effect of Acrycoat S100 on calcium alginate micropellets

Table- 6.15 Effect of the variation in the concentration of Acrycoat S100 on the physical characterization of the Calcium alginate micropellets.

Formulation Code	Conc. of Acrycoat S100 (% w/w)	Mean Diameter (µm)	Drug Entrapment Efficiency (%) (DEE)	t_{50} (min)
S1	1	594.57 ± 0.43	91.22 ± 0.34	119
S2	2	678.32 ± 0.56	86.09 ± 0.85	181
S4	4	767.25 ± 0.31	83.58 ± 0.91	278

Table- 6.16 *In- vitro* drug release data of 30% w/w Frusemide loaded pellets prepared with different concentration of Acrycoat S100.

TIME (hr)	% Cumulative Release		
	S1	S2	S4
0.5	16.21	5.82	3.63
1	37.66	13.78	8.37
2	50.71	32.15	19.16
3	76.58	49.29	26.84
4	88.37	79.88	42.93
5	100.05	87.11	59.61
6		94.64	75.87
7		101.04	88.61
8			95.79
9			101.37

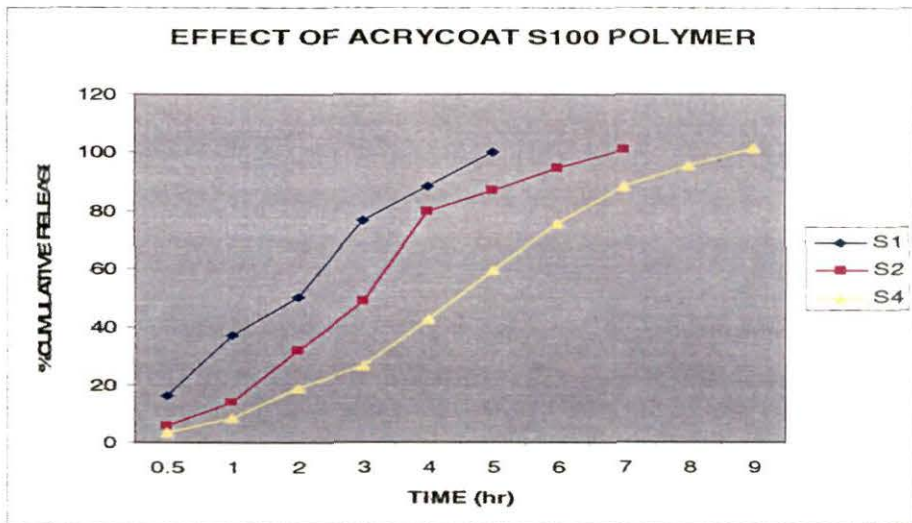


Figure: 6.12 *In-vitro* drug release profiles of micropellets prepared with Acrycoat S100 as copolymer

6.3.8.4 Effect of Methocel K15M (HPMC) on calcium alginate micropellets

Table- 6.17 Effect of the variation in the concentration of Methocel K15M (HPMC) on the physical characterization of the Calcium alginate micropellets.

Formulation Code	Conc. of Methocel K15M (% w/w)	Mean Diameter (μm)	Drug Entrapment Efficiency (%)(DEE)	t_{50} (min)
H1	1	589.25 ± 0.62	82.43 ± 0.74	117
H2	2	667.58 ± 0.56	83.74 ± 0.81	120
H4	4	759.26 ± 0.42	89.32 ± 0.91	129

Table- 6.18 *In- vitro* drug release data of 30% w/w Frusemide loaded pellets prepared with different concentration of Methocel K15M (HPMC)

TIME (hr)	% Cumulative Release		
	H1	H2	H4
0.5	14.62	11.81	10.93
1	29.37	26.47	22.83
2	52.24	50.39	48.76
3	69.18	67.71	64.82
4	78.45	76.56	72.67
5	87.23	84.12	80.31
6	92.97	90.34	88.66
7	97.78	93.41	91.12
8	99.13	96.34	93.46
9	99.94	97.89	95.68

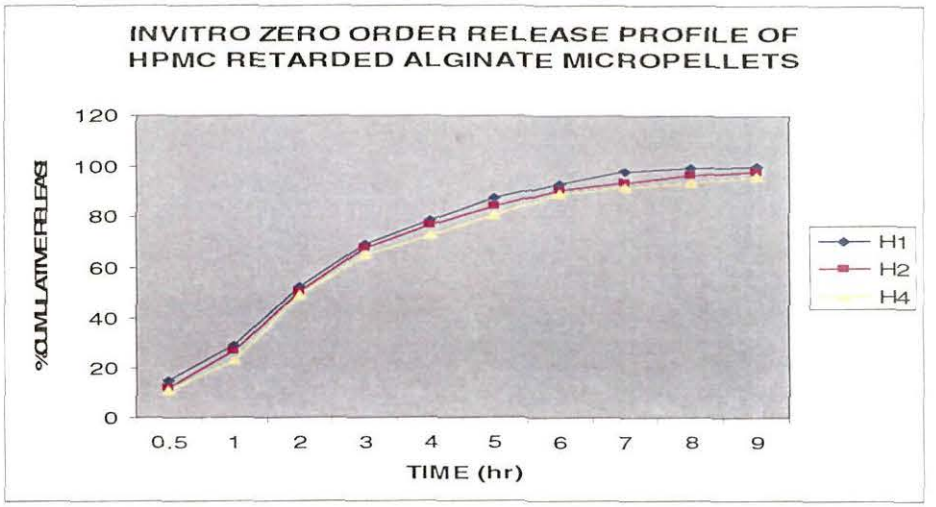


Figure: 6.13 *In-vitro* drug release profiles of micropellets prepared with Methocel K15M (HPMC) as copolymer

6.3.8.5 Effect of Surelease (Ethyl cellulose) on calcium alginate micropellets

Table- 6.19 Effect of the variation in the concentration of Surelease (Ethyl cellulose) on the physical characterization of the Calcium alginate micropellets.

Formulation Code	Conc. of Surelease (% w/w)	Mean Diameter (μm)	Drug Entrapment Efficiency (%)(DEE)	t_{50} (min)
SR1	1	601.69 ± 0.68	77.21 ± 0.77	158
SR2	2	728.14 ± 0.48	80.91 ± 0.68	187
SR4	4	776.19 ± 0.41	84.76 ± 0.59	316

Table- 6.20 *In- vitro* drug release data of 30% w/w Frusemide loaded pellets prepared with different concentration of Surelease (Ethyl cellulose)

TIME (hr)	% Cumulative Release		
	SR1	SR2	SR4
0.5	6.72	5.75	3.13
1	17.48	14.89	7.86
2	35.18	29.47	24.32
3	58.08	48.91	35.41
4	77.66	61.84	40.78
5	86.41	73.35	48.67
6	92.51	80.93	60.07
7	96.32	86.58	73.12
8	98.08	91.42	84.27
9	99.39	97.57	92.68

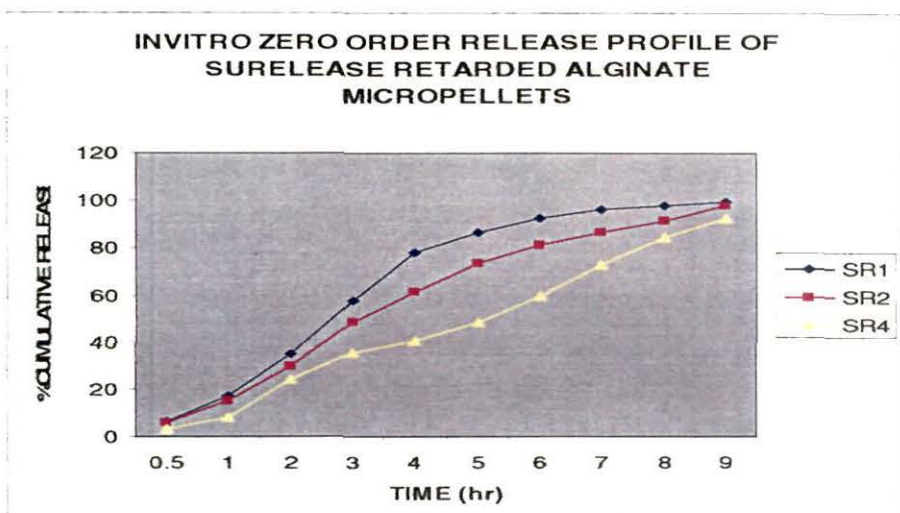


Figure: 6.14 *In-vitro* drug release profiles of micropellets prepared with Surelease (Ethyl cellulose) as copolymer

Table 6.21 Comparative study of the release kinetics in Zero order and Higuchi model for the formulations prepared using different polymers.

Formulation Code	Composition (% w/w)	Zero order		Higuchi SQRT	
		K_0	R^2	K_H	R^2
E1	Acrycoat E30D-1%	9.3247	**0.9477**	25.479	0.7939
E2	Acrycoat E30D-2%	9.0388	**0.9463**	23.909	0.7857
E4	Acrycoat E30D-4%	7.9428	**0.8407**	20.151	0.6492
L1	Acrycoat L30D-1%	18.622	**0.9519**	44.784	0.9558
L2	Acrycoat L30D–2%	14.435	**0.9776**	35.857	0.8773
L4	Acrycoat L30D–4%	10.727	**0.9123**	27.225	0.7444
S1	Acrycoat S100- 1%	17.496	**0.9844**	38.485	0.9329
S2	Acrycoat S100- 2%	13.333	**0.9438**	30.280	0.8428
S4	Acrycoat S100- 4%	10.061	**0.9467**	23.862	0.7944
H1	HPMC – 1%	12.331	0.7806	29.928	**0.9531**
H2	HPMC – 2%	11.948	0.7971	29.432	**0.9464**
H4	HPMC – 4%	11.571	0.8841	30.356	**0.9452**
SR1	Surelease- 1%	11.899	**0.8853**	26.781	0.9057
SR2	Surelease- 2%	10.718	**0.9576**	25.102	0.9131
SR4	Surelease- 4%	08.883	**0.9746**	21.482	0.8386

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

*R^2 – Correlation coefficient.

6.4 Determination of stability of the micropellets prepared using copolymers

 # Methodology - The formulations showing the best performance, with respect to *in vitro* drug 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- 6.22 Accelerated stability studies of frusemide micropellets prepared with different copolymers

TIME	S4			L4			E4			SR4			H4		
(Week)	4°C	RT	45°C	4°C	RT	45°C	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	100	100	100	100	100	100
1	98.36	98.23	87.39	98.28	98.83	97.75	86.41	97.31	84.39	99.16	97.98	87.47	99.28	98.52	89.28
2	97.38	96.29	82.54	96.93	95.97	96.13	85.13	96.62	81.11	96.79	96.61	85.18	97.57	96.93	88.42
3	94.85	94.36	77.33	93.11	94.33	95.07	84.21	94.26	78.44	94.82	94.21	83.84	96.62	95.89	86.74
4	90.52	91.72	75.94	91.37	93.78	93.89	83.37	92.57	76.41	91.39	92.22	80.71	95.09	95.37	85.33

RT--- ROOM TEMPERATURE

 # Inference - The results of the stability studies are tabulated in (Table 6.22). From the data it can be concluded that the micropellets containing copolymers at their highest concentration are comfortably stable at low (4°C) and room temperature but has a tendency to degrade at higher temperature above 45°C.

6.5 Determination of the Dissolution behavior of Marketed Conventional Frusemide Tablet (Lasix 40mg)

 # Methodology - In absence of any sustained release marketed preparation of frusemide tablets, the most highly prescribed brand **Lasix**^{R} (40 mg) was tested for its dissolution behavior and compared with that of our primary products. The tablet was kept in USP Paddle II type dissolution rate test apparatus with USP phosphate buffer pH 6.8 as the dissolution media and stirring speed was maintained at 100 rpm.

Table 6.23 ***In vitro*** **release of free drug from Conventional Frusemide tablet (Lasix 40mg)**

TIME (min)	% Cumulative Release
5	23.35
10	46.28
15	56.39
20	64.82
25	73.02
30	82.56
35	91.12
40	97.45
50	99.28

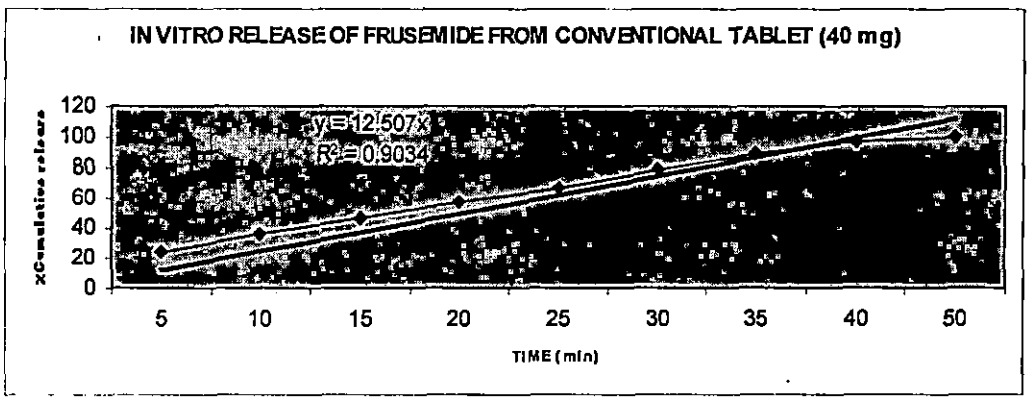


Figure: 6.16 ***In-vitro*** **release profiles of free drug from conventional tablet of Frusemide (Lasix 40mg)**

 # Inference - Nearly 100% of the drug was released from Lasix tablet within 50 minutes, whereas the researched product was successful in sustaining the release upto 9 hours. The dissolution data of the conventional tablet is shown in Table 6.23 and Figure 6.16.

6.6 Discussion

The gelled particles were cured to sufficiently hard micropellets and were 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 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. Smaller particle could be prepared by adjusting the height of the syringe from the level of counterion solution, compression force on the plunger of the syringe. The average particle size was on the highest side with Acrycoat polymers followed by Surelease. The particle size distribution was uniform and narrow. It could be estimated that with further increment in the copolymer concentration the particles would change from micro to granular level.

The scanning electron micrograph (Figure 6.15) shows the pellets being discoid in shape. As evidenced from SEM studies, surface depression was noticed at the point of contact on the drying paper. On comparison of the pellets prepared from the copolymers in highest concentration, it was evident from the photograph that more roughness of the pellet surface with Acrycoat E30D copolymers was achieved among all. Acrycoat L30D resulted in the smoothest 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 6.15 F & G), 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 6.1) act as a control and suggests that increase in total weight of the pellets makes it more spherical.

Significantly high entrapment efficiency of drug with Acrycoat based formulations confirms it being more rigid compared to the cellulose based polymers. Among the Acrylic polymers micropellets with Acrycoat L30D showed the best entrapment efficiency.

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. For all the copolymers the time of disintegration of the micropellets was

above 1 hour. The micropellets being less porous, 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 soluble only 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 showed pH dependent release.

The *in vitro* drug release data of all the formulations were fitted in Zero order and Higuchi matrix model (Table 6.21) and the rate constants and correlation coefficient were compared to get a trend in the release pattern of the drug from the formulations. From the result it is evident that the batches with Acrycoat polymers and Surelease (SR1-SR4) predominantly showed zero-order release whereas micropellets prepared with Methocel K15M (H1, H2 and H4) followed Higuchi matrix model. All the batches sustained the release of the drug for more than 8 hours as compared with the conventional tablet dosage form (Figure 6.16) except for the formulations of L30D and S100, where the release varied depending on their concentrations, within a range of 5-8 hours. 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 presence of loose drug on the surface of the micropellets the *in vitro* release profile obtained indicated a biphasic pattern i.e. initial fast release followed by a sustained pattern. Batches from Acrycoat E30D showed more prolonged action as evident from its t_{50} values when compared with other 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 (RT) and 45°C for a month (Table 6.22), the drug was found to be stable at 4°C and RT for all the formulations but showed gradual degradation at high temperatures.

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 sodium alginate, 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 entrapment efficiency and maximum prolongation of drug release. Hence, further studies was extended taking Acrycoat E30D as the optimized release controlling copolymer for the preparation of final set of formulations.

6.7 Optimized Parameters

The data obtained after performing the entire process variables were analyzed and a final conclusion was drawn (Table 6.24) to optimize the process parameters. These parameters were then kept constant in the preparation of final set of formulations and also in statistical optimization of the process with only two variables in the form of concentrations of Sodium alginate, the primary polymer, and Acrycoat E30D, the copolymer.

Table: 6.24 Optimized parameters of the process variables

SL.NO.	PARAMETERS	OPTIMIZED LEVELS
1	Concentration of Calcium chloride	5%w/v
2	Drug Loading Concentration	30%w/w
3	Drying time and mode	6 hrs in hot air oven
4	Drying Temperature	60°C
5	Curing / Hardening time	30 min
6	Stirring time and speed	30 min at 500 rpm
7	Needle size	17G
8	Height of dropping	2cm above the level of $CaCl_2$ solution
9	Syringe	Glass syringe
10	Release controlling copolymer	Acrycoat E30D

SCANNING ELECTRON MICROGRAPH

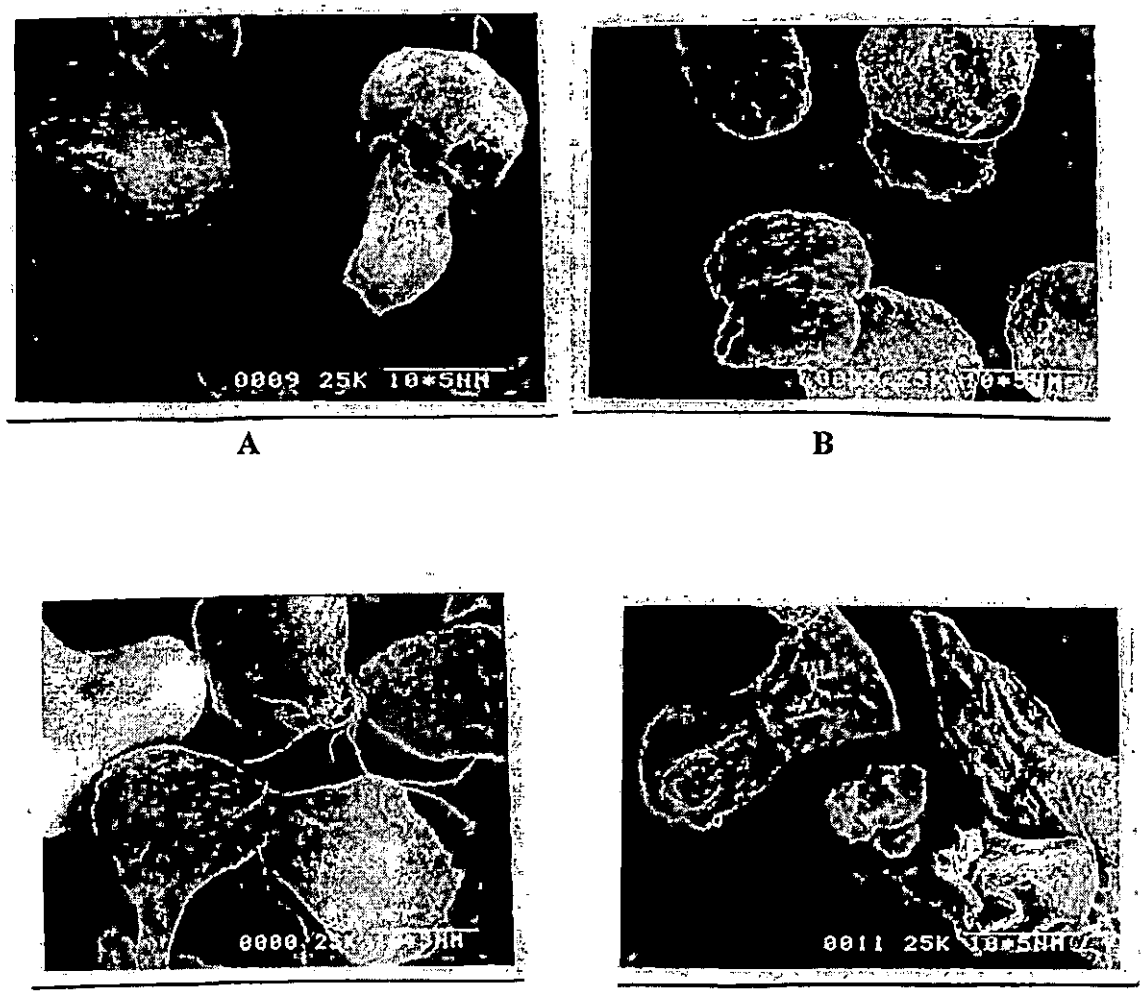


Figure 6.1 Effect of the variation in the height of dropping the drug - polymer dispersion on the morphology of the Frusemide loaded Calcium alginate micropellets.

A) 1 cm B) 2 cm C) 4 cm D) 5 cm

Magnification -- X 35 for all.

SCANNING ELECTRON MICROGRAPH

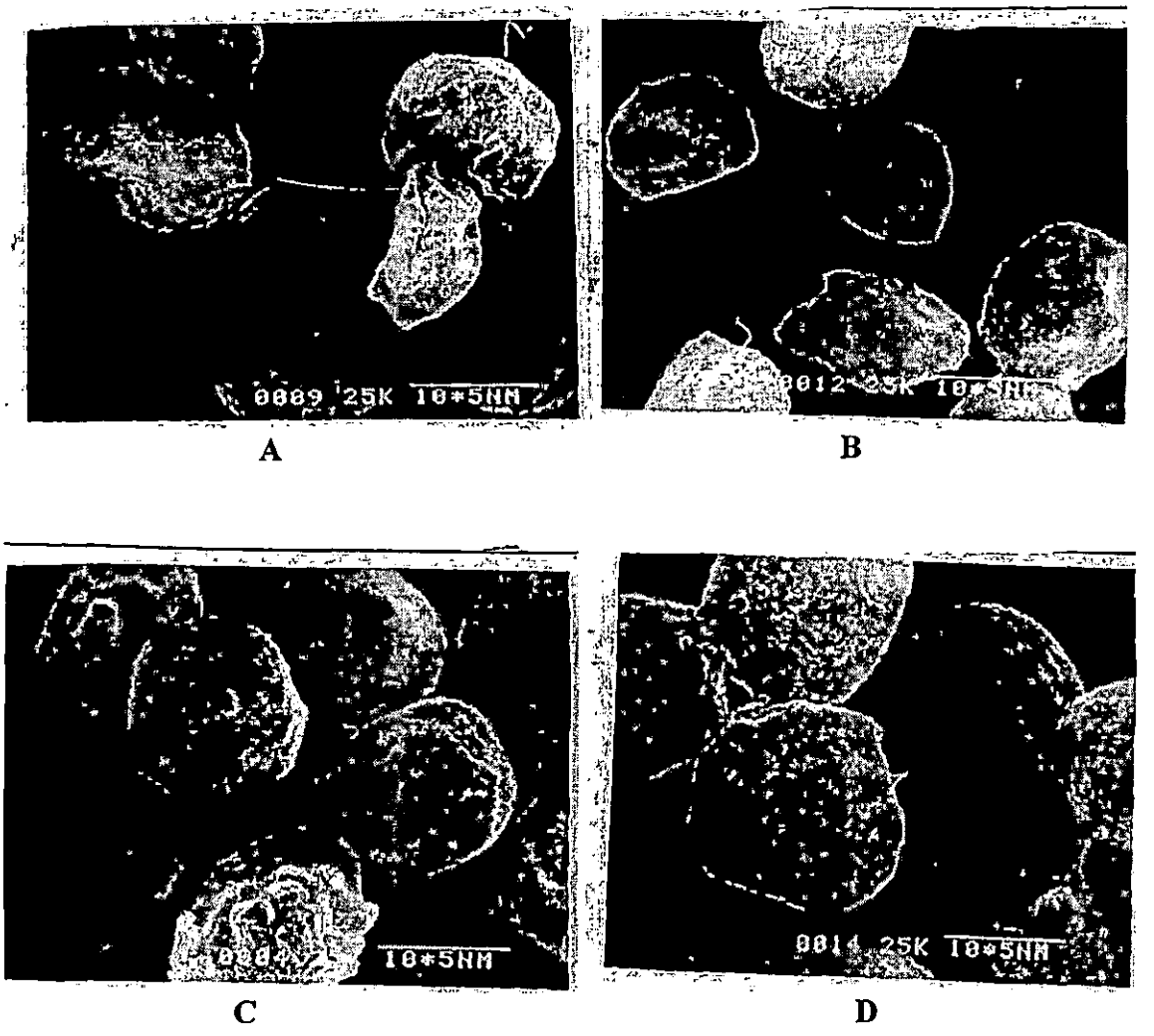
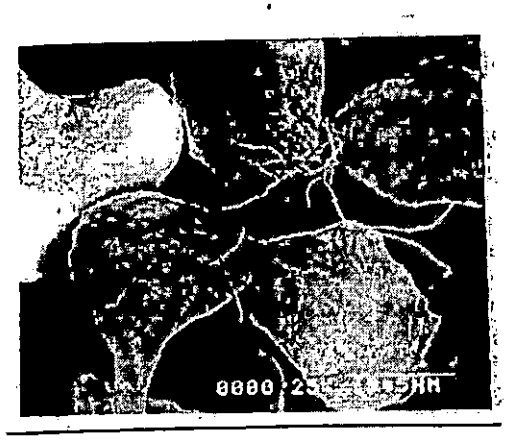


Figure 6.3 **Effect of the variation in the concentration of sodium alginate on the morphology of the Frusemide loaded Calcium alginate micropellets.**

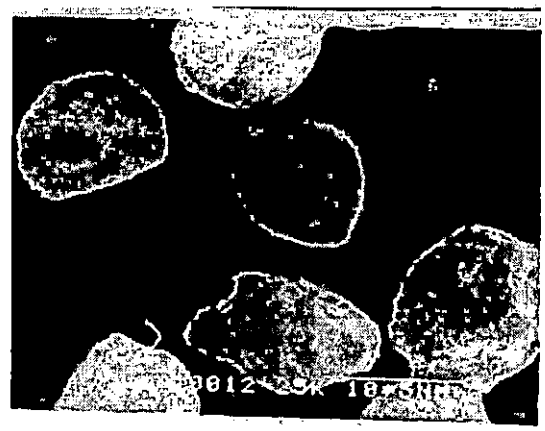
A) 1 % w/v B) 2 % w/v C) 4 % w/v D) 5 % w/v

Magnification -- X 35 for all.

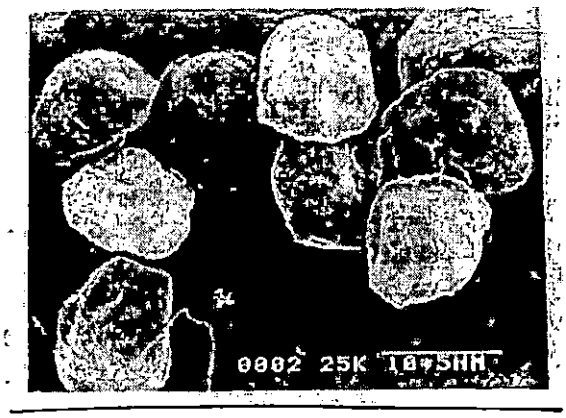
SCANNING ELECTRON MICROGRAPH



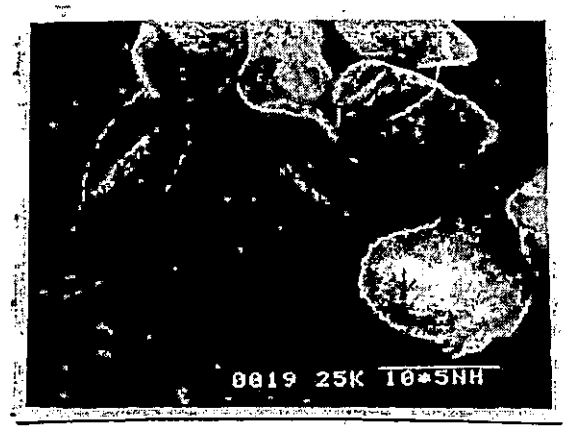
A



B



C



D

Figure 6.4 Effect of the variation in the concentration of calcium chloride on the morphology of the Frusemide loaded Calcium alginate micropellets.

A) 1 % w/v B) 2 % w/v C) 5 % w/v D) 10 % w/v

Magnification -- X 35 for all.

SCANNING ELECTRON MICROGRAPH

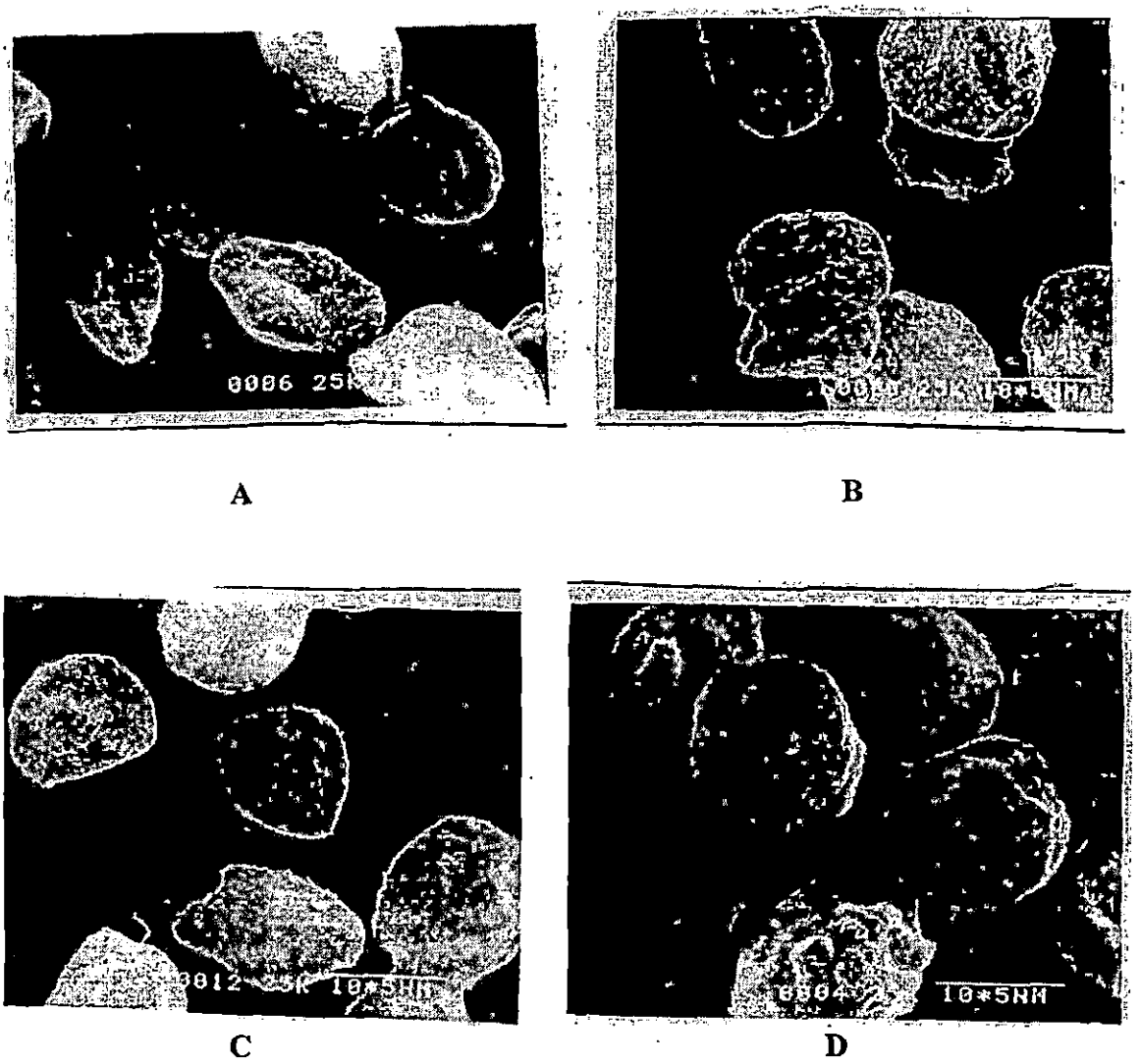
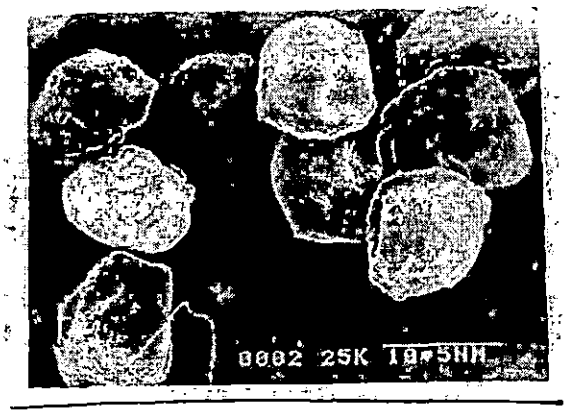


Figure 6.7 Effect of the variation in the curing time in calcium chloride solution on the morphology of the Frusemide loaded Calcium alginate micropellets.

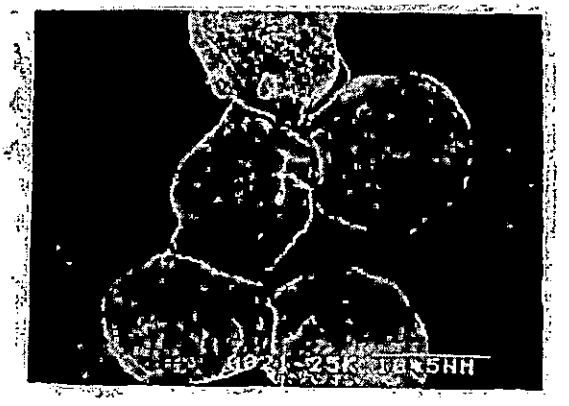
A) 5 min B) 10 min C) 15 min D) 30 min

Magnification -- X 35 for all.

SCANNING ELECTRON MICROGRAPH



A



B

C

Figure 6.9 Effect of the variation in the drug loading on the morphology of the Frusemide loaded Calcium alginate micropellets.

A) 30 % w/w B) 45 % w/w C) 60 % w/w

Magnification -- X 35 for all.

SCANNING ELECTRON MICROGRAPH

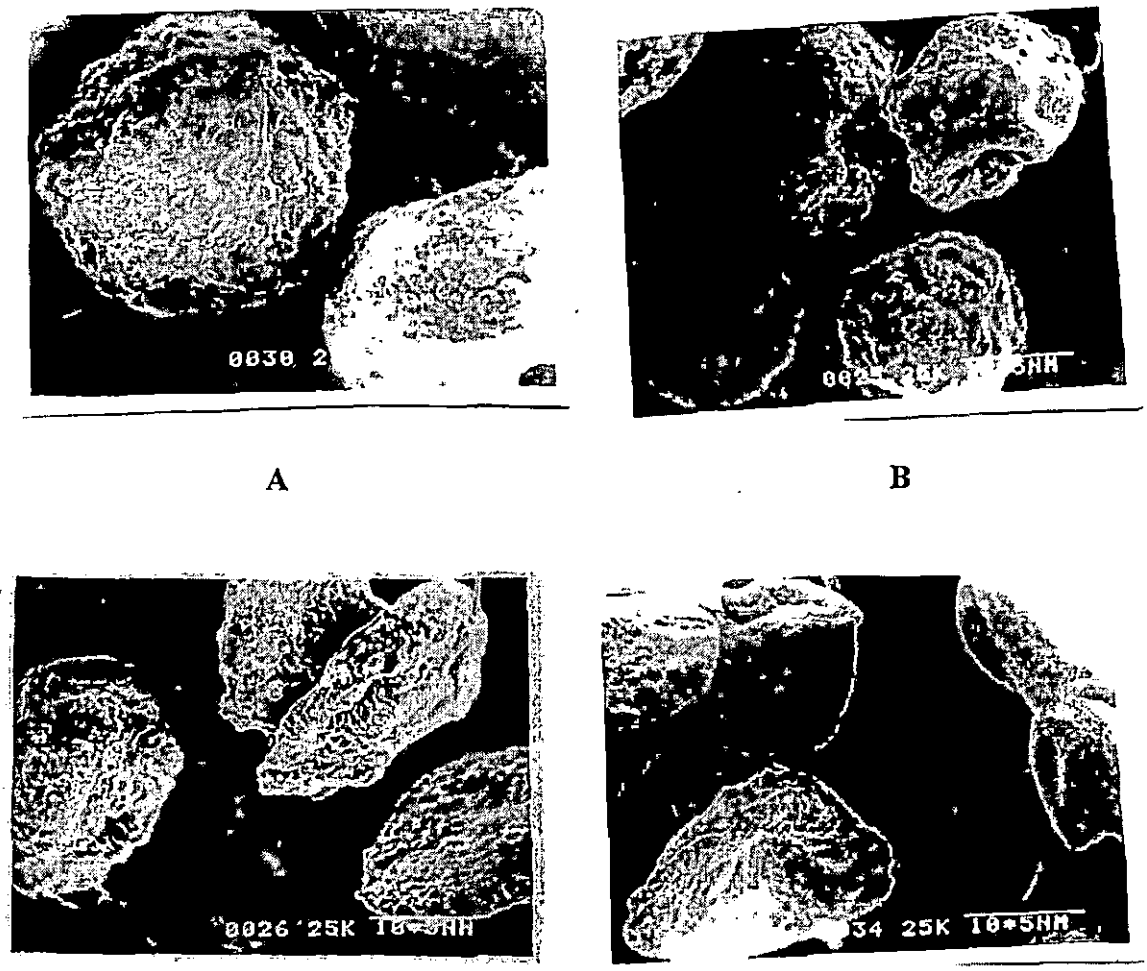
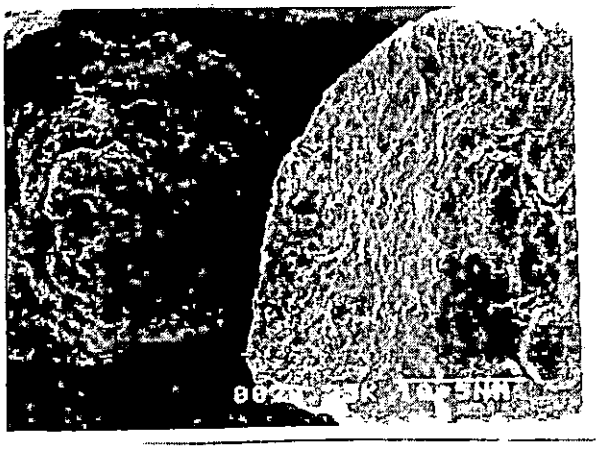


Figure 6.15 Effect of the variation of copolymers (4% w/w) on the morphology of the Frusemide loaded Calcium alginate micropellets.

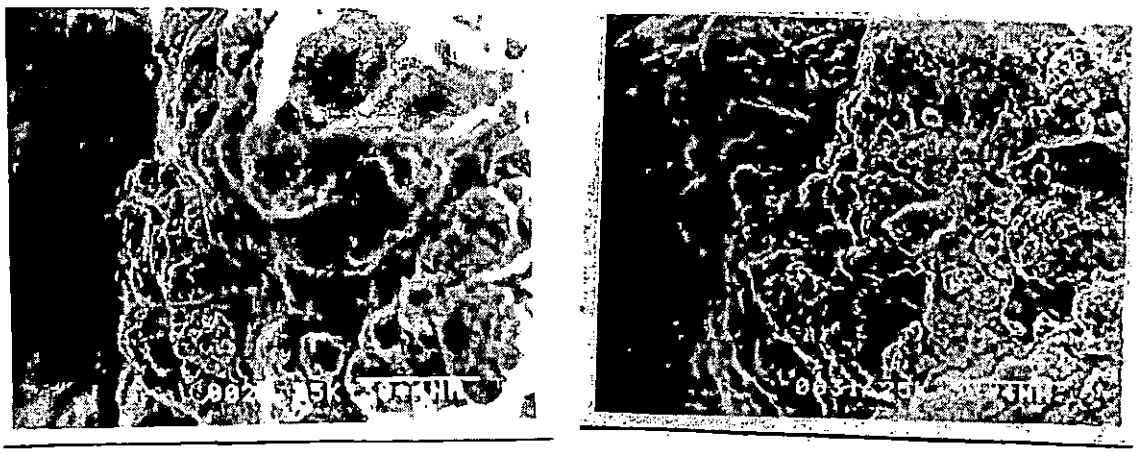
A) Acrycoat L30D B) Acrycoat S100 C) Methocel K15M D) Surelease

Magnification -- X 35 for all.

SCANNING ELECTRON MICROGRAPH



E



F G

Figure 6.15 Effect of the variation of copolymers (4% w/w) on the morphology of the Frusemide loaded Calcium alginate micropellets.

E) Acrycoat E30D F) & G) Internal Sections of micropellets with Acrycoat E30D

Magnification -- E) X 35 F) & G) X 350

6.8 REFERENCES

1. L.Y. Lim and S.C. Wan, *Drug Development & Industrial Pharmacy*, **23(10)**, 1997, 973-980.
2. R. Bodmeier and O. Paeratakul, *J.Pharmaceutical Sciences*, **78(11)**, 1989, 964-967.
3. N. Segi, T. Yotsuyanagi and K. Ikeda, *Chemical & Pharmaceutical Bulletin*, **37 (11)**, 1989, 3092-3095.
4. R. Bodmeier and H. Chen, *J. Controlled Release*, **15**, 1991, 65-77.
5. R. Bodmeier and J.Wang, *J.Pharmaceutical Sciences,* **82(2),** 1993, 191-194.
6. T. Yotsuyanagi, K. Ikeda *et al*, *Chemical & Pharmaceutical Bulletin*, **35(4)**, 1987, 1555-1563.
7. K.O.R. Lehmann and J.W. McGinity, Eds., *Aqueous Polymeric Coatings for Pharmaceutical Applications*, Marcel Dekker, New York, 1989, 153-245.
8. C.R. Steuernagel and J.W. McGinity, Eds., *Aqueous Polymeric Coatings for Pharmaceutical Applications*, Marcel Dekker, New York, 1989, 1-61