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FORMULATION AND EVALUATION OF PROBIOTIC TABLETS CONTAINING ANTI-INFLAMMATORY DRUG

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ABSTRACT

The aim of present study was to formulate and evaluate probiotic tablets containing anti-inflammatory drug Mesalazine. Matrix tablets were prepared using Sodium alginate and Hydroxypropylmethylcellulose acetate succinate (HPMCAS) as matrix forming components, with three different combinations by wet granulation method. The granules were evaluated for angle of repose, bulk density, tapped density, compressibility index and Hausners ratio. The tablets were subjected to weight variation, hardness, friability and drug content test and invitro release studies. Additionally the tablets were tested for contents of viable cell counts of probiotic lactobacilli using standard plate count (SPC) method. Invitro release studies revealed that all formulations qualified both stages for release of the drug i.e. acid stage and buffer stage 1. The release profiles were affected by variable concentrations of sodium alginate when combined with HPMC-AS. The combination at 1:2 (SA2) prevented the escape of both actives more effectively than the other two formulations at all the three stages of dissolution test. A few numbers of bacterial cells were lost in acid stage as well as in the subsequent buffer stage1. However, at the end of buffer stage 2 the viable cell count for *L .acidophilus,* were found to be 9×10^9 CFU. The combinations of HPMCAS (HF) and sodium alginate together increased acid tolerance of probiotic *lactobacilli* strain added in matrix tablets.

INTRODUCTION: Ulcerative colitis is a chronic inflammation of the large intestine (colon). It is a disease that causes inflammation and sores, called ulcers, in the lining of the rectum and colon [1]. The fetus in uterus is sterile, but on passage through the vagina during birth, it acquires microorganisms. These are rapidly added after birth and the newborn acquires its own gut microflora.

Up to 500 species of bacteria may be present in the adult human large intestine and it has been estimated that bacteria accounts for 35–50% of the volume content of the human colon [2].

The stable flora, which develops in the intestine, helps the host to resist infections, particularly in the gastrointestinal tract. The balance between "Good bacteria" and "Bad bacteria" is called as "Eubiosis" and when this balance is disturbed it is called as "Dysbiosis". The administration of probioitc products maintains the "Eubiosis" condition [3].

Probiotics is a live digestible bacterial food supplement, which beneficially affects the host by improving the intestinal microbial balance or by restoring the disturbed microbial balance [4, 5].

Presence of condition like Inflammatory Bowel Disease (Ulcerative Colitis) is one of the major reasons for disturbing microbial balance in colon. Mesalazine is considered to be the "Gold standard" drug for treatment of ulcerative colitis. The colon specific drug delivery is greatly influenced by presence of microbial flora in the intestine; hence we can't neglect the condition of dysbiosis prevailing during the IBD_S including ulcerative colitis [6]. Mesalazine is available as delayed released tablets, controlled released capsules, enteric coated tablets for oral use and rectal suppositories, enema suspension for rectal use. While probiotic bacterial preparations are available in various food products and as yogurt containing more than 10 million viable lactic acid bacteria.

In the present study an attempt has been made to combine these two very useful therapeutic agents for treatment of IBDs especially ulcerative colitis.

MATERIALS AND METHODS:

Materials: Mesalazine was Kind gift from Sarex pharma Mumbai India. HPMC-AS, and Povidone (PVP-K30) gifted by Signet chemical corporation, Mumbai. Sodium alginate was kindly supplied by Alembic Pharmaceuticals Ltd. Baroda. MRS broth (M369) & lyophilized powder of lactic acid bacteria strain (LAB) *Lactobacillus acidophilus* procured from High Media Labs Mumbai. Talc and Magnesium stearate were procured from Emcure House M.I.D.C. Pune. All other chemicals and reagents used were of analytical grade.

Preparation of matrix tablets: For preparing matrix tablets the contents of Mesalazine and lyophilized powders of bacterial cell were maintained at 250 mg and 2.5 mg respectively [7]. The accurately weighed quantities of selected polymers and drug were mixed in various proportions and mixtures were assigned different formulation codes presented in **table 1** [8].

The active ingredients Mesalazine, the polymers, Hydroxypropylmethylcellulose acetate succinate –HF and sodium alginate were passed through screen (60 #). The physical mixtures of drug, polymers, excipients, & the lyophilized powders were prepared by blending the accurately weighed quantities of each of them with Mesalazine in geometric proportions in glass mortar for 15 minutes.

TABLE 1: FORMULATIONS OF THE MATRIX TABLETS OF MESALAZINE

Sr. No.	INGREDIENTS (%W/W)	Formulation codes		
		SA1	SA2	SA3
1	Mesalazine	50	50	50
2	Lyophilized Powder	0.5	0.5	0.5
3	HPMC-AS	15	15	30
4	Sodium Alginate	15	30	15
5	PVP K-30	3	3	3
6	Magnesium state	1	1	1
8	Talc	15.50	0.5	0.5
Weight of one tablet is 500mg				

Ethnolic solutions of PVP K-30 (3% w/v) were used as binders which were added gradually to powder blends with trituration until a coherent moist mass was formed. This mass was passed through screen (22#) to get moderately coarse granules. The wet granules were dried at 35˚C for 1 hour. The dried granules were again passed through screen (44#) to obtain fine granules. The resulting granules were lubricated with magnesium stearate and then evaluated for following flow properties bulk density, tapped density, compressibility index (C.I.) & Angle of repose (θ).

The granules of each formulation type were compressed into matrix tablets using S.S. punches (diameter 13 mm flat surface) on rotary tablet press. The compression force was maintained in such a way that the hardness of resulting tablets ranged between 7-8 Kg/m^2. The batch size prepared for each formulation was of 25 tablets.

Evaluation of Matrix Tablets of Mesalazine:

- **Weight variation:** This test was performed as per procedure described in Pharmacopoeia of Indian (1996)[9] using 10 tablets of each formulation type. The tablets were weighed individually and their mean weight was calculated. The deviation of individual weight from the mean was expressed as standard deviation. The compliance of tablets with recommended allowances for variations in weight was judged on the basis of official specifications.

- **Hardness:** Hardness of three tablets of each formulation type was determined using Monsanto hardness tester following the procedure described in standard text book [10].

- **Friability:** The friability of 10 tablets of each formulation type was noted using Roche friabilator following the procedure described in standard text book [10]. The weight loss (% w/w) was calculated using following formula,

% Friability = (Loss in weight/ Initial weight) x 100

- **Drug contents:** The contents of Mesalazine were estimated using 5 tablets of individual formulations. The tablets were weighed individually, and were crushed in mortar. From this, the powder equivalent to 250 mg of Mesalazine was taken in volumetric flasks and dissolved in sufficient quantity of phosphate buffer (pH 7.2) and the final volume was made up to 100 ml. Appropriate dilutions of the resulting solutions were carried out and the contents of Mesalazine were estimated from UV absorbance of these solutions at 331 nm using previously prepared calibration curve of Mesalazine in phosphate buffer pH 7.2 [11].

- ***In vitro* release of Mesalazine from matrix tablets:** The test was conducted using three matrix tablets of each type of formulation using USP (23) dissolution apparatus (Apparatus I). The tablets of each type of matrix formulations were kept in baskets which were placed successively in below mentioned dissolution media. The dissolution apparatus was run maintaining below stated test conditions represented in **table 2**.

ABLE 2: THE EXPERIMENTAL CONDITIONS USED FOR *IN VITRO* RELEASE OF MESALAZINE FROM MATRIX TABLETS

Phases	Type and volume of dissolution medium	Speed of Rotation (rpm)	Duration (min)	λ_{max} used for recording absorbance	Volume withdrawn & frequency of withdrawn of aliquots
Phase I Acid stage	0.1N HCl 500ml pH- 3	100 rpm	120	303.0	10 ml at intervals of 30min
Phase II Buffer stage-1	Phosphate buffer 900ml pH- 6	100 rpm	60	330.0	10 ml at intervals of 30min
Phase III Buffer stage-2	Phosphate buffer 900ml pH-7.2	50 rpm	90	331.0	10 ml at intervals of 30min

- **Content of viable cell counts:** The tablets were tested for contents of viable cell counts of probiotic lactobacilli using standard plate count (SPC) method [12]. The SPC (Standard plate count) test was performed after making the serial dilutions of the revived bacterial cultures. The following scheme enlists the steps involved in the same.

Procedure for Serial Dilution Technique:

1. Aseptic transfer of 1mg dried powder of *Lactobacilli cultures* in test tubes containing sterile distilled water (9ml).
2. Aseptic transfer of 1 ml of this stock solution in to subsequent test tubes containing 9 ml of sterile distilled water resulting in the concentration of 10^{-1}.
3. Transfer of this diluted culture to subsequent test tubes to give corresponding dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}.
4. Transfer of 0.1 ml of diluted cultures i.e.10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} in to the corresponding plates of MRS medium.
5. Incubation of inoculated MRS Petri plates at $37^{o}\pm1^{o}$C for 24 hours.
6. Count of the colonies observed after incubation.

Stability Studies: Stability studies were carried out to assess the stability of formulated tablets. Tablets of selected probiotic formulations were maintained at (25°C and 60% RH) for 30 days. Tablets were evaluated for tablet characteristics and viable cell count of lactobacilli.

RESULTS AND DISCUSSION:

Evaluation of Granules: The values for loose bulk density, tapped bulk density, compressibility index and angle of repose of granules of Mesalazine prepared with probiotic lactobacilli strains and combination of HPMCAS: sodium alginate, revealed different behavior of each formulation blend (**Table 3**). All these values are still suggestive of good flowability of blends.

TABLE 3: FLOW PROPERTIES OF GRANULES OF MESALAZINE WITH PROBIOTIC LACTOBACILLI STRAINS AND INDIVIDUAL PH SENSITIVE HPMC POLYMERS AND THEIR COMBINATION WITH SODIUM ALGINATE

Code	Loose bulk density (g/ml)	Tapped bulk density (g/ml)	Carr's Compressibility Index (%)	Angle of repose(Ø)
SA1	0.138±0.0159	0.163±0.004	19.12	24.20
SA2	0.168±0.0110	0..195±0.004	14.59	27.81
SA3	0.174±0.0125	0.197±0.005	17.83	28.79

Values of loose bulk density and tapped bulk densities for Mesalazine granules containing probiotic lactobacilli strains ranged between HPMCAS: Sodium alginate *(L. acidophilus)* 0.138- 0.174. The Carr's index values ranged between 14.59-19.12. Similarly, the values of angle of repose for Mesalazine granules containing probiotic lactobacilli strains were; 24.20-28.79. All these ranges are suggestive of good flow ability of granules.

TABLE 4: CHARACTERIZATION OF MATRIX TABLETS OF MESALAZINE WITH PROBIOTIC LACTOBACILLI STRAINS

Code	Avg. Weight (mg)	Diameter (mm)	Thickness (mm)	Hardness (Kg/cm²)	Friability (%)
SA1	501±2.25	12.88	3.33	9.0	0.39
SA2	498±1.87	12.86	3.39	8.5	0.45
SA3	502±0.95	12.85	3.30	8.5	0.26

- The pharmacopoeial specifications for deviation in weight from average weight for tablets weighing more than 250 mg are ±5%. The percentage deviation in the weight of prepared tablets (weighing 500 mg) was within the specified limits for all the formulation types and hence, they complied with the test for weight variation.

- Diameter of the matrix tablets was in the range of 12.85-12.88mm. Thickness of the matrix tablets was in the range of 3.30-3.39 mm. Hardness of matrix tablets was in the range of 8-9 Kg/cm^2**.** Friability of the matrix tablets of Mesalazine formulations with probiotic lactobacilli strains ranged between 0.26-0.45 percent. The matrix tablets of different formulations possessed consistent dimensions and hardness and all of them complied with the specified limits for friability (<1%).

- ***In vitro* release of Mesalazine from matrix tablets prepared with combination of HPMCAS with sodium alginate:** All the formulations qualified both stages for release of the drug i.e. acid stage and buffer stage 1. The release profiles were affected by variable concentrations of sodium alginate when combined with HPMC-AS (**Fig. 1**). Hence, the combination at 1:2 (SA2) prevented the escape of drug more effectively than the other two formulations at all the three stages of dissolution test.

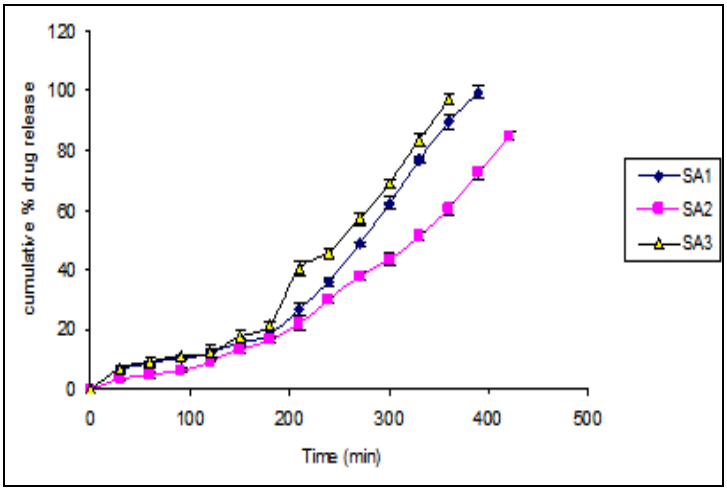

FIG-1: *IN VITRO* RELEASE OF MESALAZINE FROM MATRIX TABLETS PREPARED WITH COMBINATION OF HPMC AS (HF) AND SODIUM ALGINATE

- **Viable cell count of *lactobacillus acidophilus* from matrix tablets of Mesalazine prepared with combination of HPMCAS: Sodium alginate:** Parallel to the estimation of drug Mesalazine, viable count of probiotic cultures was also determined from the probiotic matrix tablets in the dissolution media (**Table 6**).

In case of all the three probiotic tablet formulations, a few numbers of bacterial cells were lost in acid stage as well as in the subsequent buffer stage 1. However, at the end of buffer stage 2 the viable cell counts for *L .acidophilus,* 9×10^9. The combination of HPMCAS (HF) and sodium alginate together increased acid tolerance of probiotic *lactobacilli* strains added in matrix tablets.

TABLE 5: DISSOLUTION DATA OF MATRIX TABLETS OF MESALAZINE WITH VARIABLE COMBINATION OF HPMC-AS WITH SODIUM ALGINATE

Dissolution Phase & duration	Time (min)	Cumulative (Avg.) % drug release		
		SA1	SA2	SA3
		15:15 (%)	15:30 (%)	30:15 (%)
Acid Stage pH= 3 (120min)	0	0	0	0
	30	6.57±.0.98	3.57±0.89	8.69±1.29
	60	8.38±0.66	4.83±1.28	9.27±1.73
	90	10.49±0.45	6.38±0.91	10.88±0.81
	120	**12.24±2.14**	**9.24±0.35**	**11.93±1.56**
Buffer Stage-1 pH-6 (60 min)	150	15.64±1.70	13.22±1.10	17.61±0.87
	180	**18.57±1.38**	**16.54±1.25**	**21.60±1.20**
Buffer Stage 2 pH= 7.2 (150 min)	210	26.78±1.90	22.21±2.32	40.10±2.34
	240	35.87±1.43	29.68±1.30	45.52±1.30
	270	48.52±0.88	37.96±1.43	57.05±0.95
	300	62.18±2.73	43.50±2.16	69.04±1.31
	330	76.87±1.33	51.28±1.21	83.02±2.17
	360	89.55±2.15	60.56±0.98	97.14±1.51
	390	99.41±1.98	72.37±2.18	--
	420	--	84.91±1.28	--

TABLE 6: VIABLE CELL COUNT OF *LACTOBACILLUS ACIDOPHILUS* FROM PROBIOTIC MATRIX TABLETS OF MESALAZINE FORMULATIONS PREPARED WITH HPMCAS: SODIUM ALGINATE

Dissolution phase	Viable cell count of probiotic cells in matrix tablets		
	SA1	SA2	SA3
Acid stage	10^5	10^4	10^4
Buffer stage 1	2×10^4	2×10^5	3×10^6
Buffer stage 2	8×10^9	9×10^9	8×10^{10}

Sodium alginate is sodium salt of alginic acid. It is soluble at neutral pH and hydrates to form a viscous solution at this pH. However, it is insoluble at pH below 3 and swells considerably at this pH.

Hence, the formation of hydrogel by the increasing concentration of polymer around the matrix tablets was responsible for protection of probiotic bacterial cells.

Stability Studies: The assessment of in vitro release of Mesalazine and viable cell count of the probiotic culture from the stored matrix tablets did not reveal significant alteration in these parameters over a period of 30 days.

CONCLUSION: The results of experimental studies of probiotic matrix tablets proved that the granules of showed good flow properties, tablet evaluation tests are within the acceptable limits. The release profiles were affected by variable concentrations of sodium alginate when combined with HPMC-AS.

Hence, the combination at 1:2 (SA2) prevented the escape of drug more effectively than the other two formulations at all the three stages of dissolution test. In case of probiotic matrix tablets, a few numbers of bacterial cells were lost in acid stage as well as in the subsequent buffer stage 1.

However, at the end of buffer stage 2 the viable cell counts for *L. acidophilus*, 9×10^9CFU. The combination of HPMCAS (HF) and sodium alginate together increased acid tolerance of probiotic lactobacilli strains added in matrix tablets.

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