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Research Article

DEVELOPMENT OF CELLULOSE ACETATE PHTHALATE COATED PECTIN MICROPARTICLES LOADED WITH MESALAMINE AND *SACCHAROMYCES BOULARDII* INTENDED FOR SPECIFIC COLONIC DRUG DELIVERY

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ABSTRACT

The present work was focused on delivering mesalamine along with probiotic, specifically to the colonic site. Mesalamine and probiotic were encapsulated in natural polysaccharide pectin microparticles and coated with the Cellulose acetate phthalate (CAP) as an enteric-coated polymer. The major concern of this research is to protect the drug and probiotic release from the gastric environment and target to colonic region. By using nitric oxide assay, the IC_{50} value of both probiotics (*Saccharomyces boulardii* and *Lactobacillus acidophilus*) was determined. Pectin microparticles were prepared by dehydration technique followed by coating with oil-in-oil solvent evaporation. For the drug and polymer compatibility, FTIR determination was done. The release of drug and probiotic was determined with and without rat cecal content. Furtherly pharmacokinetic studies were done to assess the drug concentration in Wistar rat's blood fluid. The nitric oxide assay confirmed that *Saccharomyces boulardii* has high nitric oxide scavenging ability. The FTIR graphs confirmed that no chemical reaction was observed within the drug and polymer. The observed *in-vitro* results of coated microparticles release have been confirmed that the coated formulation has the potential to release the drug and probiotic at the colonic site. Further pharmacokinetic studies revealed a lower value of C_{max} in the case of CAP coated microparticles formulation in comparison to uncoated ones which evidenced the lower systemic exposure of the drug.

Keywords: Ulcerative colitis, Probiotic, *Saccharomyces boulardii, Lactobacillus acidophilus,* Pectin, Colon targeted drug delivery.

Abbreviations: UC- Ulcerative colitis, S.b- *Saccharomyces boulardii*, L.a- *Lactobacillus acidophilus,* CAP- Cellulose acetate phthalate, G.I.T-Gastrointestinal tract, CFU- Colony forming units per gram, TNF-α- Tumor necrosis factor-alpha, IL-8-Interleukin-8, IL-23- Interleukin-23, PBS- Phosphate Buffer solution, SGF- Simulated gastric fluid, SIF- Simulated intestinal fluid, SCF-Simulated colonic fluid, C_{max}- Maximum drug concentration, Tmax-Time take to reach, AUC- Area under the curve, MRT-Mean residence time.

1. INTRODUCTION

The development of efficient approaches for the treatment of Ulcerative colitis (UC) is remaining a major concern for pharmaceutical industries [1]. Due to the complex physiological system of the body, it is very difficult to provide the drug specifically to the colon disease site [2]. As there is a pH variation in each compartment of GIT [3]. The conventional drugs are not efficient to reach the affected site of the colon; they get absorbed from the stomach region resulting in systemic availability of mesalamine in the blood leads to produce side effects. The observed side effects are headache, nausea, diarrhea, interstitial nephritis and hepatitis [4]. UC is the chronic relapsing disease of the colon characterized by bloody stool, weight loss and frequent urge to feces. Commonly used drugs are mesalamine, sulphasalazine, budesonide, etc [5]. Mesalamine is the first-line choice of drug for colitis management. Due to the lifestyle change, the prevalence and occurrence of UC are increasing every year. The primary symptoms of UC are loss in weight, blood in the stool, inflammation at the colonic region, swelling of rectum lining, diarrhea,

abdominal pain, discomfort feel [4]. In UC, the reduction of probiotic concentration in the colonic region has been reported by many research groups [6]. The probiotics maintain the mucus concentration which gets lower in the disease state, also act as an anti-inflammatory as well as anti-oxidant. In published reports, the researcher has concluded that the probiotics are as efficient as mesalamine for the management of UC. Also decreases inflammation by reducing the responsible proinflammatory cytokines [7]. Probiotics are live microorganisms when given in sufficient amounts, give beneficial effects to the host health [8]. The viability of probiotics becomes low due to the harsh acidic pH of the stomach. Additionally, 60% of the probiotics are killed in a harsh environment before entry into the colon [9, 10]. So there is a need to protect the viability of probiotics, encapsulation is the best method for the protection of probiotics from the acidic environment [11]. Graff *et al* [12] prepared microspheres enhance the activity of S.b. Frequently used probiotics are *Saccharomyces*, *Lactobacillus* and *Bifidobacterium* for the management of UC [4, 13, 14]. *Saccharomyces boulardii (*S.b.) is the first approved non-pathogenic yeast as probiotic for use in human use [8]. It has anti-inflammatory property work by inhibiting the mediators responsible for inflammation[15]. Dong and his research group [16] have demonstrated that S.b. was efficient as mesalazine in lessening serum inflammatory factors and preserve histological structure in dextran sodium sulfate-induced colitis in mice. Accordingly, Guslandi et al. [17] studied the effect of S.b. in UC patients; preliminary results recommended that S.b can be efficient in UC management. Thomas et al. [18] reported, S.b. inhibited (interferon-γ and TNF-α) and promoted IL-8 and transforming growth factor-β-dependent mucosal healing in patients with chronic colon inflammation. Oral administration of probiotics may improve the commensal microflora and normalize the host-microbial interaction. The research group of khoder Get et al. [8] has concluded in *Lactobacillus acidophilus* (L.a.) has anti-oxidative properties. Chen et al. [19] and his research team have concluded, that oral administration of L.a. suppressed DSS-induced colitis connected with hyper-response of the IL-23/Th17 axis. Abdin et al,[20] have concluded that L. a. probiotic could be suggested as adjuvant therapy in combination with olsalazine to attain more efficient UC management. Thakur et al. [21] has concluded that the mesalamine in combination with L.a. showed a synergistic effect in DNBS induced colitis model in Wistar rats. The combination of probiotic with mesalamine would be the best novel approach for the management of UC [22]. Various novel approaches were utilized including pro-drug, time-release approaches and microparticles, nanoparticles for maximum drug delivery to colon site among these all, the microparticles is one of the effective approach for colon-specific delivery in UC, the particle size ranging from 4-15 μm provides maximum mucoadhesion [23]. Pectin is a cell wall structural polysaccharide, dispersed richly in such fruits and vegetable sugar. Pectin is enriched in blocks of (1 → 4) linked galacturonic acid and galacturonic acid methyl ester units interrupted by single (1 → 2) linked rhamnose units [24]. Pectin has mucoadhesive properties, pectin itself has a positive effect on UC prevention [25]. It is a natural biodegradable, non-toxic polymer widely accepted for colon drug delivery. Pectin is degraded by the colon-specific enzyme (Bacteroids Bifidobacterium, Eubacterium). In previous finding coating of prepared microparticle with eudragit-S100 have been utilized for specific colon drug delivery [26]. But unfortunately, the pH of the colon has become slightly acidic in UC [27-29], So the carrier coating with eudragit-S100 is not sufficient to provide the drug release at the colon site. Therefore carrier with polysaccharides like pectin, which are microbially degraded by a colonic specific enzyme, has more potential to drug release in colon site [25]. CAP is frequently used by the pharmaceutical industry for the coating of pellets and tablets as an enteric-coated polymer [30-31]. We have prepared pectin based microparticles coated with CAP as an enteric-coated polymer. In the present study, We have evaluated the IC_{50} values of both probiotics (S.b.and L.a.). Based on the IC_{50} value S.b. was used for further formulation. We have prepared CAP coated microparticles loaded with mesalamine and S.b. for specific delivery at the colonic site.

2. MATERIAL AND METHODS

Lyophilized *Saccharomyces boulardii* and *Lactobacillus acidophilus* was procured from Hi-Tech BioSciences Pune, India. Sodium nitroprusside, Sulfanilic acid reagent and Glacial acetic acid were obtained from CDH (Central Drug House (P) Ltd, New Delhi, India. Naphthylethylenediamine and Sodium chloride was obtained from S D Fine Chemical Limited, Ambala, India. Disodium hydrogen phosphate and Potassium dihydrogen phosphate was purchased from Loba Chemie Pvt. Ltd, Mumbai, India.

2.1. Nitric Oxide assay for determination of the antioxidant potential of probiotics

Sodium nitroprusside (10 mM) was taken 2mL (0.2979gm) in PBS (pH 7.4) was taken in 0.5 mL and was then mixed with the extract. Multiple concentrations were made after that the was allowed to incubate for 25°C for around 3 h. 0.5 mL was taken out from this mixture and then added into 1.0 mL a sulfanilic acid reagent (33% in 20% glacial acetic acid) which was additional incubated at 34°C for 5 min. Finally, 1.0 mL naphthyl ethylenediamine dihydrochloride (0.1% w/v) was allowed to be mixed and incubated for 30 min at 34°C and then absorbance was checked against 540 nm [21]. IC_{50} values confirm the sample concentration, which is required to scavenge 50% of NO free radicals [32]. By utilizing the given formula IC_{50} value has been calculated.

Nitric oxide scavenged (%) = $[A_{control} - A_{test} / A_{control}] \times 100$

The IC_{50} value was achieved by making a plot between the different bacteria concentrations and scavenging activity, which is known as the total antioxidant potential required to decrease the primary NO radical concentration by 50%. Finally, the measurements were done. Where A control = Absorbance of control reaction and A test=Absorbance in the presence of the sample. The IC_{50} value was determined from the equation of the plotted graph of scavenging activity against the different concentrations of samples. The equation is- Y =mx+c , Where, y = % inhibition of NO activity (50), x = Concentration, c = constant

2.2. Method for Preparation of Pectin Microparticles

The pectin microparticles were prepared by dehydration technique as reported by Vaidya et al. [33] with necessary modifications. In this method, Mesalamine was dissolved in DMSO followed by the addition of probiotic S.b. (10^9 CFU/ml) in PBS 7.4 solution and then this suspended probiotics solution was added into mesalamine solution. In another beaker, a pectin solution was prepared and drug-probiotic dispersion was poured into it. From this dispersion, about 10 ml was dispersed in 50 mL isooctane containing a span 80 (1.0% w/v) and stirred continuously at various speeds to attain stable water/ oil emulsion. The dispersion was cooled quickly to 15°C and after that, about 50 mL of acetone was poured into it so that pectin droplets get dehydrated. Then microparticles were continuously stirred at 1000 rpm for 30 min at a temperature of 30°C to allow the complete evaporation of the solvent followed by drying and finally stored in a desiccator.

2.3. Coating of Pectin Microparticles

Pectin microparticles coating was done using the oil-in-oil solvent evaporation method as reported by Lorenzo-Lamosa et al [34] with little changes. In this method, the dispersion of 50g pectin microparticles was prepared in 10 ml of organic solvents mixture (acetone: ethanol, 9:1) containing CAP. Addition of the prepared organic phase into 70mL of light liquid paraffin containing 1% w/v span 80 was done followed by constant stirring for 3 h at 1200 rpm at room temperature to evaporate the solvent. Finally, the filtration and washing of coated microparticles were done with n-hexane. At last, the coated microparticles were filtered, dried and stored in a desiccator.

2.4. Characterization of Prepared Microparticles Formulation

2.4.1. Drug Loading and Entrapment efficiency

Drug loading and entrapment efficiency were determined as per the protocol reported somewhere else using the back-calculation method [35]. The centrifugation of microparticles dispersion was done at 12000 rpm for 30 min. The supernatant was collected in a test tube. The microparticles were washed 3 times by using water, centrifuged and supernatants were collected. The amount of per drug in the supernatant was determined using calibration curve. These parameters were estimated by the following formula.

$$\%\text{Entrapment efficiency} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

$$\%\text{Drug loading} = \frac{W_{Total} \times W_{Free}}{W_{Polymers}} \times 100$$

W_{total}, W_{free}, and $W_{polymers}$ are the weight of drug added in system, analyzed weight of the drug in supernatant and weight of polymers added in system, correspondingly.

2.4.2. Drug-Polymer Interaction

FTIR study was performed for identification and structural analysis of samples. FTIR transmittance of uncoated and coated microparticles was obtained using an FTIR spectrophotometer (Agilent). Potassium bromide (KBr) disk method was used by the addition of a small amount of sample powder with spectroscopic KBr and compressed in a vacuum press to obtain a disk. Infrared Spectrum was recorded by scanning over a wavenumber region of 400-4000 cm^{-1} using empower software [36].

2.5. *In vitro* characterization of drug-probiotic loaded microparticles

2.5.1. In-vitro drug release

In- vitro release study of prepared coated microparticles (Mesalamine and probiotic) was carried out using USP dissolution apparatus (Dissolution Test apparatus, Lab India DS 8000 instrument) type I in the 900 ml medium at 37°C at a speed of 100 rpm. The aqueous dispersion of mesalamine and probiotic microparticles was prepared immediately before it's merging into a dialysis bag of 12-14 KDa MWCO and begin the study of dissolution. Three types of dissolution media were used 1st- SGF having (pH 1.2), 2nd -SIF (pH 6.8), and 3rd-SCF (pH 7.4) with and without 2% rat cecal content was utilized to simulate the entire G.I.T transit environment. The release study was firstly performed in simulated gastric fluid (pH 1.2) for 2 h. Additionally, it was completed in simulated intestinal fluid (pH 6.8) for 3 h followed by SCF (pH 7.4) for 19 h. At various intervals of time, the samples were collected, accordingly diluted and examined by UV spectrophotometer method at 334 nm. After the sample collection, 5 ml of fresh media was placed in the dissolution media to maintain the volume [14, 35].

2.5.2. Rat cecal content medium preparation

Wistar rats (either sex) 190-260 gram were chosen for the study. The rats were obtained from Animal House of ISFCP, Moga, Punjab, India. The obtained rats were housed at ambient temperature (21±10°C) and relative humidity (55±5%) with a fixed 12h light/dark cycle. The experimental protocol was duly permitted by ISFCP/IAEC/CPCSEA/Meeting No 26/2020/Protocol No.434. Before 45 minutes of the start of dissolution studies; by spinal traction, four rats were sacrificed. After that, the rat's abdomens were opened and the cecal was ligated at both ends. Without delay, the rat cecal content was mixed in the simulated colonic medium. Finally, the solution was diluted to 2% (w/v) and transferred to the dissolution media PBS 7.4 pH for further investigation.

2.5.3. Release and viability count of S.b. from the coated microparticles during dissolution

The release and S.b. viability was verified along with coated and uncoated mesalamine-probiotic microparticles for 24 h. 10 ml of sample was taken from dissolution media. 1ml of the sample was withdrawn from the taken solution. This solution was noticeable as stock solution 10^1. From this standard solution, 1 milliliter was withdrawn and diluted with 0.05% Tween 80 and 0.1% peptone solution up to 1 to get 10^3 dilutions. The processes of serial dilutions were carried until 10^7 colony counters were obtained. After that the suspension of cells was placed into the petri plates then 10-15 ml suitable soybean casein digest agar media was transferred in each plate. These were incubated at 29±1°C under-maintained aerobic conditions for 96-120 h. To avoid misconception, this procedure was carried out three times. S.b. colonies were counter and result as CFU/g of yeast [10, 14].

2.6. *In-vivo* studies

2.6.1. Pharmacokinetic studies

Pharmacokinetic studies were carried out by following the procedure reported by Wei H *et al*. [37] with slight variation. The uncoated and coated mesalamine-probiotic microparticles were administered to rats through oral route [Wistar rats (either sex), 190-260 g] via a polyethylene cannula (diameter: 2mm) with 1ml of (1% carboxymethyl cellulose solution), at a dose correspondent to 23mg/kg. Blood samples (1.0 ml) were collected from retro orbitalis and placed in to heparinized tubes at 0.5,1,2,4,6,8,10,12,14,16 and 24 h after administration. The supernatants were obtained through this procedure, which was redissolved in 0.1ml of the mobile phase, vortexed for 3 min and centrifuged at 10,000 rpm for 3 min. Supernatants (0.02 ml) were injected for HPLC analysis of mesalamine under the conditions as explained in the next section. Further various pharmacokinetic parameters were determined by plotting plasma concentration-time graph and C_{max} and T_{max} were calculated directly. AUC was determined using the trapezoidal method followed by the calculation of MRT. The observations were expressed in terms of mean standard deviation. Statistical treatment was employed to test the determined parameters using a T-test. Also, the elimination rate constant and elimination half-life were calculated from the plasma concentration-time curve using standard formulas.

2.6.2. HPLC Conditions

The quantification of mesalamine in rat plasma was done through HPLC at different time intervals. Waters HPLC Chromatograph with UV detector and Empower software was utilized to carry out the different determinations. ODS column Phenomenex (250X 4.6 mm) was used to perform separations at room tempera-

ture. A mixture of acetonitrile, tertbutyl-ammonium chloride (0.1M), and phosphate buffer of pH 6.2 was selected as the mobile phase in a ratio of 85:15:1 v/v. Sonication, filtration and pumping of the mobile phase was done at a flow rate of 1.0ml/min. The samples were injected through Rheodyne injection and the eluted components were detected by UV detector at 330 nm.

2.7. Stability study

Stability studies of the optimized coated microparticles formulations were conceded according to the guidelines of ICH. The optimized drug-probiotic loaded micro-particles, were conducted at 25°C±2°C and 40°C±2°C,60% RH ±5%and 75±5% RH respectively. In the coated microparticles, the formulation was sealed in aluminum foils. Furthermore, significant changes were observed for 6 months. Finally, estimation of change in % drug entrapment, moisture content and percentage cumulative drug and probiotic release was evaluated [9].

3. RESULTS

3.1. Determination of antioxidant activity by using Nitric Oxide Assay

Nitric oxide assay was performed to identify the antioxidant activity of S.b. and L.a. The results were conveyed as the dose necessary to cause 50% inhibitions by probiotic (IC_{50}). The IC_{50} value of S.b and L.a was 58.06μg/ml and 71.11 μg/mL respectively. IC_{50} value between 50 μg/ml to 100 μg/mL is considered to ensure excellent intermediate antioxidant activity. The nitric oxide scavenging ability of S.b. was significantly higher than L.a. as shown in table 1 and Fig. 1 and 2. The result shows that S.b. exhibit admirable nitric oxide scavenging activity which is satisfactory for further use in the formulation.

Table 1: Concentration and percentage inhibition of nitric oxide (NO) activity of *Saccharomyces boulardii* and *Lactobacillus Acidophilus*

Concentration (μg/ml)	% inhibition of NO activity (*Saccharomyces boulardii*)	% inhibition of NO activity (*Lactobacillus Acidophilus*)
20	34.78±1.78	30.28±1.25
40	39.23±1.34	34.41±0.89
60	51.94±1.09	42.46±1.08
80	57.36±1.89	51.39±1.62
100	71.27±1.29	68.58±1.13

3.2. Drug loading and entrapment efficiency

The average drug loading capacity for mesalamine in uncoated and coated microparticles was found to be 68.47±1.2% and 69.23±1.7 respectively. The % average drug entrapment of mesalamine in uncoated and coated microparticles was found to be 82.49%±2.43 and 83.49%±3.15.

3.3. Compatibility study through IR Spectroscopy

The assessment of compatibility between drugs and excipients was carried out through infrared spectroscopy. Various vibrational frequencies were recorded for uncoated and CAP coated pectin based mesalamine microparticles. The specific IR peaks for mesalamine were originated at wave number (cm-1) at 3447.8, 1591.6, 2929.7, 1121.9 and 1384.7.for-OH,-NH,-CH,-CO and C-C stretch respectively in uncoated microparticles containing pectin and mesalamine as depicted in Fig. 3(A). A few characteristic bending peaks were also observed at 1121.9 and 767.8 corresponding to in plane and out of plane bending. In a similar fashion the vibrational frequencies were also recorded for coated microparticles containing pectin, CAP and mesalamine Fig.3 (B). These findings revealed that all the major peaks corresponding to mesalamine were there in IR spectra of uncoated as well as coated microparticles. It clearly indicated that mesalamine is fully compatible with polymers without any significant interference. The results also justified the stability of mesalamine in both the formulation without any indication of degradation.

3.4. *In-vitro* evalution of prepared micro-particles formulation

3.4.1. *In-vitro drug release*

This dissolution study was performed to mimic the conditions of the gastrointestinal tract as our main aim was to prepare colon targeted drug delivery systems of mesalamine and S.b. The prepared uncoated and coated

microparticles were placed to different simulated gastrointestinal fluids. In the case of a plain drug, burst drug release was observed in the initial 1h. More than 65% of the drug was released in the first 2 h in 0.1 N HCl (SGF) and around 90% drug was released within 6 h (SIF). In the case of uncoated microparticles, around 40% of the drug release was observed in the first 2 h in 0.1 N HCl (SGF). More than 70% of drug release was observed within 6 h (SIF). Around 90% drug release was observed within 10 h in SCF. In the case of coated microparticles, only 2-3% drug release was observed in the first 2 h in 0.1 N HCl (SGF). Due to the enteric coating of microparticles, CAP can restrict drug release in the stomach. This small quantity of drug release through this period may be due to the presence of the un-entrapped drug on the surface of the microparticles or diffusion of the drug from the outer surface of the pectin microparticles. Around 7% drug release was observed within the first 4 h. More than 35% drug release was observed within 6 h due to the removal of coating of CAP in SCF. About 70% drug release was observed within 10h. Almost the 96% drug release was accom-plished in the time frame of 24 h in SCF at pH 7.4 as showed in Fig.4.

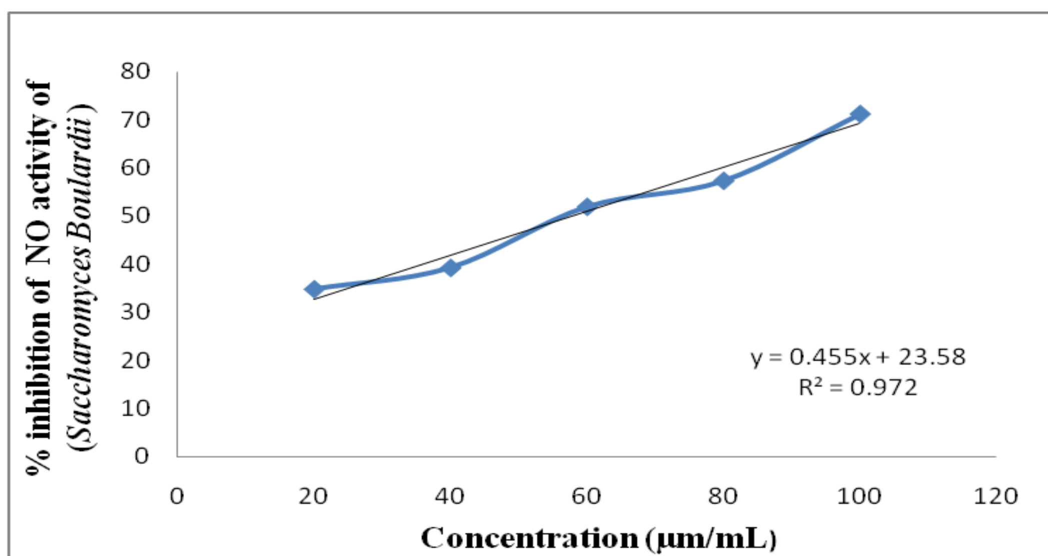


Fig. 1: Percentage inhibition of NO activity of *Saccharomyces boulardii* v/s concentration plot

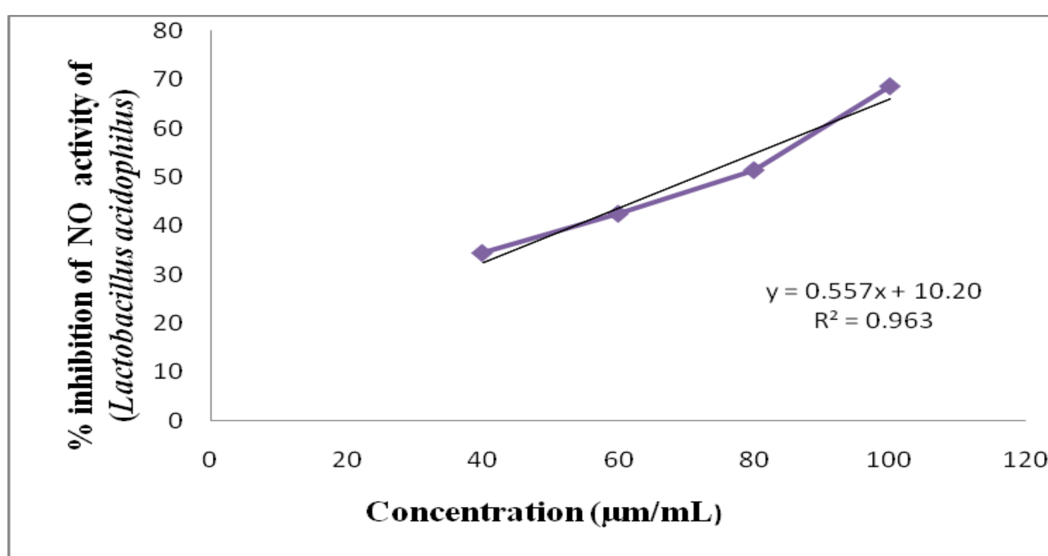


Fig. 2: Percentage inhibition of NO activity of *Lactobacillus Acidophilus* v/s concentration plot

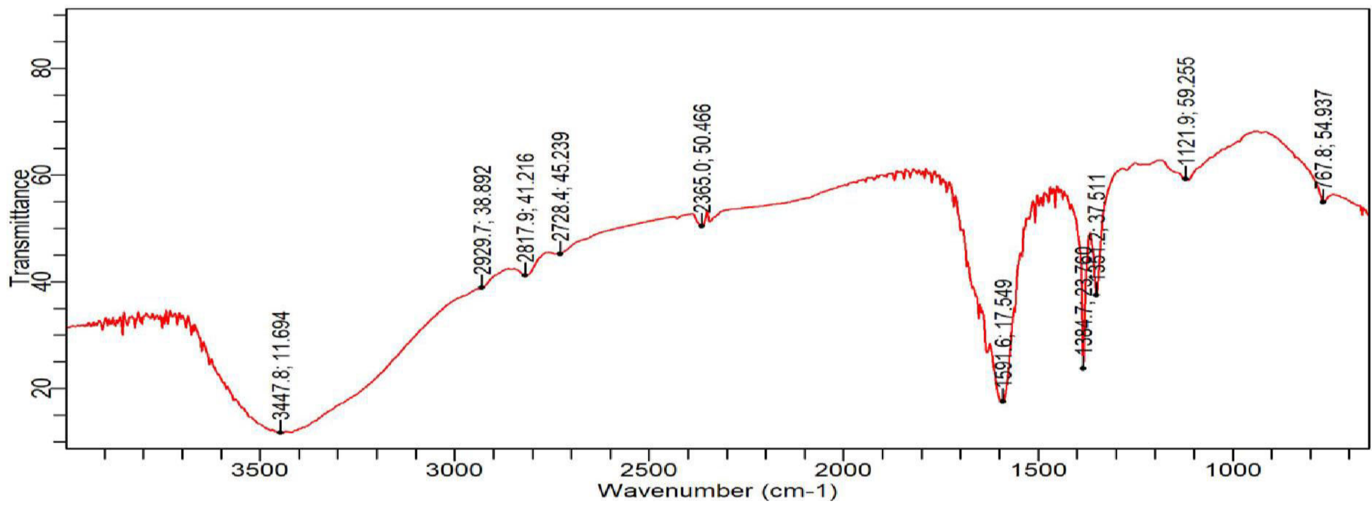


Fig.3 (A): IR wave numbers of uncoated microparticles

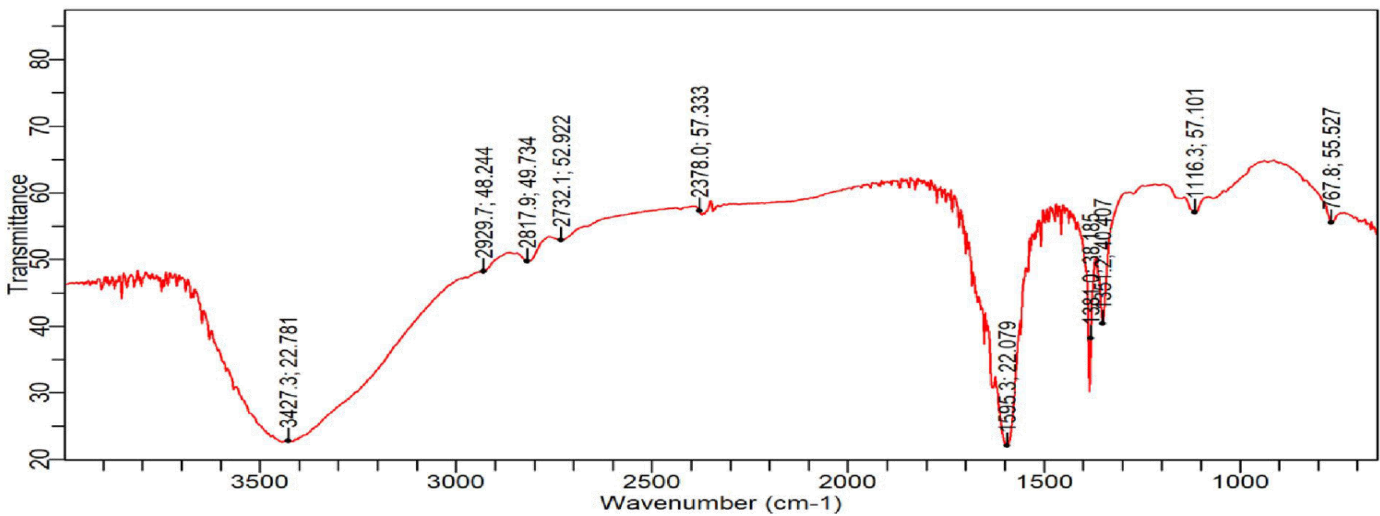
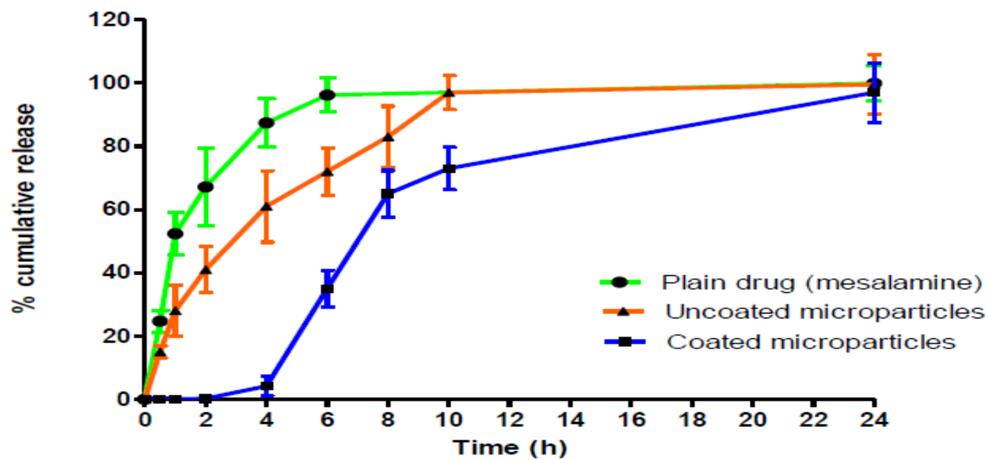


Fig. 3 (B): IR wave numbers of coated microparticles



Data are shown as mean ± SD

Fig. 4: Cumulative % drug release profiles of the plain drug, uncoated microparticles and coated microparticles in pH 1.2, phosphate buffer pH 6.8 and 7.4

3.4.2. *In -vitro drug release in the presence of rat cecal content medium*

The release of the drug and probiotic may be elevated up due to the mutual effect of the swelling and erosion of pectin polymer under the influence of favorable rat cecal content media. The Polysaccharide system of pectin can be degraded due to the enzymes present in rat cecal content [38]. In uncoated microparticles, almost 50% drug release was observed within the first 2 h in 0.1 N HCl (SGF) and more than 75% of the drug was released within the first 5 h. More than 94% of drug release was observed in 10h. In coated microparticles, only 2-3% of the drug was released in the first 2 h in 0.1 N HCl(SGF), due to the coating of microparticles with CAP. More than 30% of drug release was observed within 5h (SIF). But after changing the media, rat cecal content medium with 7.4 pH (SIF) burst drug release was observed due to the favorable environment for pectin due to the degradation of pectin by enzymes. Almost 85% of drug release was observed within 12h shown in Figure 5. From these results, it has been clear that the rat cecal medium boosted up the drug release in SCF.

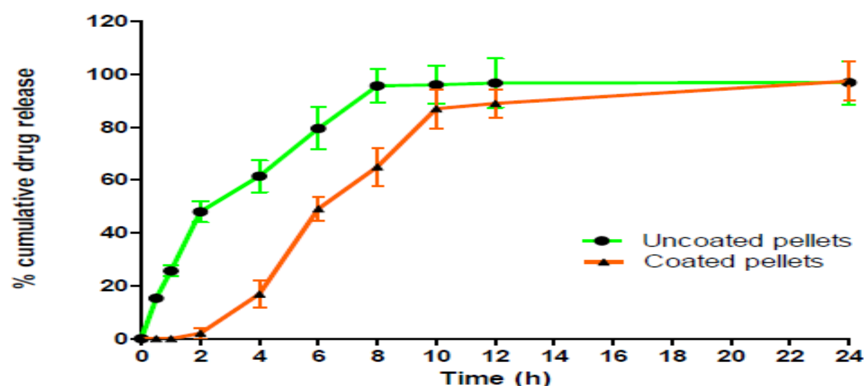
3.4.3. *In-vitro probiotic release and viability count*

The uncoated microparticles showed, near about 50% of probiotic was released in 2 h in SGF at pH 1.2. Almost 75 % of probiotic was released in SIF (pH 6.8) in 5 h uncoated microparticles. Approximately 90 % probiotic release was observed in 10h at pH of 7.4 SCF. Near about 70% of the probiotic release was observed within 8h. In the case of coated microparticles formulation in the first 2 h, in SGF at pH 1.2, only 2-4 % probiotic release was observed. At the 4th h pH 6.8, about 25% of probiotic releases were observed due to the eruption of coating polymer by SIF. Approximately 96% of probiotic release was observed within 24h as shown in Figure 6.A small rise in viable cell count was observed by a combination of drug and probiotic in pectin-based CAP coated microparticles confirmed that this combination can be used further for *in -vivo* studies. In SCF presence during the 10th h, uncoated and coated microparticles of S.b. showed 2.03×10^7 and 2.07×10^7 CFU/g viable count respectively.

3.5. *In-vivo* studies

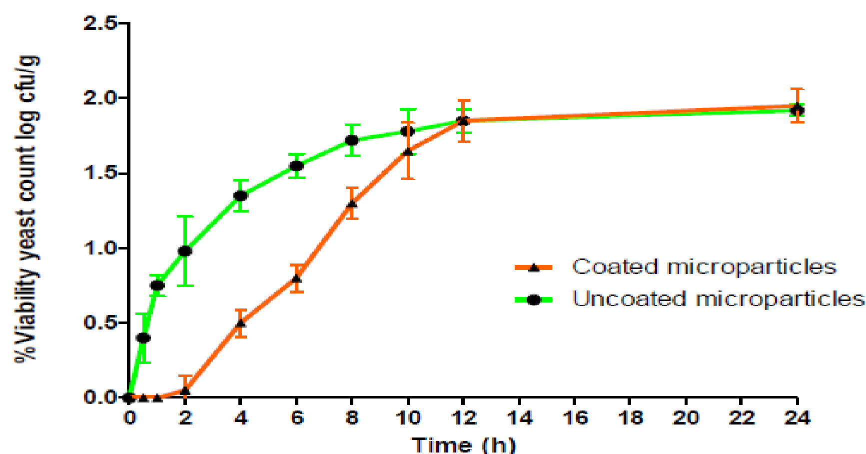
3.5.1. *Pharmacokinetics estimation*

The concentration against time profiles after administration via oral the uncoated and coated microparticles has been presented in Figure 7. After oral administration of uncoated microparticles, T_{max} was found to be 3.1±0.17, which was significantly different ($p<0.05$) from the 8.11±0.55 T_{max} obtained from the coated microparticles. The observed mean C_{max} for the uncoated microparticles (12.13±0.52 mg/mL) was much higher than that obtained from the coated microparticles (5.91±0.69 mg/mL). The determined pharmacokinetics parameters have been given in Table 2. The MRT value of drug obtained from the uncoated microparticles was about 4.3 h which was much lower than that of the coated microparticles (12.34 h). The AUC from the uncoated microparticles (34.45±1.38 μg/mL/h) was lower than that obtained from the coated microparticles (63.32±1.62μg/mL/h). Pharmacokinetic outcome founded the controlled drug release manners of the optimized microparticles formulation which recommends a more capable management of colitis by supplying more drug concentration at the colonic site and decreases the systemic drug absorption.



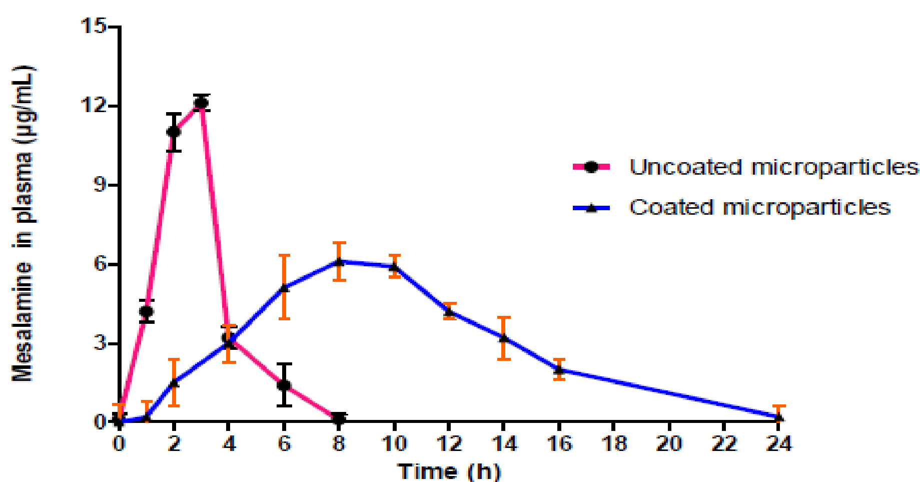
Data are shown as mean ± SD.

Fig. 5: Cumulative % drug release profiles of uncoated microparticles and coated microparticles in pH 1.2, phosphate buffer pH 6.8 and rat cecal content (pH 7.4)



Data are shown as mean ± SD.

Fig. 6: Viability count and release of S.b from uncoated microparticles and coated microparticles in pH 1.2, phosphate buffer pH 6.8 and rat pH 7.4



Data are shown as mean±SD (n=3).

Fig. 7: Plasma concentration profiles of mesalamine after oral administration of uncoated and coated microparticles in Wistar rats

Table 2: Pharmacokinetic parameters of mesalamine after oral administration of uncoated and coated microparticles

Parameters	Uncoated microparticles	Coated microparticles
C_{max} (mg/mL)	12.1	5.9
T_{max} (h)	3.1	8.1
AUC_{total} (µg/mL/h)	34.45	63.32
$T_{1/2}$	3.1	8.5
MRT (h)	4.3	12.34

3.6. Stability study

The stability study of coated microparticles was performed at temperature (25±2°C and 40±2°C) and (60±5% as well as 75±5%) respectively with relative humidity for 6 months. The changes in percentage drug entrapment, moisture content and % cumulative drug release of formulations have been shown in Table 3. There was no significant decrease in % drug entrapment, moisture content and % cumulative drug and probiotic release of formulations indicating high stability of the coated microparticles formulations as shown in Table 3.

Table 3: Stability of coated microparticles loaded with drug-probiotic after 6 month

Coated microparticles	Stability at 25±2°C				Stability at 40±2°C			
	0th day	60th day	120th day	180th day	0th day	60th day	120th day	180th day
% Drug entrapment	84.49%±3.15	84.21%±2.56	83.87%±4.51	83.38%±3.67	84.49%±3.15	84.31%±4.87	84.13%±6.23	83.12%±5.49
%Moisture content	4.98±0.36	5.35±0.56	5.74±0.18	5.98±0.36	4.98±0.36	3.96±0.48	3.06±0.31	2.17±0.43
% Cumulative drug release	75.12±1.3	74.54±1.9	74.29±2.1	73.11±3.7	75.12±1.3	75.27±2.8	74.47±2.4	74.87±3.6
% Cumulative probiotic release	95.89±2.5	95.46±3.5	95.09±1.9	94.45±3.9	95.89±2.5	95.26±4.1	94.56±4.7	94.11±3.4

4. CONCLUSION

This is the first report of the mesalamine with S.b. for specific delivery to the colon site. Wherein natural polysaccharides pectin was used for favourable degradation in the colon region only. The coating of enteric-coated CAP polymer was done which retarded the drug release in the stomach region. Based on IC_{50} value the S.b. was utilized in the formulation. The prepared coated microparticles were found to be stable as showed in FTIR graphs. In dissolution studies, the coated microparticles showed burst release after 5 h. Interestingly, the release of probiotic from the microparticles formulation was almost the same as the drug release in G.I.T. During the dissolution study a minor viability improvement of probiotic was observed in coated microparticles. The pharmacokinetic stu6dies showed a minor as well as delayed plasma concentration in the coated microparticles formulations endorsing the original hypothesis of drug exposure reduction in the non-target regions. The observed mean C_{max} for the uncoated microparticles (12.13±0.52 mg/mL) was much higher than that obtained from the coated microparticles (5.91±0.69 mg/mL).

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Declaration of competing interest

The authors declare no conflict of interest.

6. REFERENCES

1. Singh A, Kaur K, Mandal UK, Narang RK. *Curr Nanomedi* 2020;**10(3):**225-247.
2. Lee SH, Bajracharya R, Min JY, Han JW, Park BJ, et al. *Pharmaceutics*. 2020; **12(1):**52-68.
3. Kayal M, Shah S. *J Clin Med.* 2020; **9(1):**80-94.
4. Singh A, Kaur K, Kaur V, Singh G, Mandal UK, Mishra N, Narnag RK. *J Drug Del Thera* 2019; **9(3):**661-668.
5. Singh A, Singh G, Mandal UK, Narang RK. *Pharmaspire*. 2020; **12(2):**37-43.
6. Kim J, Muhammad N, Jhun BH, Yoo JW. *J Pharmac Investi*. 2016; **46(4):**377-386.
7. Singh A, Singh G, Bhattacharya S, Mishra N, Thakur A, et al. *Asian J pharma* 2020; **14(6):**1-3.
8. Khoder G, Al-Menhali AA, Al-Yassir F, Karam SM. *Experi and therc medI.*, 2016; **12(1):**3-17.
9. Kumar A, Gulati M, Singh SK, Gowthamarajan K, Prashar R, et al. *J of Drug Deliv Scien and Techn*. 2020; **60**.
10. Klayraung S, Viernstein H, Okonogi S. *International J of Pharmaceutics*. 2009; **370(2):**54-60.
11. Hua S, Marks E, Schneider JJ, Keely S. *Nanomedi: nanotech biology and medi*. 2015; **11(5):**1117-32.
12. Graff S, Hussain S, Chaumeil JC, Charrueau C. *Pharmaceutical research*. 2008; **25(6):**1290-1296.
13. Currò D, Ianiro G, Pecere S, Bibbò S, Cammarota G. *British j of pharmacology*. 2017; **174(11):**1426-49.
14. Avachat AM, Shinde AS. *Drug develop and industrial pharma*. 2016; **42(1):**80-90.
15. Sánchez B, Delgado S, Blanco Míguez A, Lourenço A, Gueimonde M, et al. *Mol nut food res.* 2017; **61(1):**1600240.
16. Dong JP, Zheng Y, Wu T, He Q, Teng GG, et al. *Chinese Medi J.*, 2019; **132(16):**1951.
17. Guslandi M, Giollo P, Testoni PA. *Eur j of gastroe & hepato*. 2003; **15(6):**697-698.
18. Thomas S, Metzke D, Schmitz J, Dörffel Y, Baumgart DC. *Ameri J of Physio-Gastro and Liver Phy*. 2011.
19. Chen L, Zou Y, Peng J, Lu F, Yin Y, et al. *J of Immuno Res*. 2015;**20**.
20. Abdin AA, Saeid EM. *J Cro and Col*. 2008; **2(4):**296-303.
21. Thakur V, Singh A, Joshi N, Mishra N. *Drug devel and indus pharma*. 2019; **45(11):**1807-1820.
22. Bayan MF, Bayan RF. *Fut J of Pharma Sci.* 2020; **6(1):**1-7.
23. Rashid M, Kaur V, Hallan SS, Sharma S, Mishra N.

Sau Pharmac J. 2016; **24**(4):458-472.

24. Ray S. *In Poly Carri for Drug Deli* 2019(pp. 737-762). Wood head Publishing 737-762.
25. Khotimchenko M. *Inter Bio Macro*. 2020; **158(1):** 1110-1124.
26. Moghimipour E, Rezaei M, Kouchak M, Fatahiasl J, Angali KA, et al. *Drug Develop Indus Pharma*., 2018; **44(5):**861-867.
27. Lee SH, Bajracharya R, Min JY, Han JW, Park BJ, et al. *Pharmaceutics*. 2020; **12(1):**68.
28. Nugent SG, Kumar D, Rampton DS, Evans DF. *Gut*. 2001; **48(4):**571-577.
29. Kaur V, Singh AS, Kaur K, Rath G. *J Drug Del and Ther*., 2020; **10(1):**111-122.
30. Maroni A, Moutaharrik S, Zema L, *State of the art*. 2017: 1027-1029.
31. Palugan L, Cerea M, Zema L, Gazzaniga A, Maroni A. *J Drug Deli Sci Tech*. 2015; **25**:1-5.
32. Eom SJ, Hwang JE, Kim KT, Paik HD. *Food sci and biotech*. 2018; **27(1):**185-91.
33. Jain A, Khare P, Agrawal RK, Jain SK. *J pharma sci*. 2009; **98(11):**4229-4236.
34. Lorenzo-Lamosa ML, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. *J f control rel*. 1998; **52(2):**109-118.
35. Kang RK, Mishr N, Rai VK. *Aaps Pharmscitech*. 2020; **21(2):**1-1.
36. Negi D, Singh A, Joshi N, Mishra N. *Anti-Cancer Agents in Medi Chem*.2020; **20(5):**589-598.
37. Mauritz JM, Morrisby RS, Hutton RS, Legge CH, Kaminski CF. *J pharmac sci*. 2010; **99(1):**385-91.
38. Jain A, Gupta Y, Jain SK. *J Pharm Pharm Sci,* 2007; **10**:86-128.