CHAPTER 8

PHYSICOCHEMICAL EVALUATIONS OF THE MICROPELLETS

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To judge the efficiency, reliability and uniformity of the micropellets evaluation of their physicochemical parameters is of paramount importance. Thus, the final nine formulations obtained were subjected to the following physicochemical studies.

8.1 General appearance of the micropellets

The wet gelled micropellets formed after instantaneous gelation, when incorporated in calcium chloride solution, were white soft mass, spherical in shape and heavier than water making them to settle down at the bottom of the beaker. After drying in open air for 30 minutes the micropellets were found to shrink in size suggesting the water loss from the micropellets. After drying the moist micropellets in an oven for 6 hours at 60° C, the resultant dry micropellets were discoid in shape rather then purely spherical. The surface which was adhered to the filter paper in the petri dish remained slightly flattened. This problem could be overcome to great extent by occasional swirling of the micropellets in the vessel of drying ensuring frequent changing of the point of contact with the petri dish. With the increase in polymer load, namely, the formulations F7, F8 and F9, more sphericity were obtained. The micropellets containing only sodium alginate (formulations F1, F2, and F3) were off white in colour whereas with the addition of Acrycoat E30D, light yellowish micropellets resulted. Micropellets prepared from high concentrations of polymers, sometimes appears to have minute tail at one end. This was due to the high viscosity of the drug – polymer dispersion, which created hinderances during extrusion from the syringe into counterion solution. This could be overcome by maintaining uniform pressure on the plunger and being more careful during the process so as to eliminate any manual error.

8.2 Particle Size Analysis[1,2]

Particle size analysis of the prepared micropellets was done with an objective to study the pattern of size distribution of the different batches of micropellets. In pharmaceutical practice particle size analysis can be done by any one of the following methods, namely:

1. Optical Microscopy
2. Sieve analysis
3. Sedimentation Method using Andreasan apparatus.
4. Conductivity method using Coulter counter.

In this study, Sieve analysis method was selected for the following obvious reasons:

1. Inexpensive
2. Simple
3. Rapid
4. Can effectively measure particles of size range between 50 – 1500 μm.
5. The method directly gives weight distribution.

Sieving Method[1,3]

The method describes the diameter of a sphere that passes through the sieve aperture as the asymmetric particle and the size is expressed as d_{sieve} or $d_{average}$. A sample of fully dried micropellets (10g) is placed on the top sieve # 16. The set of Indian Pharmacopoeial standard sieves were arranged in the order of # 16, # 22, # 30 of aperture size 1000, 710, 500 respectively. The entire set was shaken for 5 minutes in a sieve shaker. Pellets retained in each sieve were weighed and the percentage weight retained against various sieve size was recorded. The average particle size was calculated from the following formula:

$$d_{average} = \Sigma (n \times d) / \Sigma n \qquad (8.1)$$

where, n = Frequency Weight and d = Mean size. The latter is calculated by taking the average of the nominal mesh size of two corresponding sieve. The average diameter and the mode of distribution were calculated mathematically for six times and the results are tabulated in Table 8.1.

Table 8.1 Percentage frequency size distribution of frusemide micropellets by Sieve Analysis

Formulation Code	Percent weight retained on various sieves			Mean Diameter*
	# 16	# 22	# 30	(μm ± S.D.)
F 1	-	0.01	98.75	608.14 ± 0.39
F 2	2.72	16.05	81.19	655.90 ± 1.03
F 3	10.52	19.18	70.02	694.85 ± 0.72
F 4	-	0.93	99.06	608.16 ± 0.59
F 5	12.62	42.41	44.96	760.89 ± 0.51
F 6	15.79	46.15	38.05	782.78 ± 0.36
F 7	3.02	6.11	90.86	632.1 ± 0.73
F 8	12.22	16.04	71.74	693.36 ± 0.56
F 9	16.33	66.47	17.18	841.65 ± 0.48

* Results shown are 'mean ± SD', n = 6 for mean diameter; '-'signifies value < 0.01 %.

Results:

From the particle size analysis of Frusemide loaded Calcium alginate micropellets as observed form Table 8.1, it is evident that the size range of all the formulations ranged between 600 μm to 850 μm. According to the results of the size range, it is technically proved that the name micropellets for the formulation have been correctly coined. They can also be classified under microspheres as the size range of microspheres ranges from 5 μm – 1000 μm. The micropellets of first three formulations having low level (1% w/v) of Sodium alginate showed particles of least size range compared to remaining six, though formulations F7 and F8 showed similar results. Formulations F3, F6, F9 which contain high level (4 % w/w) of Acrycoat E30D showed steady increase in particle size along with different levels of sodium alginate.

In the Table 8.1, it is evident that for a particular size fraction *e.g.* # 22, maximum and minimum percentage weight retained varied widely from 0.01 % in F1 to 66.47 % in F9, which proves the flexibility of the method.

8.3 Rheological study[1,2]

To assess the flow properties of the prepared micropellets measurement of Angle of Repose method was employed. Angle of Repose is defined as the maximum angle possible between the surface of the pile of powder and the horizontal plane. The angle of repose is designated by (θ) and can be mathematically expressed as:

$$\theta = \tan^{-1}(h/r) \qquad (8.2)$$

Where, θ = Angle of Repose

h = height of the pile of micropellets, (cm)

r = radius of the base of the pile of micropellets, (cm)

The micropellets were allowed to fall freely through the stem of an inverted glass funnel from a distance of 1 cm above the horizontal flat surface. The tip of the funnel was kept at a distance of 3 mm from the peak of the pile of micropellets. The angle of repose was calculated by measuring the height (h) of the pile and the radius of the base (r) with a ruler. During the flow through the hopper and subsequently through the stem of the inverted funnel, the granules exhibit internal flow and demixing. Flow of granules is hindered on account of frictional forces between the particles. Thus, the tangent of the angle of repose is expressed as the coefficient of friction (μ).

The significance of the result of this method is lower the angle of repose, the better the flow properties as shown in Table 8.2. The result obtained, in triplicate, from the evaluation is tabulated in Table 8.3

Table 8.2 Relationship between Angle of Repose (θ) and Flow properties

Angle of Repose (θ)	Quality of Flow properties
$\theta < 25$	Excellent
$25 < \theta < 30$	Good
$30 < \theta < 40$	Flowable
$\theta > 40$	Very Poor

Table 8.3 Results of Angle of Repose (θ) of the Micropellets

Formulation Code	Angle of Repose (θ)*
F 1	16.76 ± 0.54
F 2	18.06 ± 0.96
F 3	20.11 ± 0.88
F 4	18.32 ± 0.79
F 5	20.56 ± 1.03
F 6	21.24 ± 1.97
F 7	19.52 ± 1.21
F 8	21.39 ± 1.07
F 9	22.56 ± 0.77

* Results shown are 'mean ± SD', n = 3

Results:

In the above Table 8.3, it is clearly visible that all the formulations show angle of repose in a range between 16 ° -- 23 °. On interpreting the result with the values of Table 8.2, it can be inferred that all formulations of Frusemide micropellets are free flowing and while tableting them or encapsulating in capsule shell, lubricants need not be added.

8.4 Determination of Moisture content[3] of the micropellets

To have an estimate of the stability of the micropellets and to determine the efficacy of the drying conditions adopted in the entire experimental procedures, the micropellets were subjected to moisture content study. This was achieved by placing weighed amount of micropellets at 60 ° C for 10 minutes in an Infra Red (I.R.) Moisture Balance. The micropellets were reweighed after being brought out from the I.R. Moisture balance. The difference between the initial and the final weight were calculated which gave the loss in weight. Higher the loss more amount of moisture can be assumed to be present in the micropellets. The percentage moisture content was then calculated mathematically in triplicate, using the following equation:

$$[(W_0 - W) / (W_0)] \text{ X } 100 \qquad (8.3)$$

Where, W = Final Weight; W_0 = Initial weight.

Table 8.4 Moisture Content of the Frusemide Micropellets

Formulation Code	% Moisture content*
F 1	1.71 ± 0.81
F 2	1.59 ± 0.76
F 3	1.97 ± 0.32
F 4	1.48 ± 0.48
F 5	1.44 ± 0.56
F 6	2.23 ± 0.68
F 7	1.67 ± 0.33
F 8	1.42 ± 0.54
F 9	2.32 ± 0.64

* Results shown are 'mean ± SD', n = 3

Results:

The result of the moisture content study is presented in Table 8.4. Moisture content of all the formulations was low with little variations among all the levels of polymers. This indicates the effectiveness of the drying methods since all the final formulations were dried for 6 hours at 60 ° C in hot air oven. Low moisture level also ensures high stability of the micropellets on storage reducing the chance of any hydrolytic reactions. From the result, it is evident that the formulations F3, F6 and F9, all of which contains 4% w/w Acrycoat E30D with different levels of sodium alginate showed comparatively high amount of moisture loss after drying. This may be explained as that, the presence of high concentration of acrylic polymers must have produced dense network which retained the maximum moisture within it.

8.5 Determination of Drug Content and Drug Entrapment Efficiency[4] (DEE) of the Frusemide loaded Calcium alginate micropellets

Drug content and drug entrapment efficiency of Frusemide micropellets were done to get an idea of the entrapment capacity of the polymers at different concentrations.

About 100mg of micropellets (# 30 sizes) were accurately weighed and dissolved in 25ml of Phosphate buffer (pH 7.4) and kept overnight. An aliquot from the filtrate was analyzed

spectrophotometrically, after suitable dilution, using SHIMADZU UV-VIS spectrophotometer, at 277.5nm. Reliability of the method was judged by conducting recovery analysis using known amount of drug with or without polymer. Recovery was found to be averaged at 100 ± 0.89%. Drug content of every batch was determined in triplicate for (#30) size range of micropellets and the mean ± S.D. was calculated. Drug Entrapment Efficiency (DEE) was calculated using the formula:

% DEE= (Actual drug content/ Theoretical drug content) x 100 **(8.4)**

The results of the above experiments are tabulated in Table 8.5.

Table 8.5 Drug Content and Drug entrapment Efficiency (DEE) of Micropellets

Formulation Code	Theoretical Drug Content (mg)	Actual Drug Content (mg)	Drug Entrapment Efficiency (%)*
F 1	30	28.782	95.94 ± 0.63
F 2	30	29.706	99.02 ± 0.82
F 3	30	29.841	99.47 ± 0.91
F 4	30	28.344	94.48 ± 0.48
F 5	30	28.032	93.44 ± 0.56
F 6	30	27.369	91.23 ± 0.68
F 7	30	28.863	96.21 ± 0.37
F 8	30	29.328	97.76 ± 0.56
F 9	30	29.511	98.37 ± 0.77

* Results shown are 'mean ± SD', n = 3

Results:

From the results presented in Table 8.5, it is observed that Ionotropic Gelation Technique produced micropellets of high encapsulation efficiency. The DEE value varies within a very short range from 91 to 99 % among all the nine formulations indicating very small amount of drug loss during the process. The formulations F1, F2 and F3 with low level of Sodium Alginate (1 % w/v) showed marginally higher entrapment when compared to the other two

levels. This may be explained by the fact that at higher concentration Sodium Alginate forms a rigid matrix with Calcium Chloride preventing incorporation of drug during the curing period in the Calcium Chloride solution. However the study evidenced that Sodium Alginate and Acrycoat E30D do not have much significant effect in the drug content of the formulations, since the drug content were within ± 5% of the labeled potency of the drug in all the formulations.

8.6 Loose surface crystal (LSC)[5] study

Loose surface crystal study is conducted to estimate the amount of drug present on the surface of the micropellets which may show immediate release in the dissolution media. The release mechanism of the drug is diffusion controlled which gets started after the drug present on the surface of micropellets entirely gets released. So this study indicates the initial drug concentration at the absorption site immediately after ingestion of the dosage form.

Method:

100mg of micropellets (# 30 sizes) were suspended in 100ml of Phosphate buffer (pH 6.8), as 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, after suitable dilution, using SHIMADZU UV-VIS spectrophotometer, at 277.5nm. Percentage of drug present loosely on the surface of the micropellets was obtained using following equations:

1) % LSC with respect to weight of micropellets =
(Amount of drug (mg) released after 15 min / Total weight of micropellets used in the experiment) x 100 ..... (8.5.1)

2) % LSC with respect to entrapped drug =
(Amount of drug (mg) released after 15 min / Drug content of micropellets used in the experiment) x 100 ..... (8.5.2)

Percentage of drug released with respect to entrapped drug in the sample was recorded in Table 8.6.

Table 8.6 Loose Surface Crystal study (LSC)

Formulation Code	Amount of drug released after 15 min (mg)	Amount of drug in 100mg of micropellets (mg)	LSC (%) with respect to gross wt. of the micropellets	LSC (%) with respect to entrapped drug
F 1	2.271	95.940	2.271	2.367
F 2	1.943	99.020	1.943	1.962
F 3	1.677	99.470	1.677	1.686
F 4	3.353	94.483	3.353	3.549
F 5	2.214	93.437	2.214	2.369
F 6	1.429	91.235	1.429	1.567
F 7	1.882	96.192	1.882	1.956
F 8	0.913	97.763	0.913	0.729
F 9	0.594	98.365	0.594	0.604

Results:

The values of LSC is an important parameter giving an indication of the amount of drug available on the surface of the micropellets for immediate absorption and to elicit quick onset of action. The data presented in the Table 8.6 shows that very small amount of drug ranging from 0.604% to 3.549% with respect to total entrapped drug are available loosely on the surface of the micropellets. It is also observed that with the increase in the concentration of Acrycoat E30D, there was a gradual decrease in the value of % LSC. The formulations prepared without secondary polymer (Acrycoat E30D), namely, F1, F4 and F7 reflect much higher amount of drug loosely available on the surface. Thus it can be explained by the fact that higher concentrations of both the polymers produce strong binding effect which does not allow the drug to remain free on the surface of the micropellets.

8.7 Disintegration study[4]

Disintegration study was performed in 0.1 M Hydrochloric acid and USP Phosphate buffer pH 6.8 separately in a rotating bottle apparatus. 5 micropellets of a fixed size fraction were rotated in 50 ml of the liquid medium in glass vial at 37° C at 30rpm for 2 hours. Tests were performed in triplicate.

Disintegration time (min) was observed when the micropellets were swelled and finally disintegrated and dispersed in the medium.

No swelling occurred in 0.1 M Hydrochloric acid over 24 hours. The results obtained are presented in Table 8.7.

Table 8.7 Disintegration study of frusemide micropellets in Phosphate buffer pH 6.8

Formulation Code	Disintegration time (min) *
F 1	24 ± 4.53
F 2	36 ± 5.21
F 3	52 ± 4.68
F 4	42 ± 3.85
F 5	64 ± 6.11
F 6	97 ± 4.37
F 7	73 ± 5.24
F 8	115 ± 5.98
F 9	> 120

* Results shown are 'mean ± SD', n = 3

Results:

The results of the study revealed that the ionic character of the polysaccharides showed pH-dependent disintegration of the micropellets. In Calcium alginate pellets, as reported, the carboxyl group in the alginate moiety gets ionized in the higher pH, thereby repelling each other. As a result fluid is drawn inside the pellets producing swelling and ultimately the micropellets bursts out. With the increase in the proportion of polymer and copolymer (F6

to F9) the disintegration time was delayed, as evidenced from the Table 8.7, due to dense network formation which hindered withdrawal of fluid, hence swelling of the pellets. Formulation F9 showed exceptionally high (> 2 hr) disintegration time extended beyond the testing time (2 hr), hence could not be recorded. On keeping this particular formulation for overnight, it was found to be disintegrated and dispersed in the medium. The data obtained also gives an indication of the sustained effect produced by the polymers in releasing the drug in the dissolution medium.

8.8 Studies on Drug - Polymer Interactions:

8.8.1 Study on Drug – Polymer Interaction using Infra Red (IR) Spectroscopy[3,6]

Infra Red (I.R) spectrophotometers are used for recording spectra in the region 4000 cm^{-1} to 670 cm^{-1} (2.5 μm to 15 μm) and in some cases down to 200 cm^{-1} (50 μm). Fourier Transform Spectrophotometers use polychromatic radiation and calculate the spectrum in the frequency domain from the original data by Fourier transformation. Spectrophotometers fitted with an optical system capable of producing monochromatic radiation in the measurement region may also be used. Normally the spectrum is given as a function of transmittance, the quotient of the intensity of the transmitted radiation and the incident radiation.

The primary objective of the study was to identify any potential interactions or incompatibility among the drug, Frusemide, and the polymers used in the formulation of micropellets.

The absorbance (A) is defined as the logarithm to base 10 of the reciprocal of the transmittance (T):

$$A = \log_{10}\left(\frac{1}{T}\right) = \log_{10}\left(\frac{I_0}{I}\right)$$

$T = I/I_0$,

I_0 = intensity of incident radiation,

I = intensity of transmitted radiation

Materials:

1. Potassium bromide (IR Grade) – Loba Chem, India.
2. Samples used for FTIR analysis and their corresponding IR plots:
   A. Frusemide -------- Figure 8.1
   B. Calcium alginate (Blank pellets) ------- Figure 8.2
   C. Acrycoat E30D ----- Figure 8.3
   D. Frusemide and Sodium alginate mixture ----- Figure 8.4
   E. Micropellets containing Frusemide, Sodium alginate and Acrycoat E30D and grounded and screened through (#88) I.P.sieves ---- Figure 8.5
   F. Overlapping spectra of Figure 8.1, 8.2 and 8.5 ------ Figure 8.6

Methodology:

The method adopted was **Disc Method** as per British Pharmacopoeia[6]. Substance weighing 2 mg was triturated with 300 mg of finely powdered and dried potassium bromide (KBr). These quantities are usually sufficient to give a disc of 13 mm diameter and a spectrum of suitable intensity. The mixture was grounded carefully and spreaded uniformly in a suitable die, and submitted *in vacuo* to a pressure of about 800 MPa (8 $t.cm^{-2}$). Several factors may cause the formation of faulty discs, such as insufficient or excessive grinding, humidity or other impurities in the dispersion medium and an insufficient reduction of particle size. A disc was rejected when visual examination showed lack of uniform transparency or when transmittance at about 2000 cm^{-1} (5 μm) in the absence of a specific absorption band is less than 75% without compensation.

Disc samples were analyzed in FTIR spectrophotometer (SHIMADZU FTIR - 8400S, Japan) over a range of 400- 4000 cm^{-1}. Both absorbance (A) and transmittance (T) spectra were recorded.

Results:

The IR Spectra of the materials obtained are presented in Fig. 8.1 to 8.6.From the infrared spectra (Figure 8.1 to 8.5) it is clearly evident that there was no interactions of the drug, Frusemide with the excipients used, namely, sodium alginate and Acrycoat E30D. 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 are remained undisturbed in the final formulation. This proves the fact that there is no potential incompatibility of the drug with all the polymers 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.

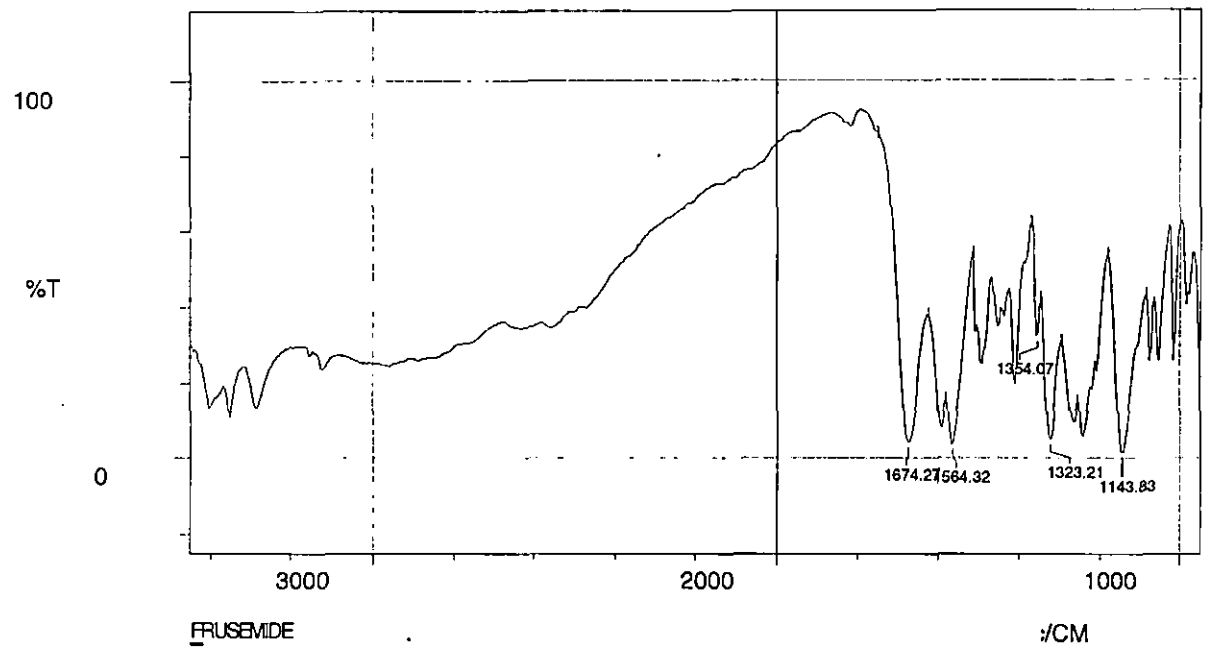

FIGURE 8.1 I R Spectrum of the Drug - FRUSEMIDE

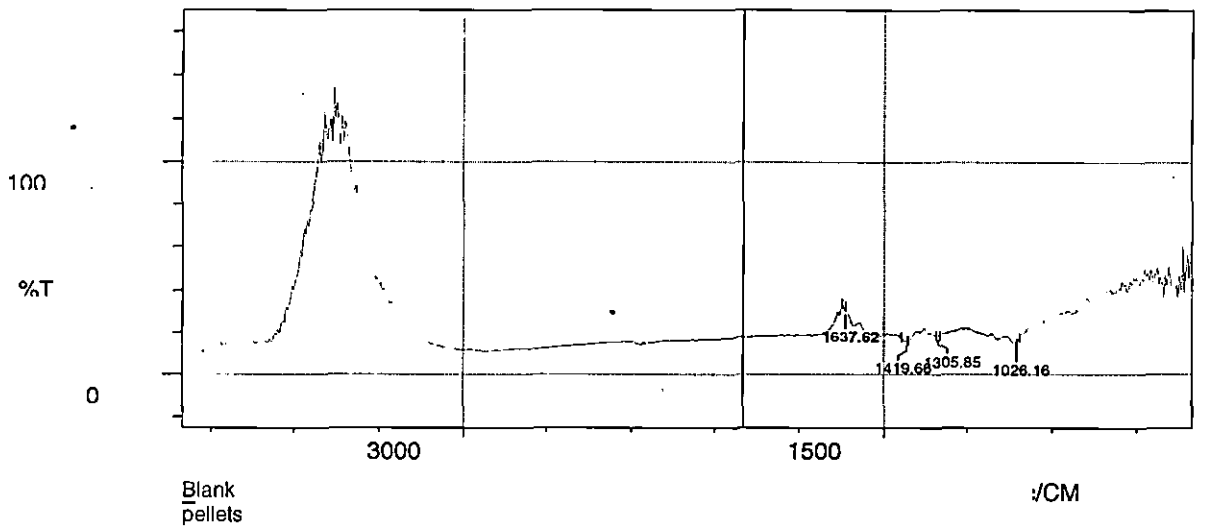

FIGURE 8.2 I R Spectrum of the Calcium Alginate pellets without drug (blank)

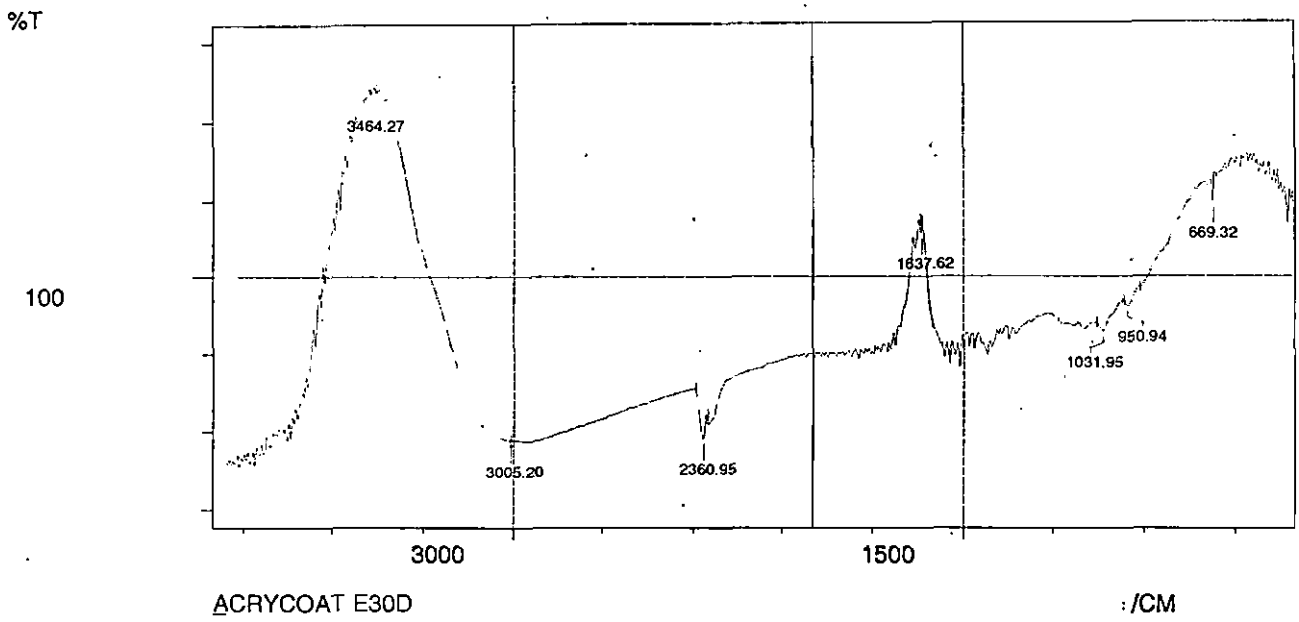

FIGURE 8.3 I R Spectrum of the Secondary Polymer – ACRYCOAT E30D

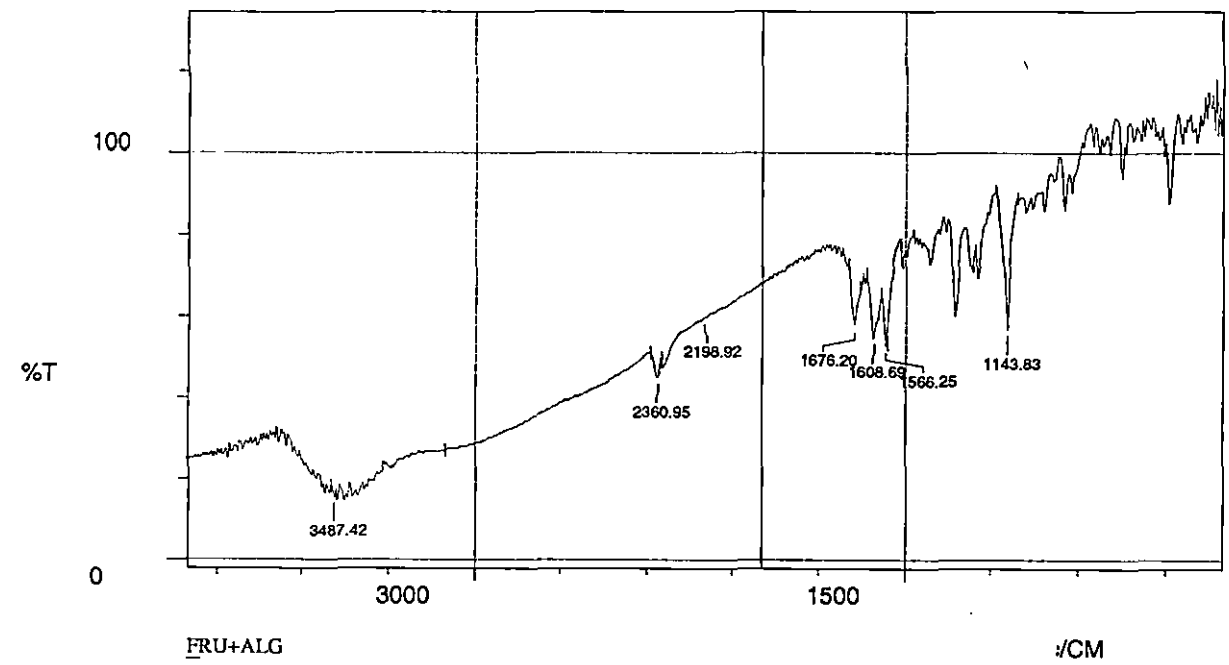

FIGURE 8.4 I R Spectrum of Frusemide + Sodium alginate micropellets

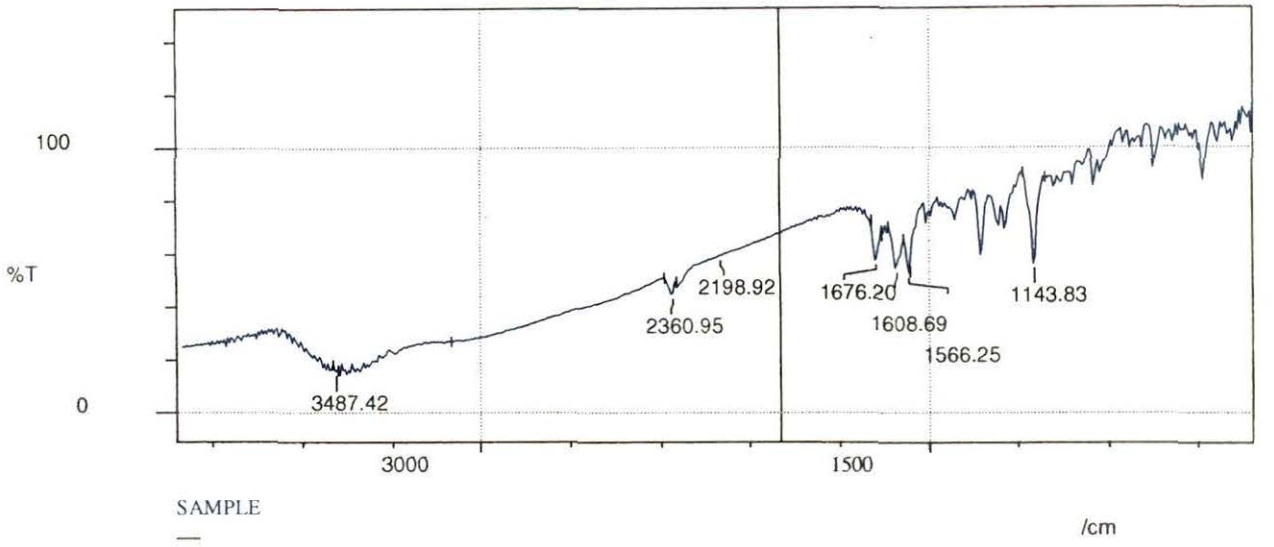

FIGURE 8.5 IR Spectrum of final micropellets containing (Frusemide+ Calcium alginate + Acrycoat E30D)

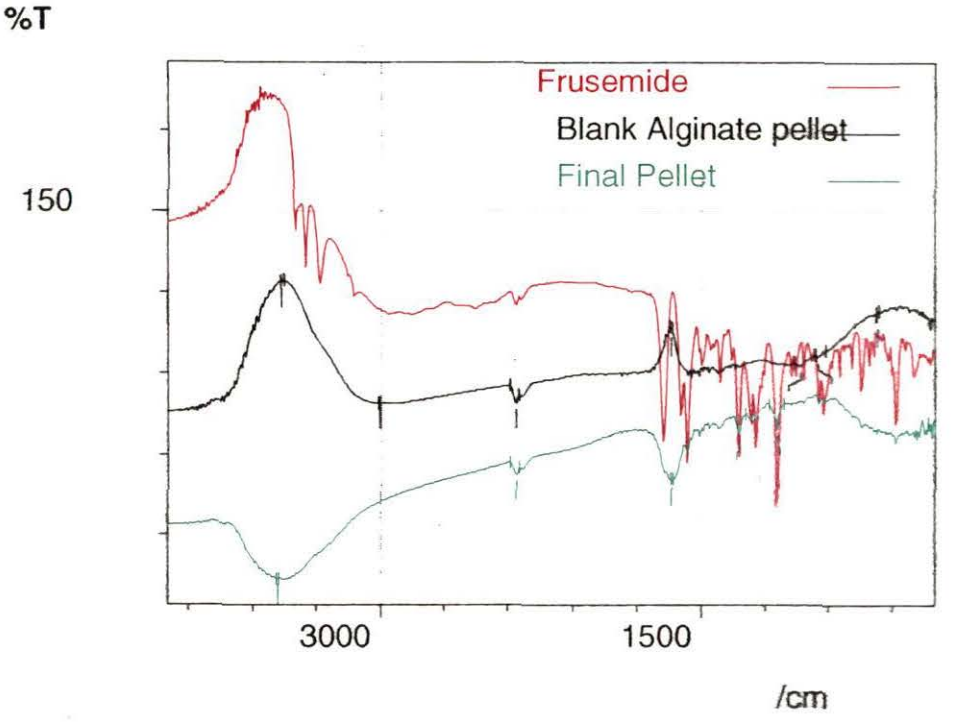

FIGURE 8.6 IR Spectra overlapped (Frusemide + Blank pellets + Final pellets)

8.8.2 Study on Drug–Polymer Interaction using Ultraviolet-Visible (UV-VIS) Spectrophotometry[3]

UltraViolet–Visible (UV-VIS) spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds. Solutions of transition metal ions can be colored (i.e., absorb visible light) because d- electrons within the metal atoms can be excited from one electronic state to another. The color of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the color of a dilute solution of copper sulphate is a very light blue; adding ammonia intensifies the color and changes the wavelength of maximum absorption (λ_{max}). Organic compounds, especially those with a high degree of conjugation, also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water soluble compounds, or ethanol for organic compounds. Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy. Ethanol absorbs very weakly at most wavelengths. While charge transfer complexes also give rise to colors, the colors are often too intense to be used for quantitative measurement. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the solution's concentration. Thus UV-VIS spectroscopy can be used to determine the concentration of a solution. It is necessary to know how quickly the absorbance changes with concentration. This can be taken from references (tables of molar extinction coefficients), or more accurately, determined from a calibration curve. A UV-VIS spectrophotometer may be used as a detector for HPLC. The presence of an analyte gives a response which can be assumed to be proportional to the concentration. For accurate results, the instrument's response to the analyte in the unknown should be compared with the response to a standard; this is very similar to the use of calibration curves. The response (e.g., peak height) for a particular concentration is known as the response factor.

An **ultraviolet-visible spectrum** is essentially a graph of light absorbance versus wavelength in a range of ultraviolet or visible regions. Such a spectrum can often be produced directly by a more sophisticated spectrophotometer, or the data can be collected one wavelength at a time by simpler instruments. Wavelength is often represented by the symbol λ. Similarly, for a given substance, a standard graph of the extinction coefficient (ε) vs. wavelength (λ) may be made or used if one is already available. Such a standard graph would be effectively "concentration-corrected" and thus independent of concentration. For

the given substance, the wavelength at which maximum absorption in the spectrum occurs is called λ_{max}, pronounced "Lambda-max".

Materials:

1. Pure drug – Supplied gift sample of Frusemide.
2. Micropellets containing calcium alginate along with the drug Frusemide.
3. Micropellets containing both calcium alginate and Acrycoat E30D along with the drug Frusemide.
3. USP Phosphate Buffer pH 6.8.

Methodology:

As discussed earlier an organic moiety shall have a fixed value of λ_{max} for a given set of conditions. This principle was employed to detect any drug – polymer interaction which may shift the λ_{max} value of the drug moiety significantly. A negligible shift shall be inferred as no chemical interaction among the drug and the polymer has taken place. On this basis, solutions at a concentration of 4 mcg/ml of pure drug sample, micropellets containing calcium alginate along with the drug Frusemide and micropellets containing both calcium alginate and Acrycoat E30D along with the drug Frusemide, were separately been prepared. All the three samples were scanned spectrophotometrically in a range between 200 to 400 nm, using SHIMADZU UV-VIS PharmSpec 1700 spectrophotometer, to determine the major wavelengths or peaks showing highest absorbance. Comparison was done among the result obtained for all the two formulations with that of the pure drug sample.

Results:

The three major peaks of the pure drug sample (Figure 8.7.1), namely, 331nm, 277.5 nm and 229.5 (Table 8.7) were not been shifted in the formulations (Figure 8.7.2 and 8.7.3), ensuring the fact that the polymers had no chemical interactions with the drug and can be said to be physico-chemically compatible with the latter.

Table 8.7 The Major Wavelengths Showing Highest Absorbance

SAMPLE	PEAK 1 (nm)	PEAK 2 (nm)	PEAK 3 (nm)
Pure Frusemide	331	277.5	229.5
Formulation (Frusemide + Calcium alginate)	330	277.5	229
Formulation (Frusemide + Calcium alginate + Acrycoat E30D)	330	277.5	230

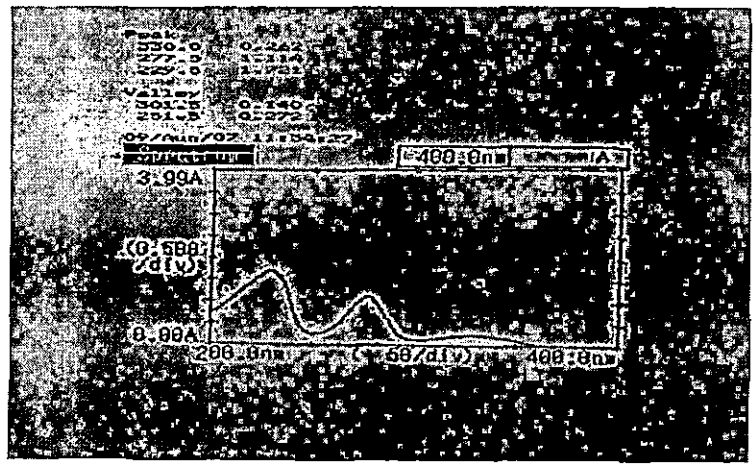

Figure 8.7.1 UV Scan report of pure Frusemide

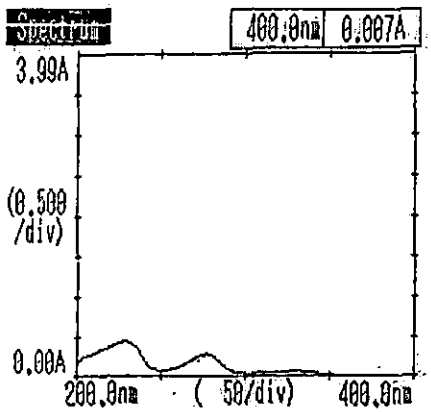

Figure 8.7.2 UV Scan report of A

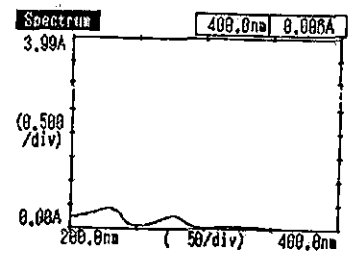

Figure 8.7.3 UV Scan Report of B

A -- Micropellets containing Frusemide + Calcium alginate

B -- Micropellets containing Frusemide + Calcium alginate + Acrycoat E30D

8.8.3 Study on Drug – Polymer Interaction using High performance Liquid Chromatography (HPLC) [3,7]

High performance liquid chromatography (HPLC), also known as high pressure liquid chromatography, is essentially a form of column chromatography in which the stationary phase consists of small particle (3-50 μm) packing contained in a column with a small bore (2-5 mm), one end of which is attached to a source of pressurized liquid eluant (mobile phase). The three forms of high performance liquid chromatography most often used are ion-exchange, partition and adsorption.

Chromatographic retention times are characteristic of the compounds but are not unique. Absolute retention times of a compound may vary from one chromatogram to the next. Comparisons are normally made in terms of relative retention, α, which is calculated from the expression: $\alpha = (t_2 - t_a / t_1 - t_a)$ where t_2 and t_1 are the retention times measured from the point of injection of the substance being examined and the reference substance respectively, determined under identical conditions on the same column, t_a is the retention time of the non-retained substance. Linear separations on the chromatogram are normally substituted for the corresponding retention volumes or times in the above expression. Where the value of t_a is small, the relative retention time, R_r, may be estimated from the expression: $R_r = t_2 / t_1$

The primary objective of the study was to identify any potential interactions or incompatibility among the drug and the polymers used in the formulation. The principle behind this identification is by comparing the retention time of both the standard drug sample and the drug extracted from the micropellets containing all the polymers. The value of $R_r \sim 1$ signifies nil or insignificant interaction.

Materials:

1. Pure drug – Supplied gift sample of Frusemide.
2. Micropellets formulation containing both calcium alginate and Acrycoat E30D along with the drug Frusemide.
3. Acetonitrile – HPLC Grade
4. Ammonium acetate – 180mM – AR Grade
5. Water - HPLC Grade

Experimental Protocol:

1. Mobile Phase – Acetonitrile + Ammonium acetate 180mM (1:1 mixture) (pH 5.4)

2. Standard sample – Pure Frusemide in Acetonitrile at a concentration of 5 mcg/ml.

3. Test sample – Micropellets equivalent to 25mg of Frusemide dissolved in Acetonitrile, sonicated for 10 minutes and diluted with the mobile phase to a concentration of 5 mcg/ml.

4. Instrument – HPLC –SHIMADZU LC-20AT / SPD-20A, JAPAN.

5. Software - SPHINCHROME™

6. Column and Length - C – 18 ; 4.6 mm.

7. Volume of Injection – 10 µl

8. Injection Flow rate - 2 ml per minute.

9. Detector – UV at a wavelength of 277.5 nm.

10. Temperature – 26 ° C

Results:

Table 8.8 Retention Time and corresponding Height and Area of the Chromatogram

SAMPLE	RETENTION TIME (min)	AREA (mV.s)	HEIGHT (mV)	AREA (%)	HEIGHT (%)
STANDARD	3.637	140.561	22.828	100	100
TEST	3.583	268.465	43.677	100	100

Chromatograms as represented in Figure 8.8.1 and 8.8.2 , and from the Table 8.8, the retention time of both Standard and Test sample was found to be very close and the value of $R_r \sim 1$, signifying no detectable interaction among the drug, Frusemide and the polymers, Calcium alginate and Acrycoat E30D was present in the formulations. This further augments the results obtained from FTIR study in **Section 8.8.1.**

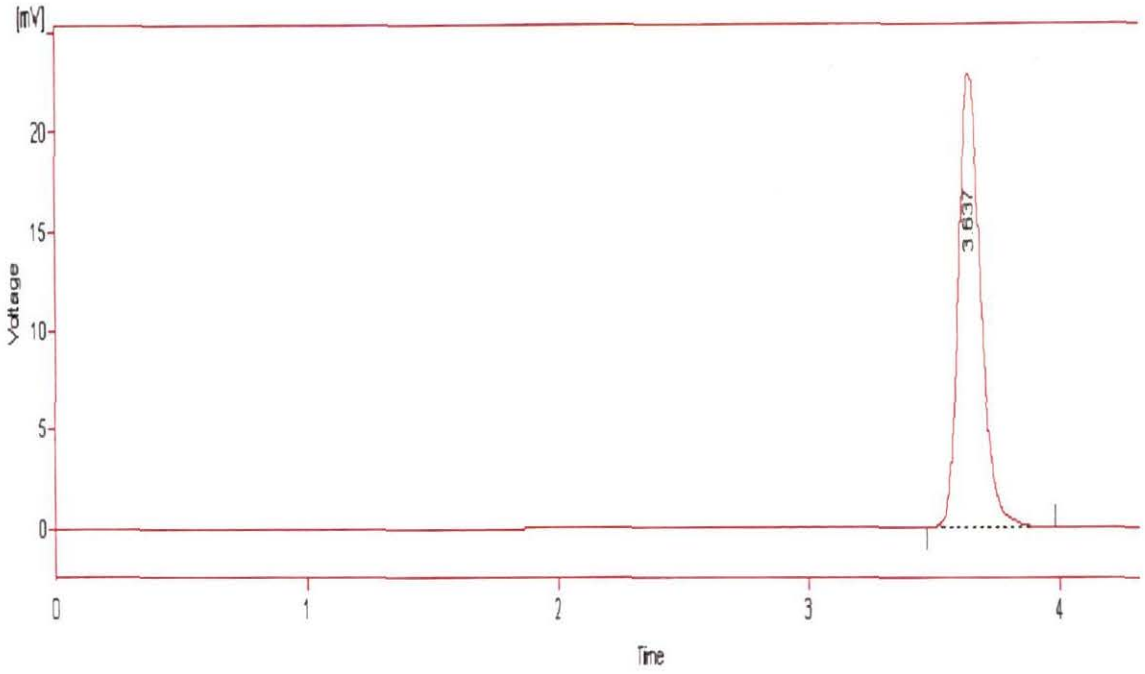

Figure 8.8.1: Chromatogram of the Standard Sample of Pure Frusemide

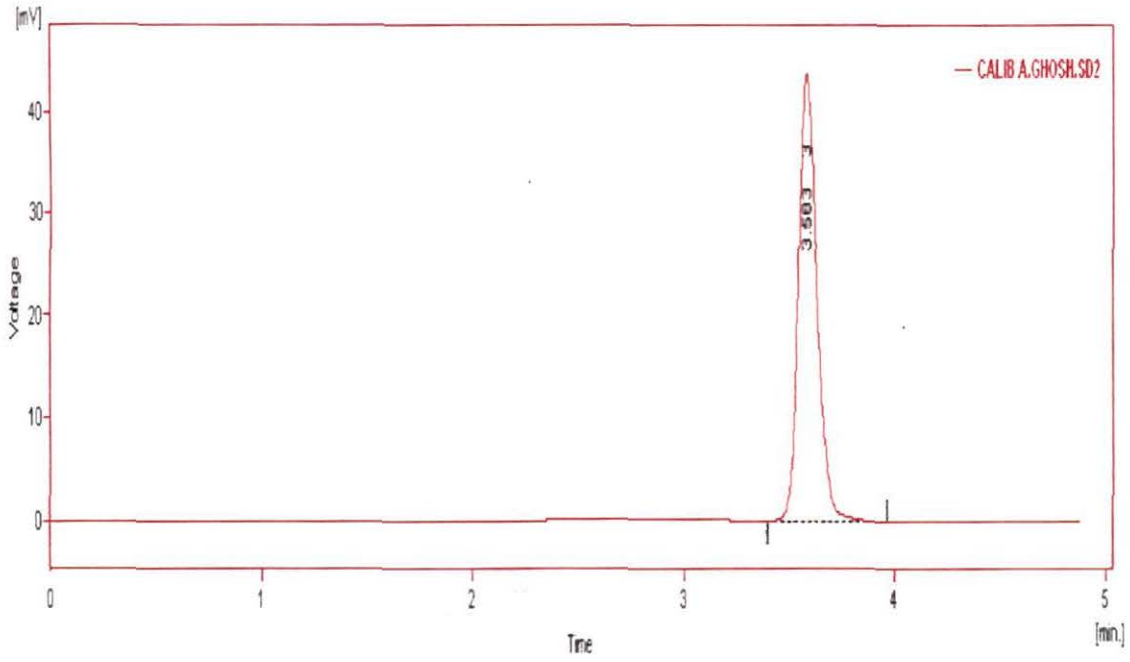

Figure 8.8.2: Chromatogram of the Test Sample containing Frusemide and Polymers

8.9 Determination of stability of the micropellets

Stability study was performed in order to assess the physicochemical stability of the formulations on storage. To achieve this, all the final nine formulations (F1-F9) were stored at 4°C and 45% Relative Humidity (RH) in refrigerator, Room Temperature (RT) and 60% RH and 45°C and 70% RH in a hot air oven, for four weeks. Every week samples were withdrawn and were assayed spectrophotometrically to measure the drug content at 277.5 nm using Phosphate buffer (pH 7.4) as blank. The percentage of residual drug assayed with respect to actual drug content is calculated and shown in (Table- 8.9).

TABLE- 8.9 Stability Studies of Frusemide Micropellets

PERCENT RESIDUAL DRUG									
TIME	**F1**			**F2**			**F3**		
(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	97.03	97.03	88.39	97.16	97.48	89.07	97.28	97.52	88.21
2	96.31	95.24	86.54	96.79	95.61	86.18	96.84	95.93	86.42
3	94.65	93.06	85.33	94.82	93.66	87.76	94.62	93.89	86.74
4	92.12	92.22	79.94	92.39	92.56	80.31	93.09	93.32	80.53
TIME	**F4**			**F5**			**F6**		
(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	98.33	97.84	88.37	98.61	98.02	87.47	98.94	98.09	88.22
2	97.41	95.89	86.44	97.53	96.16	87.18	97.45	96.13	87.42
3	94.88	94.36	86.39	95.27	94.43	85.84	95.59	94.89	86.04
4	93.52	93.72	80.86	93.67	93.91	80.94	94.44	94.37	82.36
TIME	**F7**			**F8**			**F9**		
(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	98.96	98.23	88.92	99.11	98.38	88.47	99.28	98.52	89.28
2	97.38	96.29	87.61	97.52	96.54	88.18	97.57	96.93	88.42
3	95.85	95.12	86.13	96.25	95.62	86.37	96.62	95.89	86.74
4	94.52	94.42	84.04	94.89	94.96	84.71	95.09	95.37	85.33

RT--- ROOM TEMPERATURE

Results:

From the stability studies data presented in Table 8.9, it is revealed that at lower temperatures, the micropellets remained significantly stable with a maximum loss of 10%.

At the elevated temperature the pellets showed some instability. From the results of the study it can be inferred that with the increase in net polymer concentration there is a decrease in drug degradation. Overall, the ionotropic gelation process can be reported to be successful in making stable calcium alginate micropellets containing frusemide.

8.10 Morphological study of the micropellets using Scanning Electron Microscopy (SEM)

The main objective of this study was to identify the surface topography of the micropellets along with the presence of any pores and micro channels on their surface. This study supports the data obtained from *in vitro* evaluation of the prepared micropellets and helps to describe the release behavior of the formulations.

Materials :

1. Calcium alginate (Blank pellets without drug)
2. Micropellets containing Frusemide and Sodium alginate only
3. Micropellets containing Frusemide, Sodium alginate and Acrycoat E30D

Methodology :

Morphological characterization of the micropellets was done by taking scanning electron micrograph in JEOL (Japan), JSM Model 5200 Scanning Electron Microscope. The samples were, initially, coated to 200A° thickness with gold-palladium using Pelco Model 3 sputter coater, prior to microscopy. This gold-palladium coating was done to reduce the charging effect and to impart electron conductivity property to the surface of the micropellets. 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. Cross sectional view of the desired formulations were obtained by cutting the micropellets with a razor blade. Photographs were taken within a range of 50 - 500 magnifications which are presented in Fig 8.9.1 to 8.9.25.

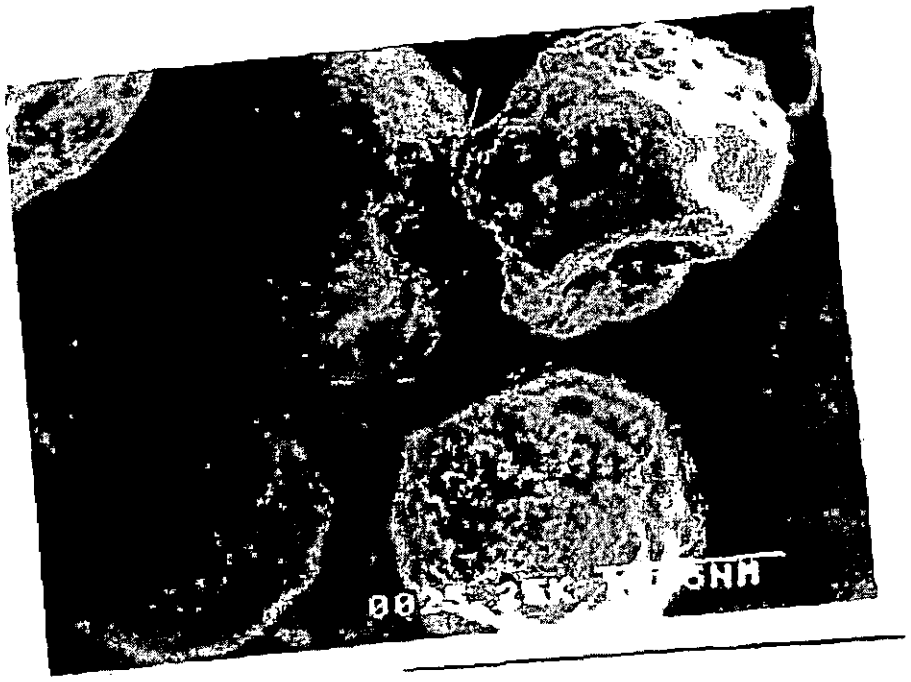

Figure 8.9.1- SEM Photograph of Frusemide micropellets (50X)

Formulation # F1

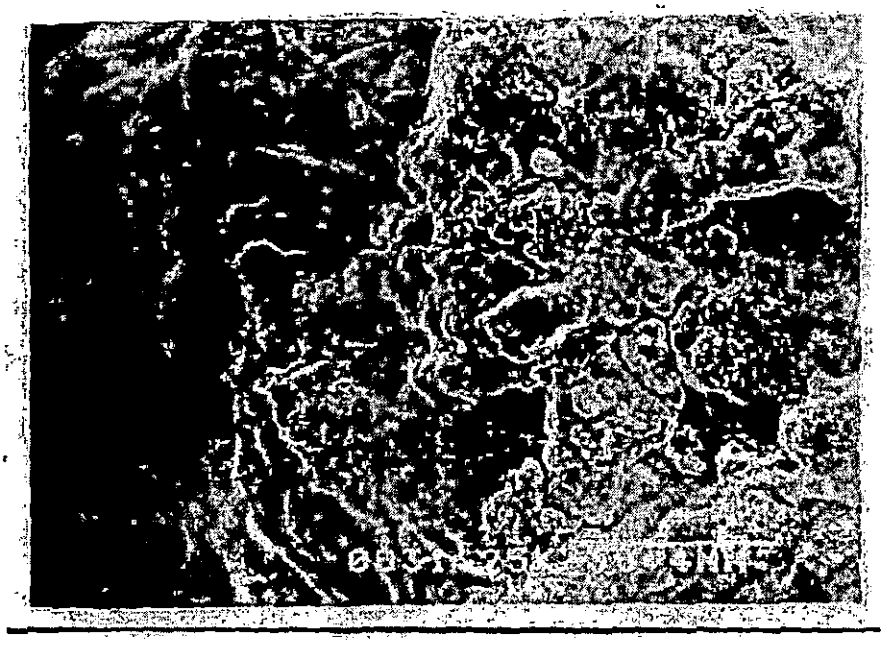

Figure 8.9.2- SEM Photograph of dissected Frusemide micropellets (350X)

Formulation # F1

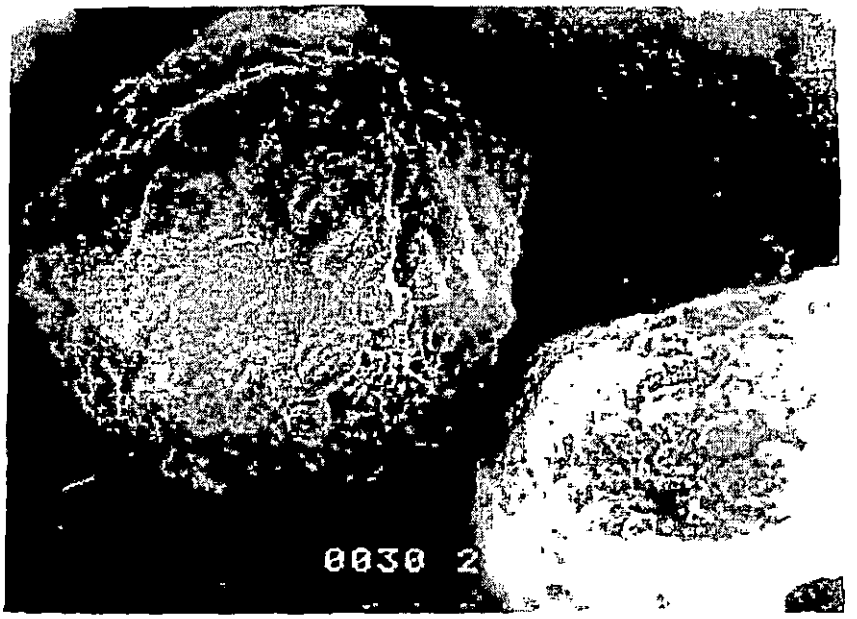

Figure 8.9.3 - SEM Photograph of Frusemide micropellets (50X)
Formulation # F2

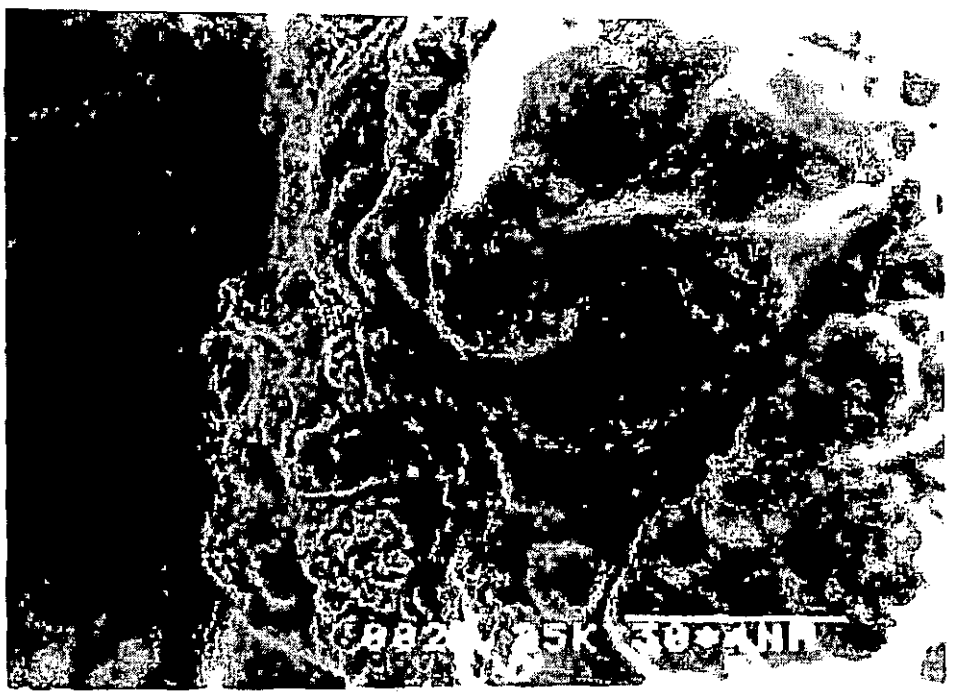

Figure 8.9.4 - SEM Photograph of dissected Frusemide micropellets (350X)
Formulation # F2

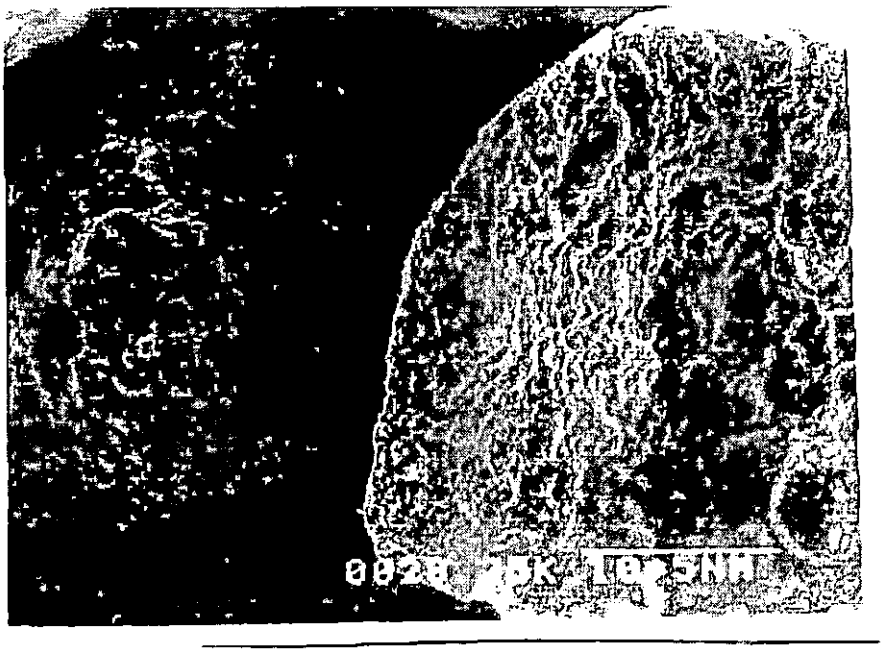

Figure 8.9.5 - SEM Photograph of Frusemide micropellets (50X)

Formulation # F3

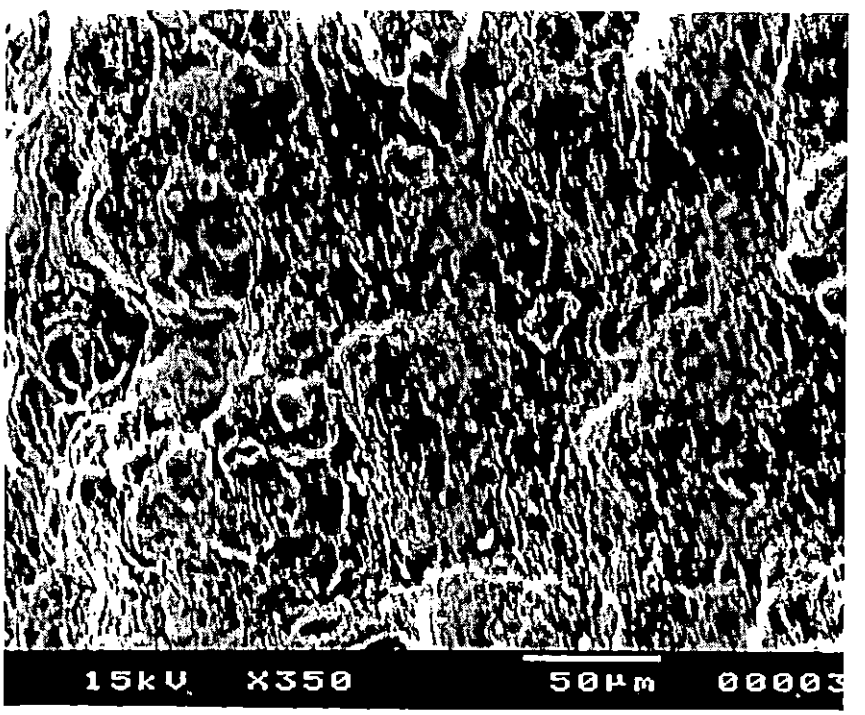

Figure 8.9.6 - SEM Photograph of dissected Frusemide micropellets (350X)

Formulation # F3

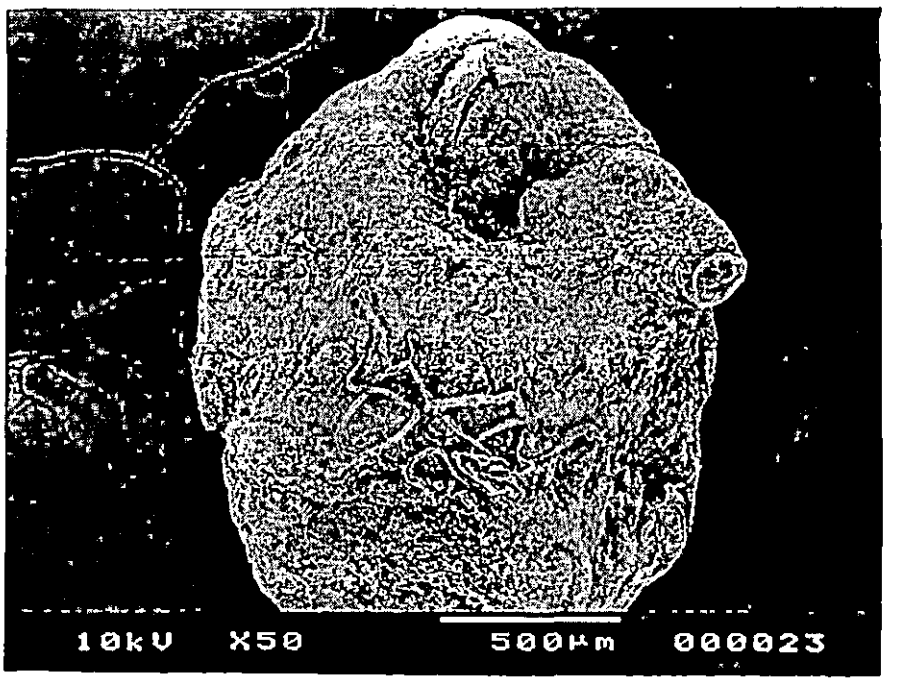

Figure 8.9.7 - SEM Photograph of Frusemide micropellets (50X)

Formulation # F4

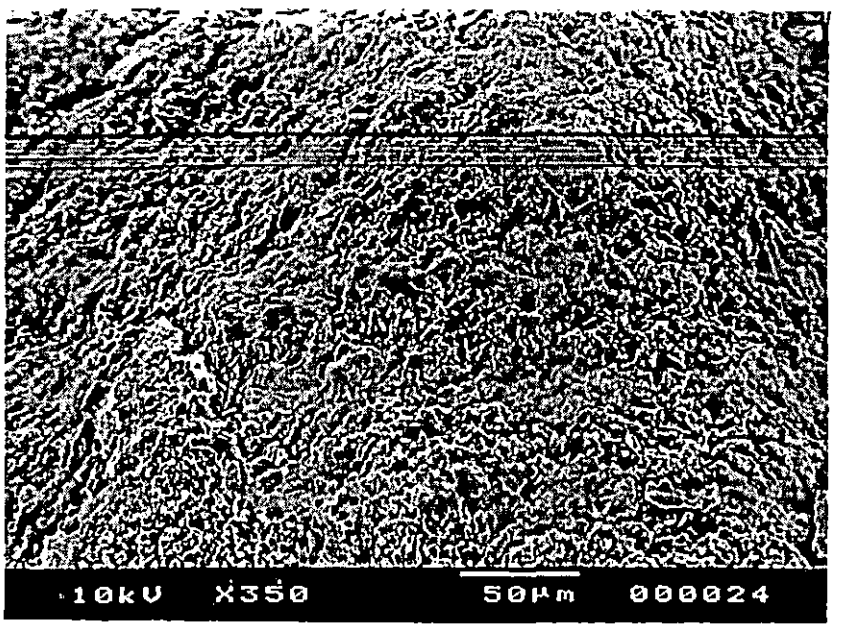

Figure 8.9.8 - SEM Photograph of Frusemide micropellets (350X)

Formulation # F4

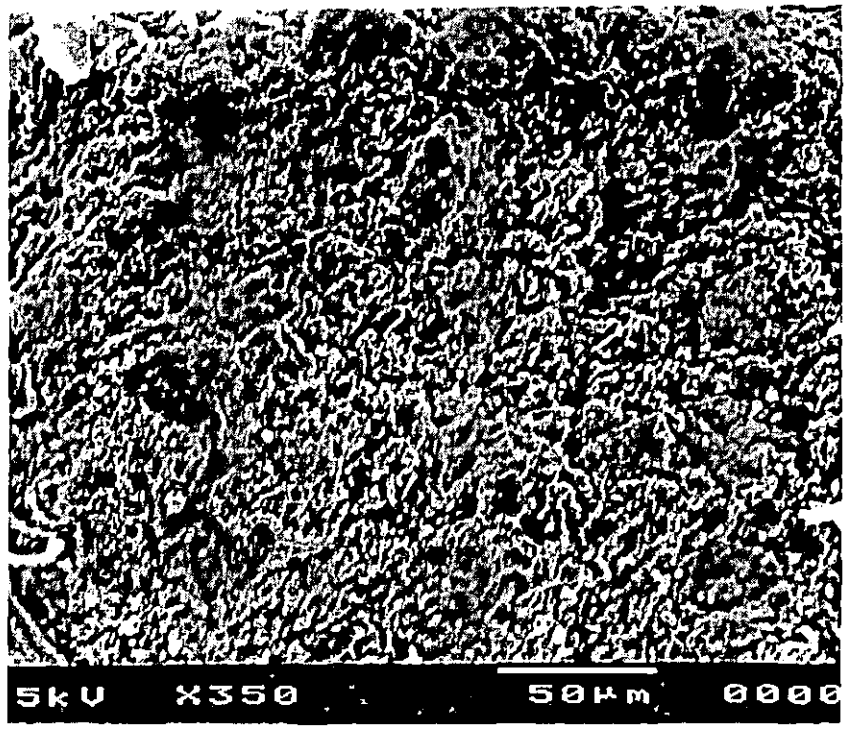

Figure 8.9.9 - SEM Photograph of dissected Frusemide micropellets (350X) Formulation # F4

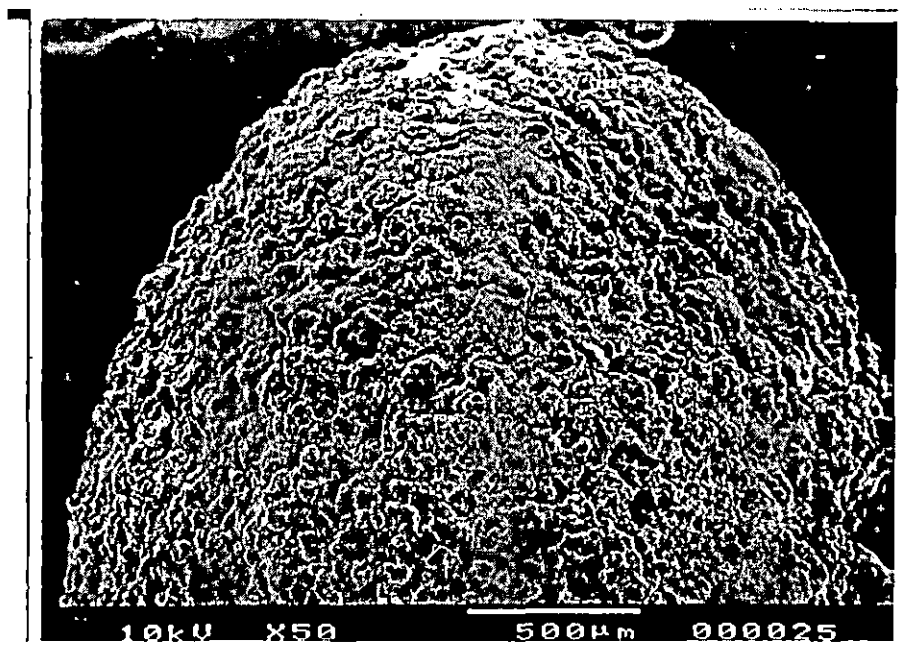

Figure 8.9.10 - SEM Photograph of Frusemide micropellets (50X) Formulation # F5

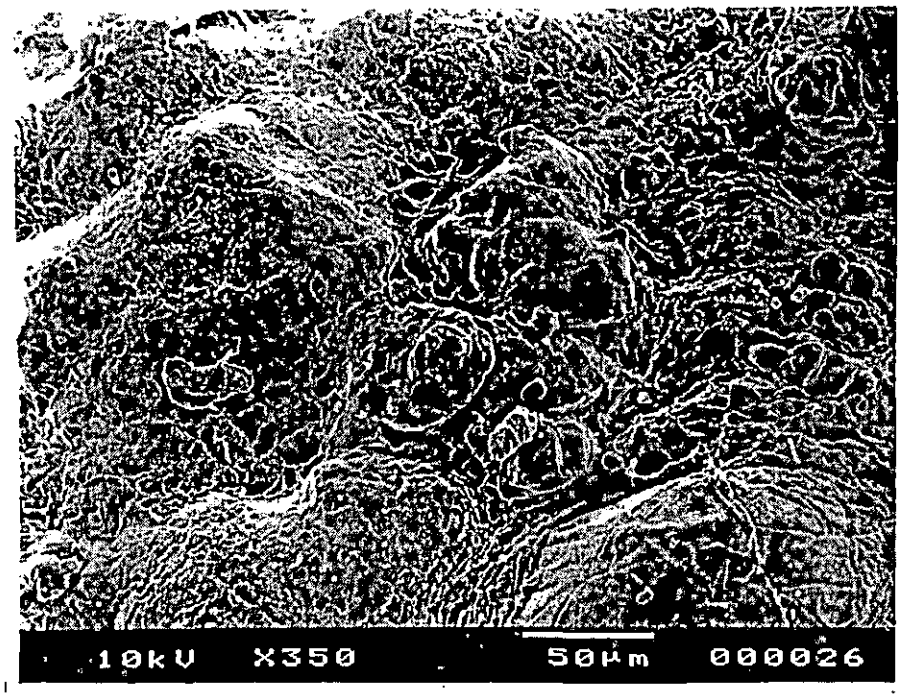

Figure 8.9.11 - SEM Photograph of Frusemide micropellets (350X)
Formulation # F5

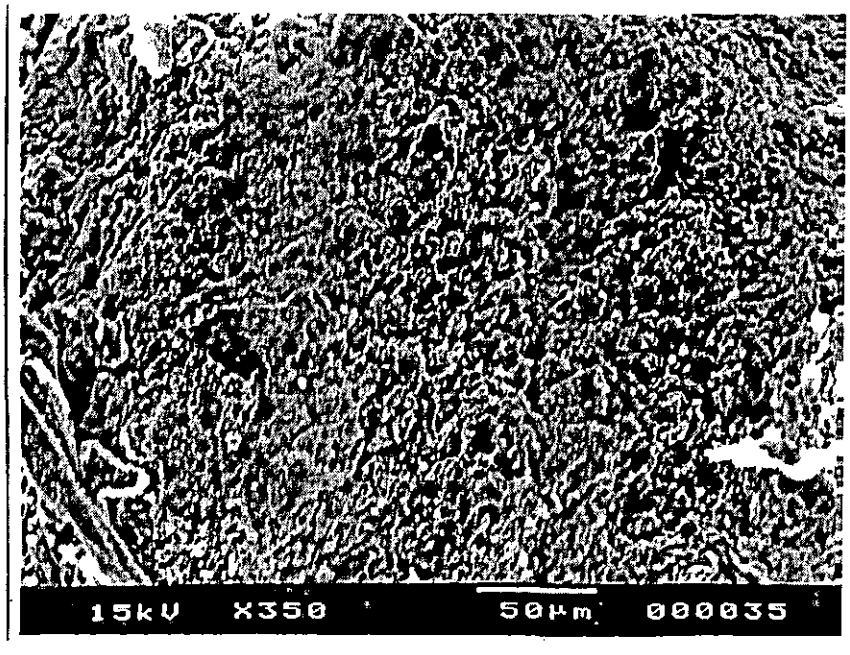

Figure 8.9.12 - SEM Photograph of dissected Frusemide micropellets (350X)
Formulation # F5

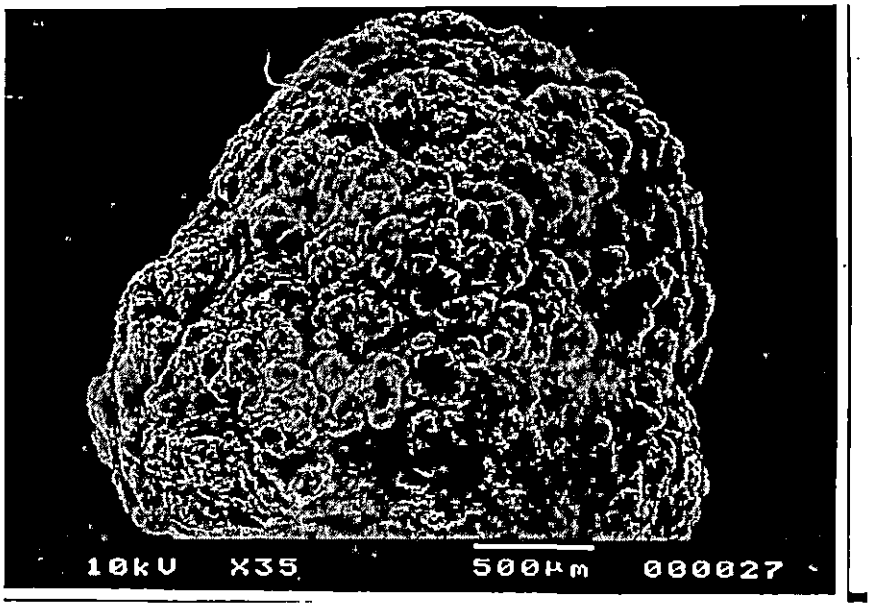

Figure 8.9.13 - SEM Photograph of Frusemide micropellets (35X)
Formulation # F6

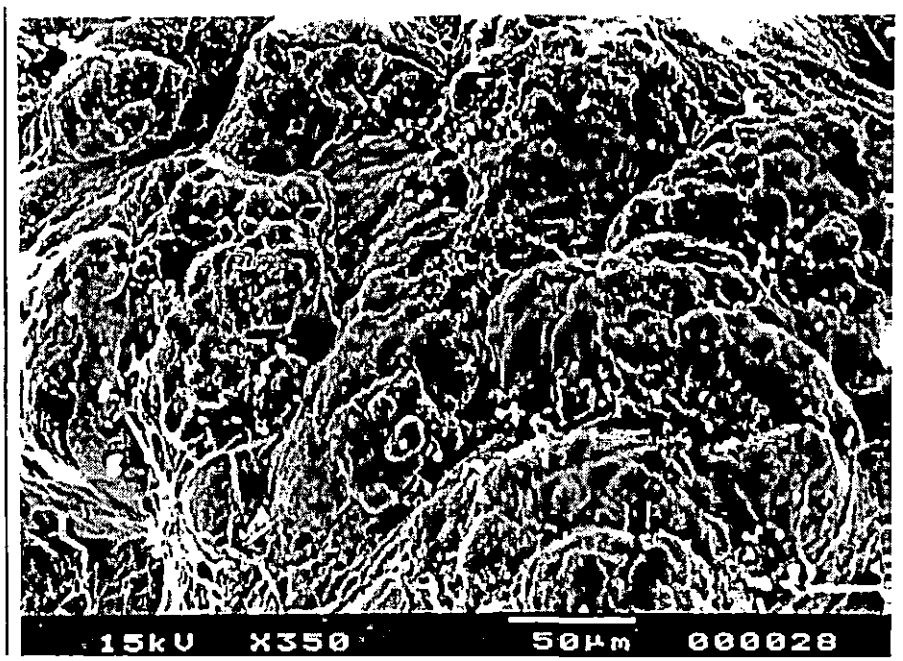

Figure 8.9.14 - SEM Photograph of Frusemide micropellets (350X)
Formulation # F6

Figure 8.9.15 - SEM Photograph of dissected Frusemide micropellets (500X) Formulation # F6

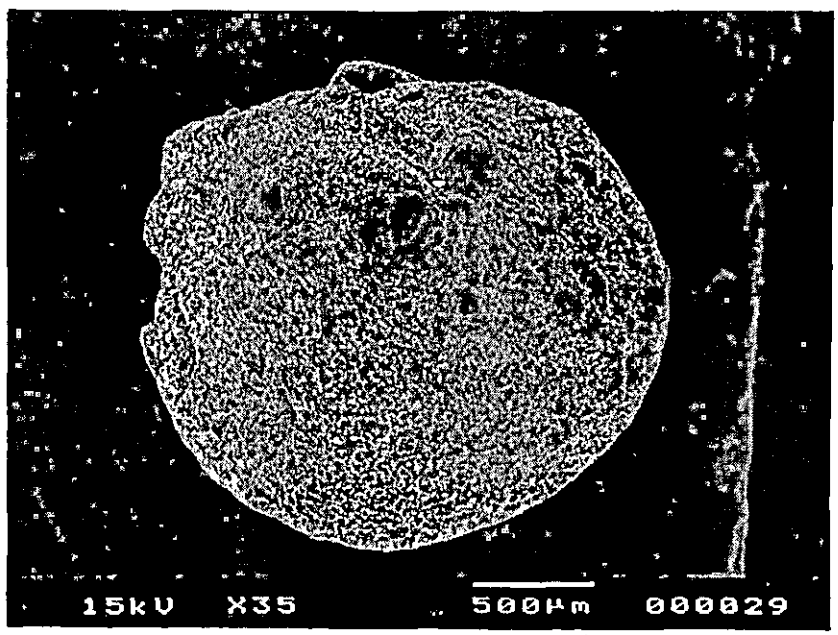

Figure 8.9.16 - SEM Photograph of Frusemide micropellets (35X) Formulation # F7

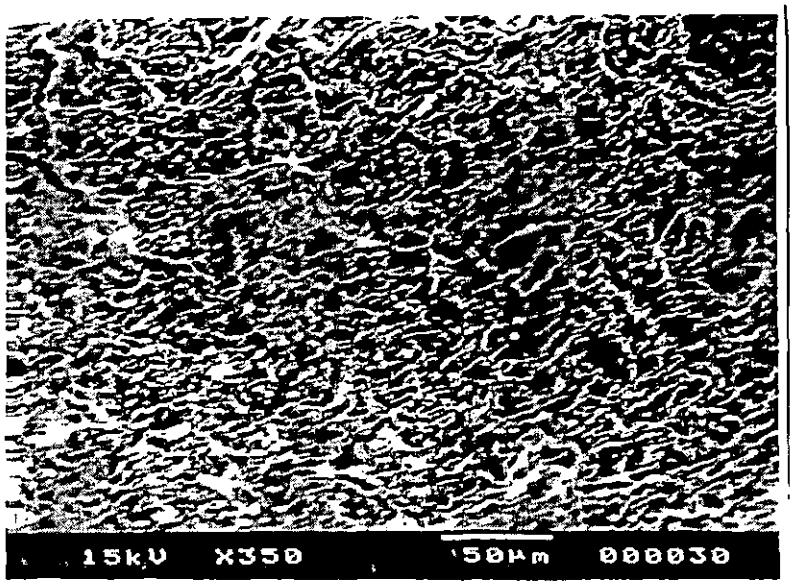

Figure 8.9.17 - SEM Photograph of Frusemide micropellets (350X)

Formulation # F7

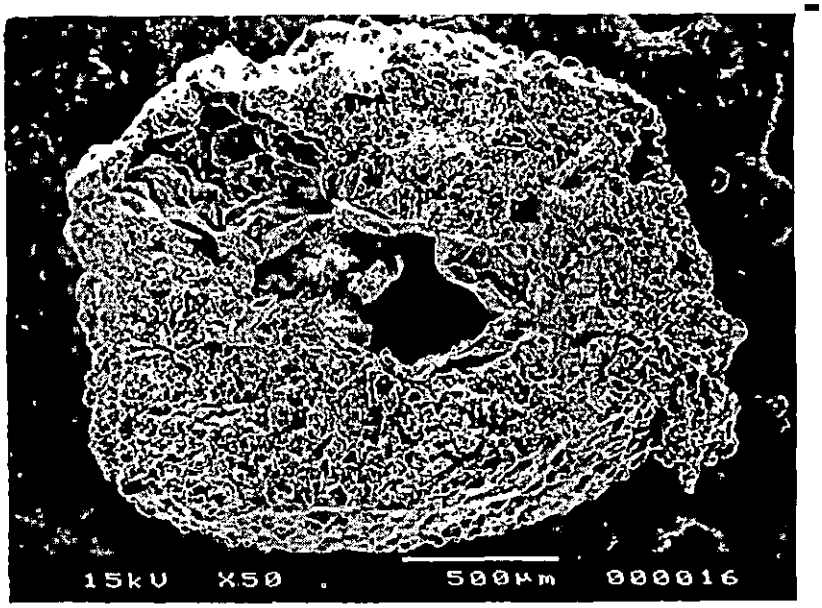

Figure 8.9.18 - SEM Photograph of dissected Frusemide micropellets (50X)
Formulation # F7

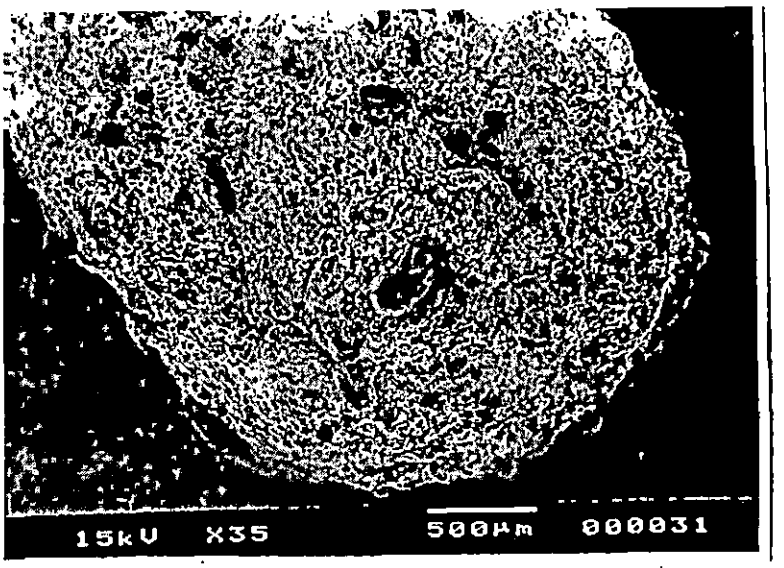

Figure 8.9.19 - SEM Photograph of Frusemide micropellets (35X)
Formulation # F8

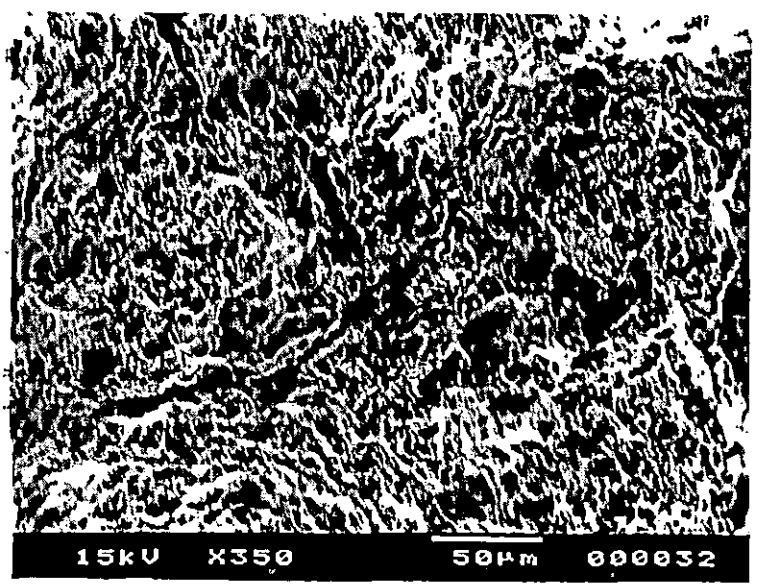

Figure 8.9.20 - SEM Photograph of Frusemide micropellets (350X)
Formulation # F8

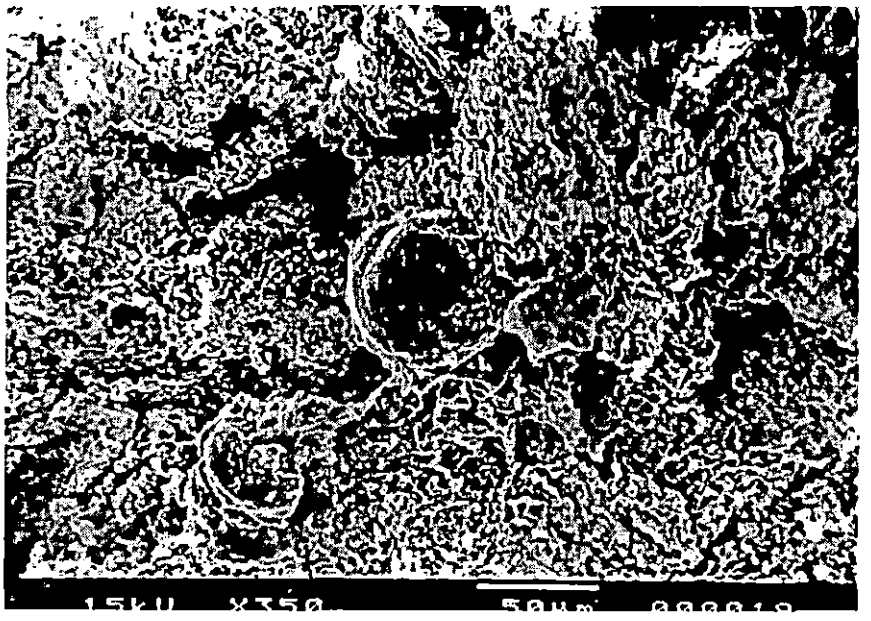

Figure 8.9.21 - SEM Photograph of dissected Frusemide micropellets (350X) Formulation # F8

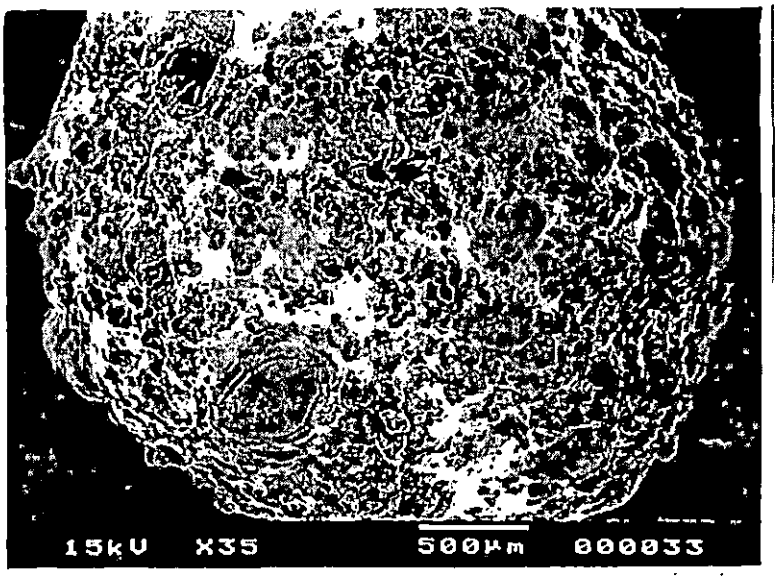

Figure 8.9.22 - SEM Photograph of Frusemide micropellets (35X) Formulation # F9

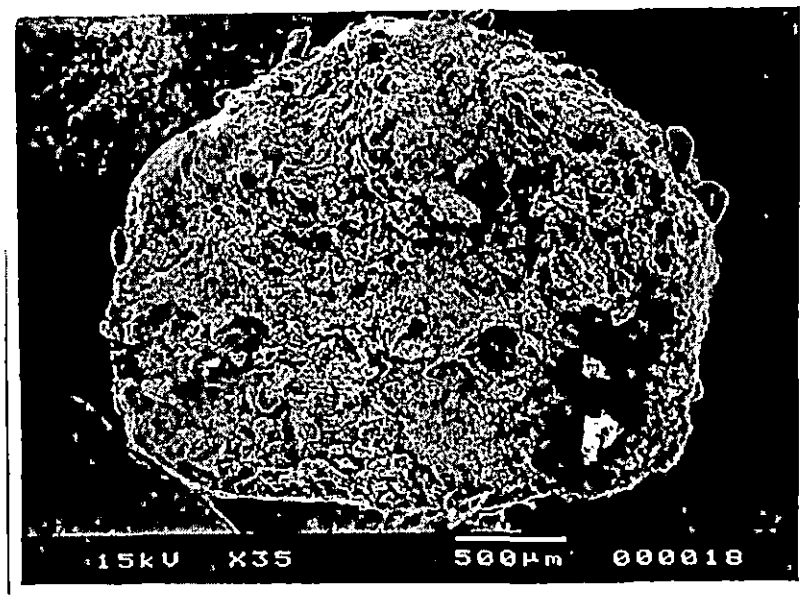

Figure 8.9.23 - SEM Photograph of dissected Frusemide micropellets (35X) Formulation # F9

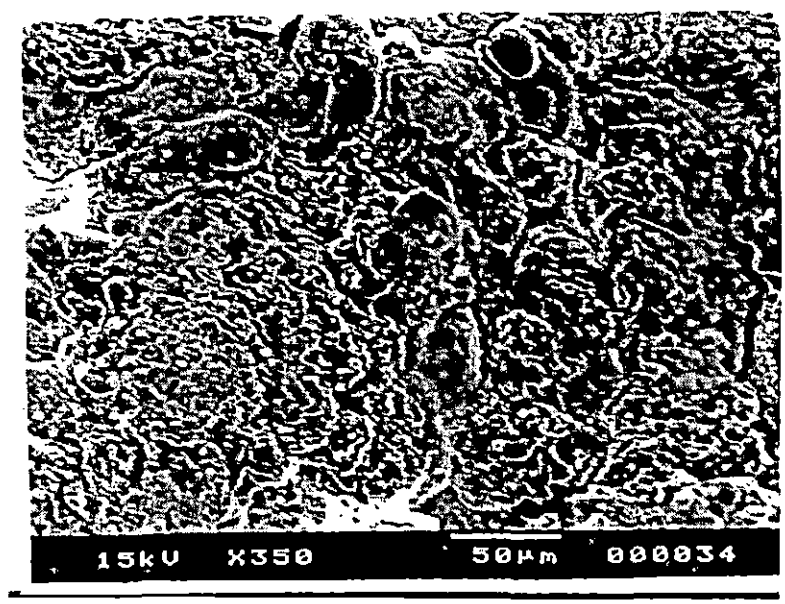

Figure 8.9.24 - SEM Photograph of Frusemide micropellets (350X) Formulation # F9

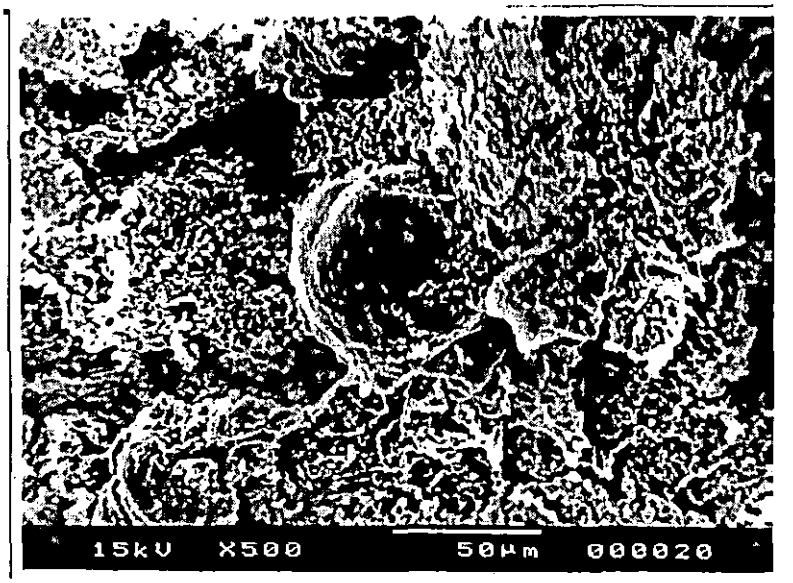

Figure 8.9.25 - SEM Photograph of dissected Frusemide micropellets (500X) Formulation # F9

Results:

The scanning electron micrograph of the final sets of micropellets presented in figure 8.9.1 to 8.9.25 showed that all the formulation produced rounded, rough pellets which cannot be defined to be purely spherical. With the increase in polymer weight the pellets took more sphericity as evident from the formulation F3 (Fig. 8.9.5 – 8.9.6), F6 (Fig. 8.9.13 – 8.9.15) and F9 (Fig. 8.9.22 – 8.9.25) containing highest level of polymer variations. When studied at higher magnification (350 X and 500 X) and when the pellets were centrally dissected and the cross sectional views were captured, the pellets showed dense network of polymers entrapping the drug. The numerous channels and pores visible in the micrograph shows the pathway through which dissolution medium can permeate into the drug-polymer matrix, swells the polymers and widens the pore diameter which in turns helps the drug molecule to diffuse from the matrix into the dissolution medium. This fact explains the sustained release nature of the formulation. Presence of free drug on the surface of the pellets supports the logic behind the loose surface crystal study reported in **Section 8.6.**

8.11 REFERENCE

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