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Formulation and In-vitro Evaluation of Mesalamine Rectal Suppositories for Ulcerative Colitis

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Conflicts of Interest
There are no conflicts to declare.

ABSTRACT

Mesalamine, also known as 5-aminosalicylic acid is an anti inflammatory drug used to treat inflammatory bowel disease like Ulcerative colitis and Crohn's disease. The objective of this study was to optimize the best formula for the rectal suppositories of mesalamine with different bases. Suppositories were prepared by fusion method using different bases (cocoa butter alone, cocoa butter: paraffin, cocoa butter: PEG 6000) in varying quantity keeping other excipients constants. Base optimization study was conducted before formulation of suppositories. All suppositories were evaluated for physical characteristics and various parameters like: displacement value, weight variation, melting point, liquefaction time, hardness test, drug content, and in-vitro release profile. All the developed formulations were within the required limits for BP 2014. However, formulation F3 which is composed of cocoa butter: PEG 6000 in the ratio of 1:1 showed better release of drug around 91.48%. Mesalamine rectal suppositories containing a new base combination of Cocoa butter with PEG 6000 have good physical properties and release profile. Since this formula is potential to be used as a better therapeutic candidate for ulcerative colitis.

Keywords: COCOA BUTTER, MESALAMINE, PARAFFIN, POLYETHYLENE GLYCOL, RECTAL SUPPOSITORIES, ULCERATIVE COLITIS.

Introduction

Suppositories are more convenient dosage form intended to deliver drugs through rectal and vaginal routes of administration. Suppositories come from the Latin word supponere, meaning 'substitute' [1]. These suppositories formed by the composition of the different excipients bases, such as cocoa butter, coconut oil, glycerinated gelatin, hydrogenated vegetable oils, hard fats, polyethylene glycols (PEGs) etc. Based on the mixture of these excipients bases

suppositories can be categories as hydrophilic based or lipophilic based. The lipophilic fat-based suppositories melt at body temperature and hence ideal for the rectal route where there is little available fluid in the lower large intestinal tract [2].

Inflammatory bowel disease (IBD) is considered as chronic, relapsing and nonspecific inflammatory disorder mainly affecting the mucosa and sub-mucosa of large and small bowel [3]. The major etiologies of IBD are genetic factors, heredity, stress, immunologic factors [4]. Common clinical manifestation of IBD is abdominal pain, diarrhea, mucosal ulceration and rectal bleeding [5]. IBD can be categorized into Crohn's disease and Ulcerative colitis having prevalence ranging from 0.1% to 0.15% in the residents of Western countries [6]. Ulcerative colitis can be termed as ulcerative proctitis when it affects rectum [7]. All of IBD patients approximately 30% of patients presented with ulcerative proctitis [8].

The major goal of drug therapy for treating IBD aims to alleviate the inflammatory process and currently employed drugs include 5-aminosalycylic acid (mesalamine), corticosteroids, immunosuppressants, and biological agents [9]. For achieving the optimal therapeutic goal, the drug delivery system should be able to present sufficient drug level into the proximal part of the colon. However, the current therapeutic strategies mostly lack such drug selectivity at targeted site [10]. Mesalamine is mostly used to treat mild to moderate cases of ulcerative colitis [7]. Oral administration of mesalamine subjected to presystemic biotransformation by enzyme present in enteric mucosa formed the inactive metabolite N-acetyl-5-aminosalycylic acid. This metabolite causes side effects such as degenerative action on the gastric mucosa [11]. Since local administration of mesalamine minimizes the upper intestinal absorption and reduces the adverse effects. Hence it will maximize drug delivery to the target site of colon and helps to enhance efficacy [12]. Previous studies reported that keeping high mucosal mesalamine concentrations in ulcerated colons improve the clinical outcome in ulcerative colitis patients [13].

In this aspect, the present study aimed to develop mesalamine formulation with different bases for the rectal route. Mesalamine suppositories would be effective to provide localized action for the ulcerative colitis. Formulation optimization can be achieved by changing the composition of bases.

Materials

An active pharmaceutical ingredient i.e. mesalamine was obtained from Asian Pharmaceuticals Pvt. Ltd. Kathmandu, Nepal. Other used excipients material; Polyethylene glycol (PEG 6000) (Yonghua Chemical Technology Co., Ltd, China), Cocoa butter (HiMedia Laboratories, Pvt, Ltd, India), Hard Paraffin (HiMedia Laboratories, Pvt, Ltd, India), Liquid Paraffin (HiMedia Laboratories, Pvt, Ltd, India), Gelatin (HiMedia Laboratories, Pvt, Ltd, India), Glycerin(HiMedia Laboratories, Pvt, Ltd, India), Tween 80(Riedel-De haen Ag seelze-Hannover, Germany), Methyl paraben (Interchimiques SA, France) were used. All the excipients used in this research are pharmaceutical grade.

Methods

Base optimization of suppositories

Base optimizations during the formulation of suppository were selected based on the three bases used i.e. cocoa butter (CB) plain, CB: Paraffin, and CB: PEG 6000 at different varying ratio 9:1 to 1:9 ratios (Table 1).

Preparation of Mesalamine suppositories [14]

Total four formulations of bullet shaped mesalamine suppositories were prepared by fusion method in aluminium mould with 24 cavities. 30 suppositories were prepared for each formulation. The details of the component of formulation are given in Table 2.

***Note:** The further study in glycerol-gelatin suppository was not carried out as drug was not properly soluble in this base. Displacement value for each formulation was calculated to get the required amount of base and API before the preparation of mixture of suppositories.

Preparation of mass mixture

Accurately weighed quantities of base and API were taken. Firstly, base was melted over water bath maintained at 80 ^{0}C. After complete melting of base, API and tween 80 was added and mixed properly using glass rod. Now, methyl paraben was added and mixed again.

Preparation of suppositories

Homogenized mixture was poured in previously cleaned and lubricated mould placed over ice to obtain bullet shaped suppositories with average weight of 3.07gm.

Preparation of phosphate buffer [15]

Placed 50ml of 0.2M potassium dihydrogen orthophosphate in 200ml volumetric flask added specified volume of 0.2M sodium hydroxide and then added reverse osmosis (RO) water to make up the volume.

0.2M Potassiun dihydrogen orthophosphate

Dissolved 27.218gm of Potassium dihydrogen orthophosphate (PDP) in distilled water and finally make up volume to 1000ml with same solvent.

0.2M sodium hydroxide (NaOH)

Dissolved 8gm NaOH flashes in distilled water and finally 100ml volume make up was done.

Phosphate buffer (PH 7.2)

Take 50ml of 0.2M PDP and 34.7ml of 0.2M NaOH in 200ml volumetric flask and dilute it with distilled water to make up the volume.

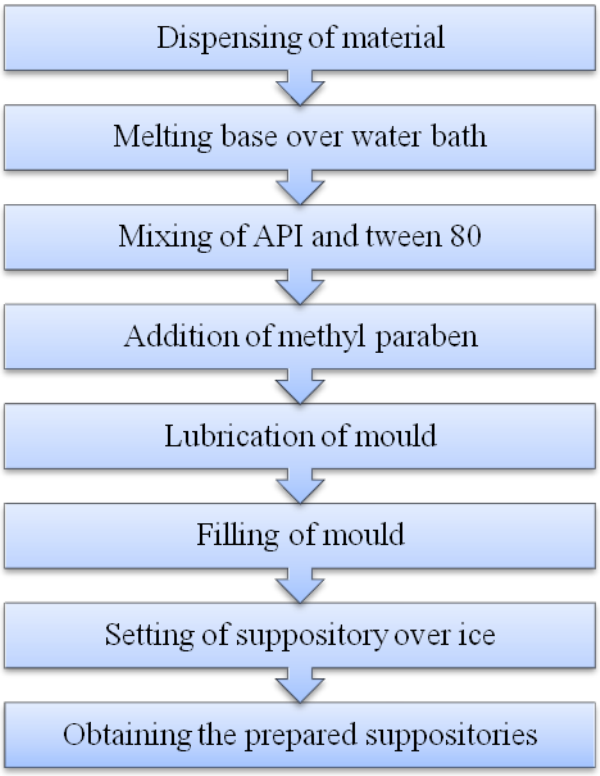

Figure 1: Flowchart showing process of preparing suppositories

UV method for drug quantification

Analysis of mesalamine by UV visible spectrophotometer (Cary 60 UV-Visible Spectorphotometer, Agilent, USA) was performed. The detection wavelength of mesalamine was recorded by UV. The detection wavelength was 330 nm, and the correlation coefficient of the calibration curve was R^2: 0.994 for concentration range of 2-12 µg/ml, indicating acceptable linearity. The samples for the calibration curve were made by using phosphate buffer saline at pH 7.2 as a solvent.

Preformulation characterization parameters

1) Displacement value [16]**:**

The displacement factor (f) means how much base is displaced by a unit weight of an active pharmaceutical ingredient. For calculating displacement value the formula containing an effervescent pair, was used.

F = [100*(E-G)/ (G*x)] +1

Where, E = blank suppository weight containing only base

G = weight of suppository with effervescent pair in a known concentration

x = effervescent pair content of the suppository in weight percentage

Equation used for calculating the weight of suppository base:

TM = E- Summation (i = 1 to n) f1*S1

Where TM means suppository base to be weighed, E is the calibration constant of the mold, f is the displacement factor of each component, and S is the weight of each component (Table 3).

Post formulation characterization parameters of suppositories

The following parameters were evaluated for the evaluation of the prepared suppositories of mesalamine.

1) General appearance:

From the prepared each formulation, 20 suppositories were randomly selected for the physical evaluation which includes color and surface characteristics. For this, each suppository was cut longitudinally and visual inspection was performed. The signs of physical deformity were fissuring, fat blooming, exudation, and migration of the active ingredients. The length and width of each suppository were measured and the mean value was calculated.

2) Weight variation:

20 suppositories of each batch weighted individually, and mean was calculated followed by calculation of the percentage deviation from the mean. Suppositories weight variation limit is no more than two suppositories should deviate by more than 5% of the average weight but should not deviate more than 7.5% [17].

3) Hardness test:

Hardness test was measured by using Monsanto hardness tester. From each batch three suppositories were randomly assigned and the average was calculated [18].

4) Melting point determination:

It was determined by using three suppositories from each batch. For this, suppository was placed in 5 ml phosphate buffer (pH 7.2) and placed in a water bath at 37ºC. The temperature for the whole suppository for the completely melting was noted [19].

5) Liquefaction time:

It indicated the time required for the suppository to liquefy under pressure similar to the rectal pressure in the presence of liquid at 37ºC. This test was done in burette with broad opening one end and a narrow on the other. For this test, burette was filled with 5 ml phosphate buffer (pH 7.2) and placed in a water bath at 37ºC. For each batch a suppository was placed inside the burette from the broad end and pushed to the narrow end. A thin glass rod was placed on the top of suppository and the time for the glass rod to penetrate the suppository was recorded as liquefaction time [20].

6) Drug content percentage:

Assay was carried out by placing one suppository in 700 ml of phosphate buffer pH 7 maintained at 37ºC till melted. 1 ml of sample was withdrawn and diluted to 50 ml with phosphate buffer. Then the content of mesalamine was determined by using UV visible spectrophotometer by measuring absorbance of diluted sample at 330 nm [21].

7) In-vitro release profile:

The in-vitro releases of different formulation of mesalamine suppository were studied using dissolution test apparatus type I (Paddle) (LABINDIA, Martix TechnoChem, India). For each batch, a suppository was placed in 900 ml phosphate buffer (pH 7.2, temperature 37ºC) at a 75 rpm. Aliquots of 10 ml were collected at predetermined time intervals, filtered through Whatman No. 1 filter paper and used for the quantitative determination of mesalamine using UV visible spectrophotometer at 330 nm. Each sample was replaced with 10 ml fresh buffer. The cumulative percentage of drug release was calculated and plotted versus time [21].

Result and discussion

The treatment of IBD in the clinical setting is mainly used by the anti-inflammatory drugs, especially NSAIDs [22]. NSAIDs cause the GI side effects, since it leads to the further development of the search of alternative therapeutic pathways of drug delivery. Since, rectal route offers the less side effects and ease of administration to other routes [23]. Mesalamine also cause severe GI side effects so its rectal drug formulation is necessary.

Before formulation of the mesalamine suppositories the dummy suppositories prepared using cocoa butter (CB) alone were translucent gelly like consistency with irregular surface. They lacked physical strength upon exposure to room temperature. Suppositories prepared with varying ratio of CB and paraffin was opaque with regular surface. The rigidity of suppositories at room temperature was found to be increased with increased concentration of paraffin.

Similarly, combination of CB and PEG 6000 produced opaque suppositories with satisfactory hardness at room temperature. For the further formulation CB plain was also chosen as a standard suppository base. Based on observed hardness, rigidity and optimal melting point and liquefaction time, the base composition with CB: Paraffin (8:2) and CB: PEG 6000 (1:1) were chosen for further formulation development (Table 1).

Table 1: Base optimization studies

Base component (Ratio)	**Base optimization parameters**		
	Hardness (Kg/m^2)	**Melting Point (^{o}C)**	**Liquefaction Time (minutes)**
CB Plain			
	3.2	31	40
CB : Paraffin			
9:1	3.2	38	40
8:2	4.5	41	45
7:3	4.5	48	86
6:4	4.8	48	Did not liquefy till 120 minutes
5:5	4.9	51	Did not liquefy till 120 minutes
4:6	5.0	51	Did not liquefy till 120 minutes
3:7	5.2	54	Did not liquefy till 120 minutes
2:8	5.5	55	Did not liquefy till 120 minutes
1:9	5.5	58	Did not liquefy till 120 minutes
CB: PEG 6000			
9:1	3.6	31	3
8:2	3.8	31	3
7:3	3.8	33	10
6:4	4.1	34	10
5:5	4.5	38	15
4:6	4.5	44	17
3:7	4.9	46	32
2:8	5.0	55	45
1:9	5.5	60	48

After the base optimization, different suppository batches (F1, F2, and F3) were formulated for the rectal delivery by using mesalamine as active ingredients as shown in Table 2.

Table 2: Formulation of Mesalamine Suppositories

S. No.	Ingredients	Formulation Codes		
		F1	F2	F3
1.	Mesalamine (g)	7	7	7
2.	Cocoa butter (g)	54.65	47.04	28.13
3.	Hard paraffin (g)	-	10.76	-
4.	PEG 6000 (g)	-	-	28.13
5.	Tween 80 (mL)	5	5	5
6.	Methyl paraben (mL)	1	1	1

Note: F1:- Cocoa butter plain only (CB Plain); F2:- CB: Paraffin (8:2); F3:- CB: PEG 6000 (1:2)
F1, F2, and F3 are three formulation containing different bases.

After formulation all three batches were inspected for physical inspection. All the prepared suppositories were yellowish in color with smooth shiny surfaces; no cracking was seen because smoothness the surface is important to ensure ease of administration [24]. Uniformity of suppositories color shows that proper mixing of active ingredient in the formulations. Longitudinally cutting pieces of suppositories does not show any pitting, exudation, fissuring, and fat blooming. All three formulated suppositories were uniform in length and width.

All three formulation shows displacement value near one. Weight variation of all three different batches was within the required limits of British Pharmacopoeia (BP) 2014. Furthermore, drug content percentage was evaluated for both three formulations and market formulation were complies with BP 2014 standards [25].

The melting temperature of the formulation were measured and compared to the marketed formulation, and results are demonstrated in the Table 3. Melting point of the F1 was relatively lower than that of other formulation which may be due to the plain cocoa butter formulations gradually melted at room temperature. On the other hand, liquefaction time of formulation F2 is comparable to marketed formulation (MF). Liquefaction time of F3 is fastest liquefaction time which may be due to the hydrophilicity characters of poly ethylene glycol.

For handling, insertion, packaging, and transportation without cracking suppositories should optimum hardness [26]. All formulated suppositories were hard and able to withstand pressure higher than 2 kg/cm^2.

Table 3: Formulation characterization parameters (Pre and Post) of the tested suppositories

S. No.	Parameters studied	Formulation			MF
		F1	F2	F3	
1.	Displacement value	0.97±0.01	0.902±0.04	1.13±0.02	NT
2.	Weight variation (g)	3.061±0.006	3.107±0.005	3.190±0.007	NT
3.	Melting point (°C)	33±1.06	38±1.23	36±1.01	42±1.5
4.	Liquefaction time (minutes)	40±0.02	46±0.04	16±0.01	54±0.005
5.	Hardness test (Kg/cm^2)	3.7±0.12	4.8±0.11	5.0±0.10	4.5±0.09
6.	Drug content (%)	98.60±0.2	93.21±0.5	102.8±0.4	97.17±0.1

*All data are expressed as mean ± SD (n=3). MF = Marketed formulation, NT = Not test

In vitro drug release

In suppositories, drug release profile was affected by various factors such as type of base used, the compatibility between the drug and the base, chemical nature of the additives used during formulations.

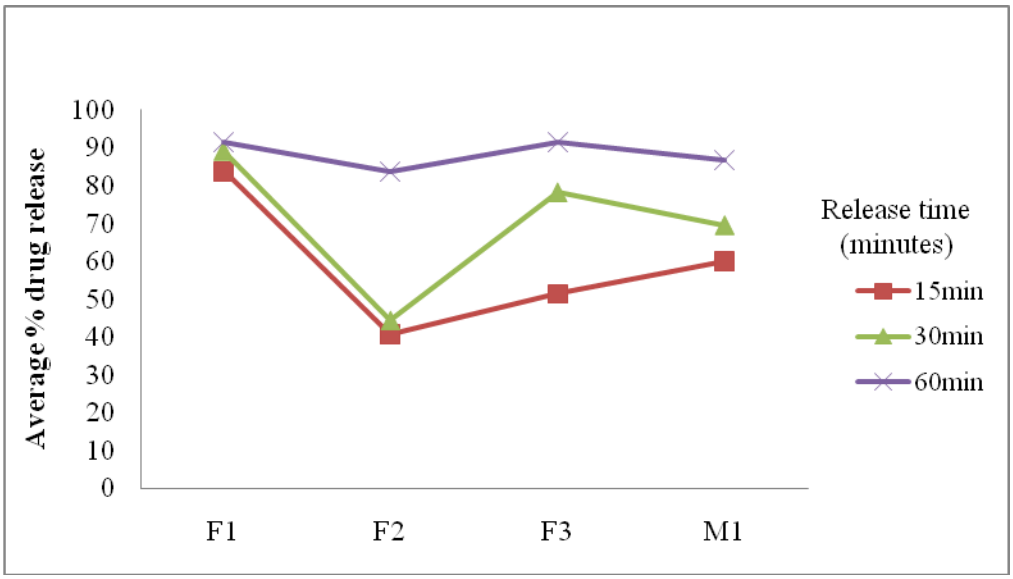

Figure 2: Mesalamine release profile from different suppository formulations

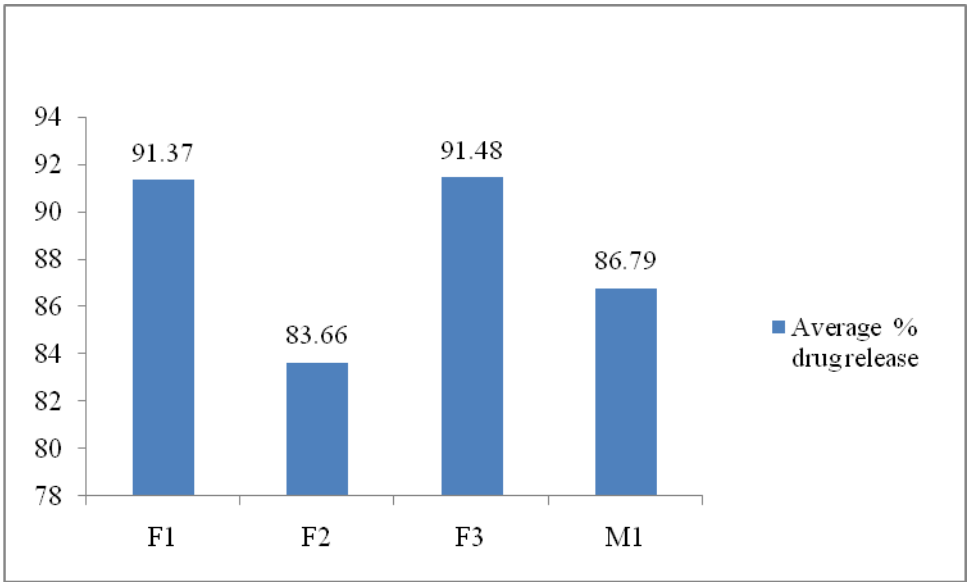

Figure 3: Average % total drug release from different formulation of suppositories

In-vitro drug release of F3 and F1 was almost equal with above 90% release within 60 minutes. Upon analysis of release profile, F1 released almost 80% and F3 released around 40% drug within 15 minutes and around 42% at 30 minutes. Greater release with Cocoa butter plain can be defended by its lower melting point in F1 whereas hydrophilicity of PEG 6000 is responsible for F3. Similarly hydrophobicity of paraffin may be the reason behind slower and lower release drug profile as shown in Figure 2. All the formulation showed greater than 80% release which is mandatory for suppositories as shown in Figure 3.

Conclusion

In conclusion, cocoa butter and combination of cocoa butter can be the ideal base for suppositories preparation of mesalamine. Combination of PEG 6000 with cocoa butter can be a better suppository base than that of combination with paraffin compared on the basis of pharmacopeial evaluation parameters. Although assay and drug release was satisfactory with cocoa butter, it can be an optimum base due to its low melting point. Among combination of bases with cocoa butter, PEG 6000 was found to have agreeable pharmacopeial evaluation parameters than that of paraffin. Since our present work has proposed cocoa butter: PEG 6000 (1:1) combination base can be optimum to formulate mesalamine suppositories.

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