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Formulation and evaluation of Prednisolone retention enema as dispersible tablet and vehicle for the treatment of ulcerative colitis

Chidambaram Santhanamariammal[1,2,3], Thenrajan Raja Sekharan[1,*], Benedict Lydia[2], Pillappan Ramkumar[2]

[1]Department of Pharmaceutics, SankaralingamBhuvaneswari College of Pharmacy, Anaikuttam-626130, Sivakasi, Tamil Nadu, India
[2]Fourrts (India) Laboratories Pvt. Limited, Kelambakkam-603103, Kanchipuram District, Tamil Nadu, India
[3]Research Associate-I, Formulation Research and Development, Apex Laboratories Pvt. Limited, Chennai, Tamil Nadu, India.
*corresponding author e-mail address:***rajasekharant2k@gmail.com***

ABSTRACT

Ulcerative colitis is a chronic idiopathic inflammatory bowel disease that causes chronic inflammation and damage in the gastrointestinal (GI) tract that typically presents in the second or third decade of life with bloody diarrhoea and abdominal cramps. The objective of the present work was to formulate and evaluate of Prednisolone retention enema as a dispersible tablet and it is suspended in suitable vehicle for the treatment of ulcerative colitis. In the present work, an attempt has been made to formulate and evaluate Prednisolone retention enema as a dispersible tablet by using three different methods (direct compression (F1 to F3, wet granulation (F4 and F5) and slugging method (F6)). Prepared dispersible tablets were administered with the help of vehicle for the effective treatment of ulcerative colitis. Dispersible tablets were evaluated for various parameters. In vitro drug release and microbiological evaluation studies were performed to the best formulation in rectal suspension. Then the best formulation was subjected to carry out for stability studies at three different temperatures. Among the six formulations, F6 formulation showed better results. FT-IR study showed that there was no interaction between a drug and excipients. In vitro drug release and microbiological evaluation studies were performed for F6 formulation in rectal suspension. About 99.06 % of the drug was released at 60 min. and also it was found to be microbiologically stable. Results from the stability studies showed that F6 formulation alone and with rectal suspension was stable for a period of 90 days. It was concluded that F6 formulation in rectal suspension possesses a promising future for the treatment of ulcerative colitis.

Keywords: *Prednisolone, ulcerative colitis, dispersible tablet, vehicle solution and retention enema.*

1. INTRODUCTION

Ulcerative colitis (UC) was described in the year 1800 by Samuel Wilks. It is characterized by continuous colonic mucosal inflammation that extends proximally from the rectum. It is a chronic disease that typically presents in the second or third decade of life [1]. The etiology is unknown. Risk factors include a history of recent infection with *Salmonella* or *Campylobactor* [2]. UC is more prevalent than Crohn's disease. North America and Northern Europe have the highest incidence and prevalence rates of ulcerative colitis, with incidence varying from 9 to 20 cases per 1 lakh person-years and prevalence rates from 156 to 291 cases per 1 lakh people. Rates are lowest in the southern hemisphere and eastern countries [3]. UC may be insidious, with gradual onset of symptoms or the first attack may be acute and fulminate. There are 5 types of UC. They are Proctitis **(**Limited to rectum), Proctosigmoiditis (Involves rectum and sigmoid colon i.e. Lower segment), Distal colitis (Extends from rectum and entire left colon), Extensive colitis (Involves more than half the colon or the entire colon) and Pancolitis (Affects the entire large intestine).More mild symptoms include a progressive loosening of the stool, abdominal cramping and diarrhoea. As the disease progresses from mild to more severe, the patient may also experience weight loss, fatigue, loss of appetite that may result in nutrient deficiencies, mucus in the stool, severe rectal bleeding, fever and anemia [4]. Several categories of drugs may be effective in treating UC. The type of drugs taken will be depending on the severity of the disease condition. Sulfasalazine, Mesalamine, Balsalazide, Olsalazine, Prednisolone and Budesonide are drugs which are most commonly used to treat UC, which are available in the different dosage forms like tablets, capsules, suppositories and enema (in solution, emulsion and suspension form) [5-11]. Rectal administration is not often the first route of choice; but it becomes a good alternative when the oral route is inadvisable. Relatively low cost and lack of technical difficulties make rectal drug administration attractive when compared to parenteral therapy [12]. An enema is a method of administration and may involve solutions, suspensions, emulsions, foams and gels. Corticosteroids can be administered as retention enemas as adjunctive treatment of some patients with ulcerative colitis [13]. Powders/tablets intended for the preparation of rectal solutions or suspensions are single-dose preparations that are dissolved or dispersed in water or other suitable solvents at the time of administration [14]. Dispersible tablets are uncoated or film-coated tablets that can be dispersed in liquid before administration giving a homogenous dispersion [15-17]. The objective of this study was (i) to formulate dispersible tablet and vehicle as enema formulation, (ii) to select excipients and find any incompatibility between the drug and excipients, (iii) to formulate Prednisolone dispersible tablet and vehicle, (iv) to perform the precompression evaluation and tablets evaluation parameters, (v) to select the best formulation of dispersible tablet, (vi) to prepare rectal suspension using best

formulation of dispersible tablet, (vii) to perform *in-vitro* drug release study for rectal suspension, (viii) to conduct Microbiology studies for the rectal suspension, (ix) to perform stability studies for the selected formulation of dispersible tablet.

2. EXPERIMENTAL SECTION

Materials and methods.

Prednisolone was obtained as a gift sample from Fourrts India Laboratory, Chennai. Riboflavin-5-sodium phosphate was obtained from Supriya Life Science Mumbai. Microcrystalline cellulose (MCC) PH101 and PH 112 was obtained from Wei Ming pharmaceuticals, Taiwan. Crospovidone was procured from Nanhang industrial& co., China. Maize starch was obtained from RidhiSidhi Pharmaceutical Pvt Ltd, New Delhi. Colloidal silicon dioxide was obtained from Wacker Silicones, Mumbai. Lactose (DCL-21) was purchased from Cabot sanmar Ltd, Chennai. Lactose monohydrate DMV Fonterra excipients, New Zealand. Magnesium stearate was procured from Amishi Drugs & Chemical, Gujarat. Purified talc was obtained from Gangotri Inorganic (P) Ltd, Gujarat. Sodium chloride was procured from Avantor Performance materials India ltd, Haryana. Methyl hydroxybenzoate was obtained from Rasula Pharmaceutical, Hyderabad. Propyl hydroxybenzoate was procured from Alta Laboratories, Mumbai. Sodium carboxymethyl cellulose obtained from Jalan cellulose Co Ltd., India. Propylene glycol was purchased from Manali Petrochemicals Ltd., Chennai. Purified water was purchased from Andavar&Co, Chennai. All the above excipients and other chemicals used in these formulations are of analytical grade.

Methods.

Formulation development. Three methods were used for the formulation of Prednisolone dispersible tablets. Tablets were prepared by using direct compression in F1 to F3 formulations, wet granulation is used in F4 and F5 formulation and slugging method is used in formulation F6. The details of the excipients used in the formulations were mentioned in Table 1.

Table 1. Formulation of Prednisolone Dispersible Tablets.

Ingredients (mg)	Quantity of ingredients (mg/tab)					
	Direct compression			Wet granulation		Slugging method
	F1	F2	F3	F4	F5	F6
Prednisolone	20	20	20	20	20	20
Riboflavin -5 sodium phosphate	3	3	3	3	3	3
MCC (PH112)	40	40	45	-	-	40
MCC(PH102)	-	-	-	40	40	-
Crospovidone	15	15	20	15	35	15
Maize starch	4	4	7	28	28	4
Maize starch Paste	-	-	-	2	2	-
Colloidal anhydrous silica	6	8	12	2	2	6
Lactose (DCL 21)	36	36	37	-	-	36
Lactose Monohydrate	-	-	-	34	34	-
Methyl hydroxybenzoate	-	-	-	0.4	0.4	-
Propyl hydroxybenzoate	-	-	-	0.1	0.1	-
Magnesium stearate	4	4	4	4	4	4
Purified talc	2	2	2	1.5	1.5	2
Total weight (mg)	**130**	**132**	**150**	**150**	**170**	**130**

Direct Compression Method. The specified quantity of prednisolone and other all excipients were accurately weighed. Weighed Prednisolone and MCC (PH112) were sifted using 30 # mesh. Riboflavin-5-sodium phosphate, crospovidone, maize starch, and lactose (DCL21) were sifted through 40 # mesh. Colloidal anhydrous silica was passed through 60 # mesh sieve. The sifted powders were mixed in a polythene bag for ten minutes. The above sifted granules were lubricated using magnesium stearate and purified talc, which was sifted through 60 # mesh size, mixed for 5 minutes in a polythene bag. Then final lubricated blend is compressed at an average weight of 130 mg (F1), 132 mg (F2) and 150 mg (F3) using punch size 7.1 mm (F1 and F2) and 8 mm (F3).

Wet Granulation Method. The specified quantity of Prednisolone and all other excipients were accurately weighed. Individually Prednisolone and maize starch were sifted using 60 # mesh. Riboflavin -5- sodium phosphate, crospovidone, MCC (PH 102) and lactose monohydrate were sifted through 40 # mesh. Colloidal anhydrous silica was passed through 60 # mesh sieve. The sifted powders were mixed in a polythene bag for ten minutes. The binder solution of starch mucilage paste was prepared by maize starch in purified water having methyl hydroxybenzoate and propyl hydroxybenzoate under stirring condition. The prepared mucilage was added slowly to the dry mixed powder and granulated using the kneading method. The wet mass was dried at a temperature of 60°C until the LOD (loss on drying) of granules is reached less than 1%. The dried granules were sifted through 20 # mesh. The above dried granules were lubricated using magnesium stearate and talc was sifted through 60 # mesh size, mixed for 5 minutes in a polythene bag. Then final lubricated blend was compressed at an average weight of 150 mg (F4) and 170 mg (F5) using the punch size of 8 mm.

Slugging Method. The specified quantity of Prednisolone and other all excipients were accurately weighed. Individually Prednisolone and MCC (PH112) were sifted using 30 # mesh. Riboflavin -5- sodium phosphate, crospovidone, maize starch, and

lactose (DCL21) were sifted through 40 # mesh. Colloidal anhydrous silica was passed through 60 # mesh sieve. The sifted powders were mixed in a polythene bag for ten minutes. The above sifted granules were lubricated using Magnesium stearate and purified talc which is sifted through 60 # mesh size, mixed for 5 minutes in a polythene bag. Then final lubricated blend was compressed into tablets using the large punch size of 20 mm. The compressed tablets were crushed into granules using mortar and pestle. Those granules were passed through initially by 16 # mesh then by 20 # mesh and finally passed through 30 # sieve mesh to got uniformed granules. The collected granules were again compressed into a tablet at an average weight of 130 mg (F6) using the punch size of 7.1 mm.

Preparation of Vehicle for Prednisolone Dispersible Tablet. Accurately weighed sodium chloride was dissolved in purified water. Separately sodium carboxymethyl cellulose was dissolved in propylene glycol then poured into the sodium chloride mixture. Methyl hydroxybenzoate and propyl hydroxybenzoate were dissolved in hot purified water and added into the above mixture. Finally, the volume of the solution was made up to 100 ml with purified water. The quantities of vehicle solution were given in Table 2.

Evaluation of Precompression Parameters.

Angle of repose. Accurately weighed prednisolone was taken in a beaker. It was allowed to flow through the funnel freely on the surface of the paper to form a cone shaped pile. The diameter of the cone (d) and the height (h) of the pile was noted. From the diameter, radius (r) was calculated. The angle of repose (θ) was calculated using the following formula [18].

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

Where,

θ = Angle of repose

H = Height of the cone

R = Radius of the cone

Bulk density (D_b). Weighed quantity of prednisolone was transferred into a 50 ml measuring cylinder without tapping during transfer the volume occupied by the granules was measured. Bulk density (D_b) was measured by using formula [19].

$$D_b = m/Vo$$

Where,

D_b= bulk density

M= mass of the blend

Vo= untapped volume

Tapped density (D_t). Weighed quantity of Prednisolone was taken into a 50 ml measuring cylinder. Then cylinder was subjected to 50 taps in tapped density tester (Electro lab USP II). According to the U.S.P, the blend was subjected for 50 taps; the percentage volume variation was calculated by using the following formula [19].

$$D_t = m/Vt$$

Where,

D_t= tapped density

M= mass of the blend

Vt = Tapped volume

Compressibility index. The compressibility index of the Prednisolone was determined by the Carr's compressibility index with the help of bulk density and tapped density value using the following formula [20].

$$\text{Compressibility index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio. Hausner's ratio of the Prednisolone was determined by the ratio of tapped density and bulk density using the following formula [20].

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Moisture content. Approximately weighed 1 gm of prednisolone and placed into a plate on the moisture balance. The temperature was set to be 60^0C. Then the moisture content present in the Prednisolone was measured in percentage [21].

Evaluation of Prednisolone Dispersible Tablets.

General appearance. The tablets should be free from cracks, depression, pinholes etc. the colour and polish of the tablets should be uniform on whole surface. The tablets were examined externally under a biconvex lens for surface, cracks, depressions, pinholes, colour, polish, etc.

Hardness test or crushing strength. Hardness of the tablet is defined as the force required in breaking a tablet in a diametric compression test. In this test, a tablet was laced between two anvils, force was applied to the anvils and the crushing strength that just causes the tablet to break is recorded. Hence hardness is sometimes referred to as "Crushing Strength". It was measured using Monsanto tablet hardness tester. The values were expressed in kg/cm^2.

Thickness. Thickness mainly depends up on die filling, physical properties of material to be compressed under compression force. The thickness of the tablets was measured by using Digital Vernier callipers. The thickness was denoted in millimeter.

Weight variation test. Weigh 20 tablets individually weighed and the average weight was calculated. Not more than two of the individual weights deviate from the average weight by not more than the ±7.5% percentage deviation. The percentage deviation of the tablets was calculated by using the following formula,

$$\text{Percentage deviation} = \frac{\text{weight of tablet (mg)} - \text{Average weight of tablets}}{\text{Average weight of tablets}} \times 100$$

Friability. The friability of tablets was determined by using Roche friabilator. Twenty tablets were weighed and placed in friabilator and rotated at 25 rpm for 4 minutes. Then the tablets were taken out, de-dusted and reweighed. The percentage friability of the tablets was calculated by the formula.

$$\text{Percentage Friability}(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Disintegration time. The test was carried out on six tablet using 900 ml distilled water at 37°C±2°C was used as disintegration media and the time in seconds taken for complete disintegration of the tablets with no palpable mass remaining in the apparatus was

measured in seconds. Also, measure the time of disintegration in vehicle solution.

Wetting time. A piece of tissue paper (10.75×12 mm) folded twice was placed in a culture dish (d=6.5 cm) containing 6 ml of water. A tablet was placed on the paper and the time for complete wetting was noted.

Water absorption ratio. A piece of tissue paper folded twice was placed in a small petri dish containing 6 ml of water. A tablet was placed on the tissue paper and allowed to completely wet. The wetted tablet was then weighed. The water absorption ratio (R) was calculated by using the following formula.

$$R = \frac{W2 - W1}{W1} \times 100$$

Where,

R = Water absorption ratio

W2 = Weight after wetting

W1 = Weight before wetting

***In-vitro* dispersion time.** Tablet was added to 10 ml of distilled water at 37±0.5°C. The time required for complete dispersion of a tablet was measured.

Uniformity of dispersion. The fineness of dispersion test was done by two tablets in 100 ml of water and stir gently until completely dispersed. A smooth dispersion was obtained which passes through a sieve screen with a nominal mesh aperture of 710 mm (sieve no. 22) [22-30].

Drug content estimation (By UV). The absorbance of 10 µg/ ml of both the standard and sample preparations were measured at 246 nm using diluents (methanol: purified water, 30:70) as blank. The content of Prednisolone present per tablet was calculated by using the following expression [31,32].

$$Drug\ content = \frac{absorbance\ of sample}{absorbance\ of\ standard} \times \frac{weight\ of\ standard}{100} \times \frac{1}{100} \times \frac{5}{10} \times \frac{100}{weight\ of\ sample} \times \frac{100}{1} \times \frac{10}{5} \times \frac{purity\ ot\ standard}{average\ weight} \times 100$$

$$Assay = \frac{drug\ content}{label\ claim} \times 100$$

IR spectral analysis. Compatibility studies were assured by FT-IR studies. The pure drug sample and the complete formula of formulation were chosen for the study. The FT-IR spectra's of the above samples were studied after a period of 30 days from the preparation of the mixtures, to facilitate prompt detection of incompatibility. The spectra's were obtained by preparing potassium bromide (KBr) pellets under dry condition by using pellet press. The spectra of the crude drug sample and that of the drug-excipients mixtures were compared to check the incompatibility problems, if any.

Preparation of rectal suspension. One Prednisolone dispersible tablet from F6 formulation was placed into a container containing 100 ml of vehicle solution. Shake well for 2-3 minutes, a yellow colour suspension was obtained.

Evaluation of rectal suspension.

Determination of pH value. The pH determination was carried out by the electrode was immersed in the solution in pH meter [33].

Determination of viscosity. The viscosity of the solution was measured by using Spindle 61, 50 RPM in Brook field DV- E viscometer [33].

***In-vitro* release study.** The release of Prednisolone from suspension was studied in 900 ml of phosphate buffer pH 7.4 as dissolution medium in a U.S.P type II dissolution (paddle) apparatus at 50 rpm and 37±0.5°C temperatures. 10 ml of suspension was placed in a bowl. 5 ml of sample was withdrawn at every 10 minutes interval up to one hour; the solution was filtered through membrane filter and make up to 10 ml with pH 7.4 phosphate buffer. The sample was analysed at 246 nm in UV double beam spectrophotometer. pH 7.6 phosphate buffer as a blank. The percentage drug release was calculated by using the following formula [34,35].

$$\%\ \text{of drug release} = \frac{\text{sample absorbance} \times \text{weight of std} \times \text{dilution factor} \times \text{purity of std} \times 100}{\text{std absorbance} \times \text{weight of sample} \times \text{label claim of drug}}$$

Microbiological Evaluation. The formulated suspensions were evaluated for microbial limit test. The following tests were performed based on the I.P. procedure. They are Total aerobic microbial count, Total combined yeast and mold count and Test for specified microorganisms such as *Escherichia coli, Salmonella typhi, Staphylococcus aureus*and *Pseudomonas aeruginosa* [36].

Accelerated Stability Studies. Stability studies were aimed at determining the result of aging and storage under various conditions on the formulated dispersible tablet. It was carried out to evaluate the stability of F6 formulations after storing at different temperatures for a period of 3 months. The prepared tablets were kept at three different temperatures such as 4±2°C, 28±2°C and 45±2°C for 3 months. Every month the tablets were evaluated for all the physical parameters. The *in-vitro* drug release studies were determined by UV double beam spectrophotometer [37,38].

Table 2. Formulation of Vehicle for Suspending Prednisolone Dispersible Tablet.

Ingredients	Quantity (mg/100 ml) for 1 tablet
Sodium chloride	90
Methyl hydroxybenzoate	230
Propyl hydroxybenzoate	34
Sodium carboxy methyl cellulose	100
Propylene glycol	5000
Purified water	To 100 ml

3. RESULTS SECTION

Evaluation of Precompression Parameters. The Prednisolone powders/granules were evaluated for different parameters and the results are given in Table 3.

Angle of repose of the powder blend was found to be between 32°27′ to 44°26′. Angle of repose for F1 to F3 formulations was within the limit of 41° to 45° and their flow properties were passable. F4 and F5 formulations 36° to 40° and

their flow properties were fair. In F6 formulation, the angle of repose is within the limit of 30° to 35°. This indicates the granules having good flow property.

Bulk density for F1 to F3 formulations was found to be between 0.332 and 0.396 (gm/ml), F4 and F5 formulations between 0.398 and 0.411 (gm/ml) and F6 formulation 0.557 (gm/ml). The values were found to be high for Slugging method than direct compression method and wet granulation method. This may be due to an increase in void space observed in granules.

Tapped density was found to be between 0.436 and 0.511 (gm/ml) for F1 to F3 formulations, 0.512 and 0.526 (gm/ml) for F4 and F5 formulations and 0.647 (gm/ml) for F6 formulations. The values of the direct compression method and wet granulation method were found to be decreased when compared to slugging method since the granules size were larger than the powder blends. So it does not settle down easily like powders in case of direct compression method.

Compressibility index was found to be in the range of 21.70 to 23.85 % for F1 to F3 formulations, 22.20 and 21.86 % for F4 and F5 formulations, 13.91% for F6 formulation. From the observed values the flow type is passable in direct compression and wet granulation method and good for slugging method.

Hausner's ratio was found to be between 1.27 to 1.31 for F1 to F3 formulations, 1.28 and 1.27 for F4 and F5 formulations and 1.16 for F6 formulation. From the observed values the flow type is passable for direct compression method and fair for wet granulation method. In slugging method, the flow property was good.

Moisture content for the F1 to F6 formulations was found to be between 0.7 to 1.0 % the moisture content of all formulations were within the limit (1%).

Table 3. Evaluation of Formulated Prednisolone Powders/Granules.

Formulation No.	Angle of repose (degree)	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr's Index (%)	Hausner's ratio	Moisture content (%)
F1	$40^{o}75'$	0.332	0.436	23.85	1.31	0.89
F2	$41^{o}15'$	0.391	0.511	23.48	1.30	0.80
F3	$44^{o}26'$	0.396	0.506	21.70	1.27	0.86
F4	$37^{o}12'$	0.398	0.512	22.20	1.21	0.85
F5	$36^{o}24'$	0.411	0.526	21.86	1.22	0.76
F6	$32^{o}27'$	0.557	0.647	13.91	1.16	0.70

Table 4. Evaluation of Prednisolone tablets.

Parameters	F1	F2	F3	F4	F5	F6
Hardness [a] (kg/cm^2)	2.95±0.37	3.40±0.46	2.55±0.69	3.80±0.42	4.35±0.41	2.30±0.42
Thickness [a] (mm)	2.93±0.03	2.82±0.04	3.42±0.02	3.01±0.05	3.62±0.06	2.97±0.01
Weight variation [b] (mg)	131.5±8.40	133 ±9.50	151±12.50	150±1.09	170±1.05	130±0.50
Friability [a*] (%)	0.23±0.03	0.24±0.05	0.27±0.04	0.26±0.06	0.25±0.07	0.11±0.01
Disintegration time [c*] (sec.)	53.80±2.59	67.00±2.45	72.20±2.86	180.60±1.95	187.40±1.95	51.00±2.07
Wetting time* (sec.)	50.0±1.00	57.6±1.14	55.2±1.48	61.0±1.00	63.0±1.58	46.20±0.84
Water absorption ratio* (%)	54.70±0.25	49.93±0.77	50.77±0.77	51.04±0.06	50.10±0.04	52.99±0.08
***In-vitro* dispersion time* (min)**	2.06±0.03	2.15±0.19	2.33±0.43	2.49±0.37	2.55±0.06	1.32±0.01
Uniformity of dispersion	Pass	Pass	Pass	Pass	Pass	Pass
Drug content [d] (%)	98.50	99.20	99.43	99.86	99.95	99.99

Note: a = 10; b = 20; c = 6; d = 15

* Values are expressed as mean ± SD, n = 5

Evaluation of Prednisolone Dispersible Tablets. The Prednisolone dispersible tablets were evaluated for different parameters and the results are given in Table 4. F1 formulation had high weight variation due to the poor flow property of powder. So the quantity of glidant was increased in the F2 formulation. Then also the weight variation was high due to the poor flow property of powder. So the weight of the tablet was increased up to 150 mg by increasing the excipients in F3 formulation. This formulation also had high weight variation because the flow of powder was poor. In F4 formulation, wet granulation method was chosen to avoid the weight variation. In this method the flow property was found to be fair and the weight variation was within the limits. But the disintegration time and in-vitro dispersion time was found to be high. That's why the number of excipients was increased from 150 mg to 170 mg in F5 formulation. Here also the same problem occurs, disintegration time and in-vitro dispersion time was somewhat more. Then slugging method was preferred for F6 formulation. The average weight of one tablet was reduced to 130 mg. The granules obtained are of uniform in size. The flow property of the granules was found to be good.

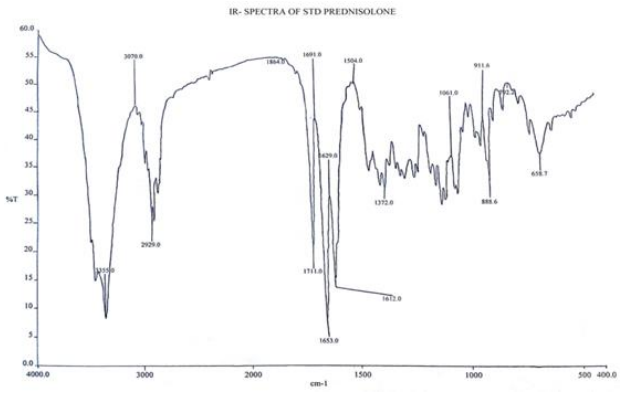

Figure 1.FT-IR Spectrum of Pure Prednisolone.

Disintegration time and in vitro dispersion time was reduced when compared to F5 formulation. Uniformity of dispersion of all the formulations was found to be passing. With the above data's F6 formulation was selected as a best

formulation. Because its passes all parameters. So this F6 formulation may suitable as a dispersible tablet formulation for rectal suspension formulation.

FT-IR Spectrum of Pure Prednisolone and F6 formulations were shown in Figure 1 and 2. From the IR spectral analysis, the result reveals that there was no interaction between the drug and excipients.

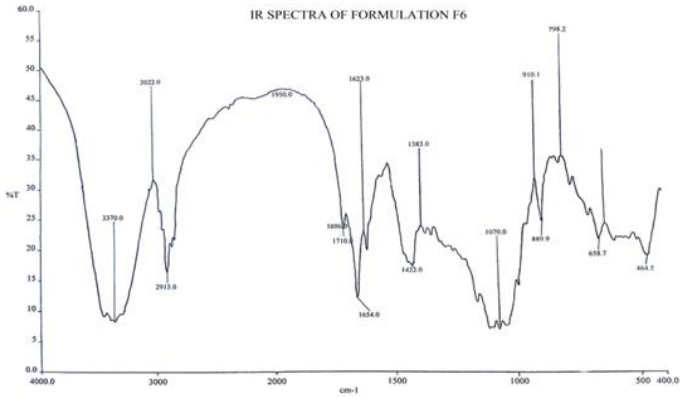

Figure 2.FT- IR Spectrum of F6 formulation.

The pH of F6 formulation in rectal suspension was found to be 6.2 and the viscosity was 55.9. The data was given in the Table 5.

The in vitro release study graph for the F6 formulation in rectal suspension was shown in the Figure 3. The percentage drug release of Prednisolone from F6 formulation in rectal suspension was 99.07 ± 0.02 % at 60 minutes.

Table 5. Evaluation of F6 formulation in Rectal Suspension**.**

Parameters	F6 in rectal suspension
pH	6.2
Viscosity (cps)	55.9

From the above data, the total aerobic viable count and total combined yeast count were observed that the cfu limits of formulated rectal suspension formulations were found to be within the limits. All the specified microorganisms were found to be absent. Thus the rectal suspension formulation was microbiologically stable.

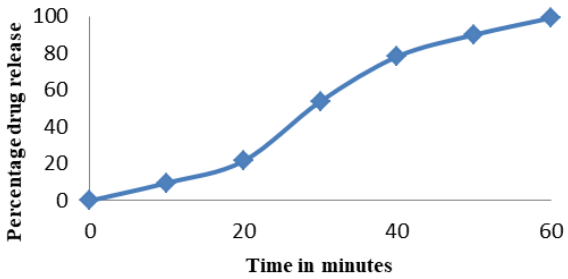

Figure 3. Graph for in-vitro release study of F6 formulation in rectal suspension.

Table 6. Microbial Limit test for F6 formulation in Rectal Suspension.

S. No.	Test/ specified microorganisms	Limits	Report
1	Total aerobic viable count	NMT 1000 cfu/ml	30 cfu/ml
2	Total combined yeast and mould count	NMT 100 cfu/ml	10 cfu/ml
3	*Escherichia coli*	Absent/ml	Absent
4	*Salmonella*	Absent/ml	Absent
5	*Pseudomonas aeruginosa*	Absent/ml	Absent
6	*Staphylococcus aureus*	Absent/ml	Absent

*cfu – Colony Forming Units

According to the procedure the stability study of Prednisolone dispersible tablet was carried out at storage condition for 4°±2°C, 28°±2°C and 45°±2°C with a period of three months. The results revel that there was no change in all physical parameters. The report for the microbial limit test for F6 formulation in rectal suspension against specific microorganisms were given in the Table 6.

The drug release of Prednisolone from the rectal suspension has no changes for a period of three months even storing at three different temperatures. The stability study data for F6 formulation, tablets evaluation studies were shown in Table 7 and its *in vitro* drug release studies were shown in Table 8

Table 7. Stability study for F6 formulation.

Parameters	Initial period	$4^0 \pm 2^0$ C			$28^0 \pm 2^0$ C			$45^0 \pm 2^0$ C		
		1st month	2nd month	3rd month	1st month	2nd month	3rd month	1st month	2nd month	3rd month
Colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Hardness[a] (kg/cm^2)	2.30 ± 0.42	2.30 ± 0.29	2.31 ± 0.09	2.30 ± 0.38	2.30 ± 0.31	2.32 ± 0.01	2.30 ± 0.45	2.31 ± 0.09	2.32 ± 0.09	2.30 ± 0.09
Thickness[a] (mm)	2.97 ± 0.01	2.97 ± 0.04	2.96 ± 0.05	2.97 ± 0.10	2.97 ± 0.03	2.96 ± 0.28	2.96 ± 0.36	2.97 ± 0.05	2.96 ± 0.08	2.97 ± 0.02
Weight variation[b] (mg)	130.00 ± 0.50	129.00 ± 0.40	130.00 ± 0.10	131.00 ± 0.05	130.00 ± 0.60	130.00 ± 0.18	130.00 ± 0.10	129.00 ± 0.50	130.00 ± 0.20	131.00 ± 0.10
Friability[a*] (%)	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.11	0.11 ± 0.15	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.04	0.12 ± 0.01	0.10 ± 0.01	0.11 ± 0.05
Disintegration time[c*] (sec)	51.00 ± 2.07	50.30 ± 1.20	49.80 ± 1.44	49.90 ± 1.12	50.10 ± 1.30	49.90 ± 2.04	50.10 ± 1.00	50.10 ±1.20	49.40 ± 3.44	49.90 ± 1.20
Wetting time[*] (sec.)	46.20 ± 0.84	46.30 ± 0.04	45.90 ± 0.13	46.10 ± 0.01	46.50 ± 0.04	44.80 ± 0.54	46.10 ± 0.61	46.50 ±0.14	45.80 ± 0.04	47.10 ± 0.01
Water absorption ratio[*] (%)	52.99 ± 0.08	51.99 ± 0.17	52.02 ± 0.05	52.05 ± 0.10	51.79 ± 0.07	51.54 ± 0.65	52.19 ± 0.10	51.99 ±0.07	52.54 ± 0.05	52.49 ± 0.10
***In-vitro* dispersion time[*] (min.)**	1.32 ± 0.01	1.31 ± 0.05	1.31 ± 0.03	1.31 ± 0.18	1.32 ± 0.10	1.32 ± 0.08	1.31 ± 0.23	1.32 ± 0.05	1.32 ± 0.03	1.31 ± 0.28
Drug content[d] (%)	99.99	99.98	99.98	99.98	99.99	99.98	99.98	99.98	99.97	99.96

Note: a = 10; b = 20; c = 6; d = 15

*Values are expressed as mean ± SD, n = 5

Table 8. Stability study for *in-vitro* drug release of Prednisolone from F6 formulation in Rectal Suspension.

S. No.	Time in minutes	Initial period	$4^0 \pm 2^0$ C			$28^0 \pm 2^0$ C			$45^0 \pm 2^0$ C		
			1st month	2nd month	3rd month	1st month	2nd month	3rd month	1st month	2nd month	3rd month
1	10	9.63	9.62	9.61	9.59	9.64	9.62	9.60	9.65	9.60	9.58
2	20	21.56	21.25	21.49	21.58	22.15	21.78	21.54	22.05	21.69	21.48

3	30	53.87	54.05	53.78	53.84	54.06	54.25	53.94	54.15	53.95	53.74
4	40	78.19	78.44	78.38	78.15	78.74	78.54	78.32	78.94	78.68	78.25
5	50	89.95	88.81	89.23	89.99	88.78	89.73	89.92	88.9	89.65	89.78
6	60	99.06	99.06	99.06	99.05	99.06	99.05	99.05	99.05	99.05	99.04

4. CONCLUSIONS

Formulation and evaluation of Prednisolone retention enema as a dispersible tablet and vehicle for the effective treatment of Ulcerative Colitis was successfully carried out. Preformulation studies, formulation of Prednisolone dispersible tablets, evaluation parameters, in-vitro release study, microbiology test and accelerated stability studies were performed. From the all above observations it was concluded that the formulation F6 by slugging method was better one compared to the other formulations. Thus it can be concluded that the Prednisolone retention enema as dispersible tablet and vehicle possesses promising future delivery of rectal formulation of drugs in suspension form for the effective treatment of ulcerative Colitis.

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6. ACKNOWLEDGEMENTS

Fourrts (India) Laboratories Pvt. Limited, Kelambakkam-603103, Kanchipuram District, Tamil Nadu, India.Department of Pharmaceutics, SankaralingamBhuvaneswari College of Pharmacy, Anaikuttam-626130, Sivakasi, Tamil Nadu, India.