ISSN 2063-5346

FABRICATING COATED PECTIN-BASED RESVERATROL LOADED MICROPARTICLES BY BOX-BEHNKEN DESIGN OPTIMIZATION OF FOR COLONIC DELIVERY AND *IN VIVO* EVALUATION THEREOF

Puneet Kumar [*1], Dr. Shivali Singla [1], Dr. Sachin Goyal [1]

[1] School of Pharmacy, Abhilashi University, Chail-Chowk, Mandi (H.P.) – 175028, India

Corresponding author:Puneet Kumar , School of Pharmacy, Abhilashi University, Chail-Chowk, Mandi – 175028, Himachal Pradesh, India, Email: btpharma2011@gmail.com

Article History: Received: 01.02.2023 **Revised:** 07.03.2023 **Accepted:** 10.04.2023

Abstract

Purpose: The present work deals with the formulation and optimization of pectin-based colon specific microsphere formulation containing Resveratrol followed by comprehensive *in vitro* and *in vivo* evaluations thereof. **Methods:** Pectin based microparticles containing Resveratrol were prepared and optimised using Box-Behnken design and coated with Cellulose acetate phthalate. The formulations were evaluated by *in vitro* dissolution test in simulated gastric, intestinal, and colonic fluid with and without 4% w/v rat caecal matter. Drug-excipients compatibility study was also performed by FTIR spectrometric analysis. Drug release pattern was evaluated by mathematical modelling. *In vivo* pharmacokinetic study was also performed and the optimised formulation was evaluated for efficacy in a TNBS rat model of ulcerative colitis *in vivo.* **Results:** Box-Behnken design assisted successful formulation of optimised microspheres and the formulated microspheres were within the official limits. FTIR study showed no well-defined chemical interaction between drug and excipients. *In vitro* studies showed MF4 and MF5 as the best formulations. Hence *in vitro* study in 4% w/v rat caecal matter was performed which revealed greater drug release in that environment. Statistical analysis by unpaired student’s t test also supported the above-mentioned findings ($p<0.0001$). Mathematical modelling showed that release pattern follows the Peppas model. *In vivo* pharmacokinetic study demonstrated improved and favourable pharmacokinetic parameters for the microspheres. In TNBS induced colitis model, the microsphere formulation demonstrated significant improvement in TNBS induced colitis. The rats treated with microsphere formulation in different doses (10 mg or 20 mg/kg) via oral route and sulfasalazine (500 mg/kg) showed remarkable dose-dependent improvement of mentioned symptoms after 7 days of treatment. **Conclusion:** The study showed that the pectin-based CAP coated microparticles were synthesized and optimized successfully and can be used successfully for colon specific drug delivery system.

KEYWORDS: Pectin; Resveratrol; Colon Targeted; Microparticle; Rat Caecal Matter.

1. INTRODUCTION

Colon targeted drug delivery has achieved great importance as a targeted drug delivery system. Colon specific diseases are often inefficiently managed by oral therapy, because most orally administered drugs are absorbed before arriving in the colon.[1] Therefore colon-specific drug delivery systems, which can deliver drugs to the lower gastrointestinal tract without releasing them in the upper gastrointestinal tract, can be expected to decrease the side effects of the drugs and improve the quality of life of patients suffering from colon-specific diseases. Many colon-specific drug delivery systems have been investigated, not only to treat the colonic diseases, but also to improve the bioavailability of drugs.[1]

Various approaches have been reported during the last decade to develop new methodologies for colon site specific drug release, including pH sensitive delivery systems, gastrointestinal transit time of various formulation (time controlled release) ,microbially controlled release.[2] Colonic micro flora is increasingly recognized as preferable triggering component in the design of colon specific drug delivery systems, since the abrupt increase of the bacterial population and corresponding enzyme activities in the colon represent a continuous event independent of GI transit time. It has been well established, that the colon contains over 400 distinct species of bacteria having a population of 10^{11}-10^{12} CFU /ml.[3] The primary sources of carbon and energy for these bacteria is the fermentation of polysaccharide present in dietary residues.[3] The most promising colon-specific drug delivery systems are those based on the enzymatic action of colonic bacteria on polysaccharides.[4]

Based upon this natural phenomenon, colon specific drug delivery using pectin further coated with cellulose acetate phthalate (CAP) can be a promising technique for colonic drug delivery. Coming from renewable sources, pectin also have frequently economical advantage over other synthetic polymers.[5] The drug release from the system activated by colonic micro flora appears to be more suitable with regard to selectivity. Exploiting the use of this pectin based systems for colonic drug carrier means that issues of safety, toxicity and availability are simplified.[6], [7], [8, 9]

The purpose of the present study was to formulate colon specific microparticles using the pectin and CAP to target the colon. Resveratrol was used as model drug as it is absorbed throughout the gastrointestinal tract [10] and recent studies has also proved its efficacy in the treatment of colorectal cancer as well as ulcerative colitis.[11-13] The microparticles were tested for its efficacy by performing *in vitro* dissolution test with and without 4% w/v rat caecal matter. *In vivo* pharmacokinetic study and *in vivo* pharmacodynamic study in TNBS animal model were also performed for evaluation of the efficacy of the optimised formulation.

2. MATERIALS AND METHODS

2.1 Materials

Resveratrol was received as a gift sample from M Sea Pharmaceuticals Pvt. Ltd., Paonta-Sahib, India. Disodium hydrogen phosphate and Potassium dihydrogen phosphate was purchased from Loba Chemie Pvt. Ltd, Mumbai, India. Cellulose Acetate phthalate (CAP), Sodium metabisulphite and other excipients used to were of standard pharmaceutical grade and all other chemical reagents used were of analytical grade.

2.2 Methods

2.2.1 Drug and excipients compatibility study

The compatibility between drug, excipients and polymers was investigated using FTIR spectrophotometer (Shimadzu

FTIR 8400S,USA) according to potassium bromide pellet method.[14]

2.2.2 Formulation of colon targeted microparticles

The pectin microspheres were prepared using the method reported by Esposito et al. [15] with slight modifications. Briefly, the drug-polymer solution in distilled water (10 mL) was dispersed in 50 mL isooctane containing span 80 and the dispersion was continuously stirred at varied speed to obtain stable water/oil emulsion. The dispersion was rapidly cooled to 108°C and then 50 mL of acetone was added, in order to dehydrate the pectin droplets. The formulation was continuously stirred at 1000 rpm for 30 min at 30°C for complete solvent evaporation. Microspheres were freeze-dried and kept in airtight container [15, 16].

2.2.2.1 Optimization of uncoated microsphere

Systematic optimization of uncoated microsphere was done by Box-Behnken design [17] (BBD) employing three critical factors, pectin concentration (A); Stirring speed (B) and Span 80 concentration (C). Particle size (µm), entrapment efficiency (EE %) and recovery (%) were the key CQAs. A total seventeen runs were given by the design expert software for the uncoated microsphere to be prepared and evaluated for response variable such as particle size (µm), EE (%) and recovery (%).

2.2.2.2 Coating of Pectin Microparticles

Pectin microparticles coating was done using the oil-in-oil solvent evaporation method as reported by Lorenzo-Lamosa et al., (1998) with little changes [18]. 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.

2.2.3 Characterization microspheres

Percentage Yield

After preparation of microspheres by the above stated method the Yield (%) of the batches was calculated by the following formula [19]:

$$\text{Yield}(\%) = \frac{\text{Weight of nanoparticles obtained}}{\text{Weight of drug and polymer used for nanoparticles preparation}} * 100$$

Micromeritics properties

The flow properties of prepared microspheres were measured by using various factors like angle of repose, bulk density, tapped density, Carr's Index and Hausner's, ratio. These were measured by using following equations [20].

Drug Loading and Entrapment Efficiency Determination

Drug loading and entrapment efficiency were determined as per the protocol reported somewhere else using the back-calculation method. 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 [21, 22]. According to the following equations, drug loading and entrapment efficiency percentages were calculated:

$$Actual\ Drug\ loading(\%) = \frac{\text{Amount of drug present in microparticles}}{\text{weighed of microparticles sample analysed}} x100$$

$$Entrapment\ efficiency(\%) = \frac{\text{Amount of drug present in microparticles}}{\text{Amount of initial drug}} x100$$

Particle Size Analysis

The particle size and size distribution of the microspheres were measured by using Malvern NANO ZS90. The instrument is equipped with a solid-state laser, which employ dynamic light scattering (DLS).

Appropriate amount of the dried microspheres from each formulation was suspended in double distilled water and was sonicated for an appropriate period prior to the measurement. The resulted homogeneous suspension was then determined for the average hydrodynamic particle size, and size distribution [23, 24].

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)

The morphology of microspheres was examined using TEM (JEM CX 100) operating at 200kv capable of point-to-point resolution. Amalgamation of bright field imaging at magnification and of diverse modes was used to disclose the form and size of polymeric nanoparticle. The sample was done by negative staining. First the sample was allowed to dry on carbon coated grid and then negatively stained with 2% aqueous uranyl acetate solution [23, 25].The shape and surface morphology of the microspheres was examined using Scanning Electron Microscopy Hitachi SEM (S-3600N). Suitable amount of sample of nanoparticles was mounted on metal stubs, with a double-sided adhesive carbon tape and was splited with a razor blade. The samples were sputter-coated with gold under argon atmosphere for secondary electron emissive SEM and observed for morphology [23, 25].

***In-vitro* drug release study**

In vitro drug release study is one of the imperative analytical implements which can predict or provide basic information of the delivery system and its several characteristics. The release of drug from a controlled & targeted drug delivery system like microspheres is of particular significance and through it release mechanism and kinetics of the drug can be known. There has been significant advancement in the design and development of particulate systems but unfortunately still there is no new updated compendial or regulatory standard available for its *in vitro* drug release study. Many challenges are faced while opting both compendial and non-compendial *in vitro* release methods [26, 27].

For successful colon specific drug delivery, the drug needs to be protected from absorption from the environment of the upper gastrointestinal tract and then be abruptly released into the colon. Hence continuous efforts have been made on designing colon targeted drug delivery systems with improved site specificity and versatile drug release kinetics to fulfil different therapeutic needs.[28] In order to evaluate this parameter *in vitro* dissolution study of the formulations was performed in the upper gastrointestinal tract followed by colon for further 5 h. For *in vitro* evaluation of colon specific drug delivery systems, the ideal dissolution should closely mimic the *in vivo* conditions with regard to pH and types of enzymes. Dissolution testing of colonic delivery systems with conventional basket method has usually been conducted in different buffers for different periods of time that the colon specific delivery system might encounter *in vivo*.[3] In the present study, *in vitro* dissolution study was performed in 900 ml of (SGF) pH 1.2 for 2 hrs, pH7.4 (SIF) for 3 hrs and pH 6.8 (SCF) for 5 hrs. About 1 ml of sample was withdrawn at regular interval of time and diluted to 10 ml in volumetric flask .The apparatus was maintained at 37 0.5 ± 0.5°C and at 100 revolution per minute [29]. Finally, the drug concentrations in samples were measured in triplicates using UV-spectrophotometer (Shimadzu-UV1700 spectrometer, Shimadzu, Japan) at 303 nm.

Preparation of Rat caecal content medium

The IAEC (Institutional Animal Ethics Committee), Bilwal Medchem and Research Laboratory Pvt. Ltd., Vidhyadhar Nagar, Jaipur, Rajasthan according to the guidelines of CPCSEA (Committee for the purpose of Control and Supervision on Experiments on Animals) approved the

present experimental protocol (Reg. No.-2005/PO/RcBT/S/18/CPCSEA).

Accordingly, albino rats weighing 150-200g maintained on normal animal feed were used for the preparation of rat caecal content medium, induced with 1% w/v solution of pectin for 7 days. Thirty minutes before commencement of studies, 6 rats were killed, abdomen opened, the caeca isolated, ligated at both ends, cut loose and immediately transferred to pH 6.8 dissolution medium previously bubbled with CO_2. The caecal bags were opened; their contents were individually weighed, mixed and then suspended in pH 6.8 dissolution medium to give required caecal dilution of 4% w/v. As the caecum is naturally anaerobic, all these operations were carried out under supply of CO_2.

In vitro drug release study with 4%w/v rat caecal matter

To overcome the limitation of conventional dissolution testing, rat caecal contents have been widely utilized as alternative dissolution medium because of the similarity of human and rodent colonic micro flora. For example, the average log10 viable count of *Bacteriodes* and *Bifido bacteria*, two numerically predominant polysaccharide-degrading bacteria, is 8.0 and 7.0, and 8.0 and 8.2 in human large intestine and rat caecum respectively. Another advantage of utilizing rat caecal contents is the relatively easy availability of rats.[3]

On the basis of performance of *in vitro* dissolution study in SGF, SIF and SCF for 10 h, the best formulation/s which gave lowest cumulative release in SGF and SIF, but highest cumulative release in SCF was selected for further *in vitro* dissolution study with 4%w/v rat caecal matter.[30, 31] Initial studies were carried out at pH 1.2 for 2 h, after this ,the dissolution medium was changed to pH 7.4 for 3 h followed by pH 6.8 containing 4%w/v rat caecal matter and the dissolution was continued until the completion of 10 h. The dissolution was performed in six stages USPII dissolution rate apparatus, but slight modification was made to it. A beaker (capacity 250 ml, internal diameter 55 mm) containing 100 ml of dissolution medium was immersed in the water containing in 1000 ml dissolution flask, which was in turn, in the water bath of the apparatus. The microparticles were placed in the baskets of the apparatus and immersed in the dissolution medium containing 4% w/v caecal contents. The experiments were carried out with continuous CO_2 supply into the beakers to simulate anaerobic environment of the caecum.[32]

Mathematical treatment of the *in vitro* release kinetics

In order to understand its pharmacokinetic models, it is necessary to evaluate the mechanism by which drug is released from microspheres/pellets as well as its corresponding kinetics. Data obtained from the *in vitro* drug release studies were analysed using a range of kinetic equations such as zero order, first order, etc., and graphs were compiled based on these equations. In order to determine the value of r^2 and k, a regression analysis was performed on the corresponding linear plots [33]. The release kinetics of the best formulation/s was analysed according to zero order, first order kinetics, Higuchi and Korsmeyer-Peppas model. The correlation coefficients (r^2) were calculated for linearity.[34]

Statistical analysis of the *in vitro* release data

The *in vitro* release data of the best formulation/s with and without 4% w/v rat caecal matter was subjected to statistical analysis by student's 't' test to find out the extent increase in the release rate [35].

In vivo Pharmacokinetic studies

Male Wistar rats, weighing 250–300 g, were obtained from Bilwal Medchem and Research Laboratory Pvt. Ltd., Vidhyadhar Nagar, Jaipur, Rajasthan, India. All animals used in the experiments received care in compliance with the guidelines of

The Committee for the Purpose of Control and Supervision of Experiments on Animals. The experimental protocol was approved by the Institutional Ethical Committee, Bilwal Medchem and Research Laboratory Pvt. Ltd., Vidhyadhar Nagar, Jaipur, Rajasthan, India (approval no. CPCSEA – 2005/PO/RcBT/S/18/CPCSEA). The rats were randomly divided into two groups (n = 8) receiving plain Resveratrol suspended in 0.5% sodium CMC and optimized coated microspheres/pellet, respectively. Both the groups were further divided into two subgroups for blood withdrawals at alternate time points. The rats were fasted 12 h prior to dosing having free access to water. The dose of Resveratrol in both the groups was kept to 10 mg/kg p.o. Formulation was administered orally along with water with the help of infant feeding tube (No. 8) due to large particle size. Blood (0.5 ml) was withdrawn from both the groups at 5 min, 15 min, 30 min, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 25, 30 and 48 h through retro orbital plexus under anaesthesia. The blood was collected in the micro centrifuge tubes containing EDTA. Plasma samples (supernatant) were collected by centrifuging the blood samples at 5000 rpm for 10 min and stored at -20 0C until HPLC analysis. The pharmacokinetic parameters such as Cmax, Tmax were obtained from plasma concentration versus time curve whereas AUC0–48h was calculated by linear trapezoidal rule and compared between plain drug and optimized coated microsphere/pellet formulation.

In vivo Pharmacodynamic studies: TNBS induced colitis

Study Design and Induction of Colitis

The Laboratory Animal Center of Bilwal Medchem and Research Laboratory Pvt. Ltd., Vidhyadhar Nagar, Jaipur, Rajasthan, India with a pathogen-free environment, constant temperature (23 ± 2), and acceptable humidity (55 ± 5%) provided us with 48 male Wistar rats (aged 4-6 weeks, 150–200 grams) supplied with a balanced diet along with free access to water. The rats were fasted with free access to water for 24 h before induction of colitis. After rats were anesthetized with ketamine (50 mg/kg i.p), the rubber-tipped gavage needle was inserted into the anus of rats (7 cm) and 1 ml solution of 2,4,6-trinitrobenzenesulfonic acid (TNBS, 150 mg/kg dissolved in ethanol) was slowly injected into the colon while the control group received only ethanol. Animals were held in the head down position for 30 seconds and then returned to their cages [36-38]. Later, water and food were available. 12 hours after colitis induction, the treatments were started and continued one a day for six consecutive days. The effectiveness of treatment was assessed by clinical, macroscopic, and histopathological assessments. The rats' general conditions were assessed daily.

Experimental Animals.

A total of 48 Wister rats were divided into 6 groups (8 rats per group, n = 8). Group I: Healthy control group (Normal control); Group II: TNBS-induced colitis untreated rats (Positive control); Groups III: TNBS-induced colitis treated rats who received Free Resveratrol 10 mg/kg orally Groups IV: TNBS-induced colitis treated rats who received MF 10 mg/kg orally; Groups V: TNBS-induced colitis treated rats who received MF 20 mg/kg orally and Groups VI: TNBS-induced colitis treated rats who received Sulfasalazine (treated control)

Macroscopic and histopathological scoring

The dosage and period of treatments were accompanied by daily body weights, gross stool evaluation for visible and/or occult bleeding. On the last day of the experiment (7th), the degree of colonic inflammation and damage was scored (Table 1.) as described by Morris et al. with slight modifications [39, 40]. After the 10 cm of distal colon segments of slaughtered rats have been dissected (under the guide of

anaesthesia), flushed with cool saline, and weighted, histologic assessment of colitis was performed by a drug blinded expert pathologist. The histopathological assessment of damage was determined according to the numerical grading score introduced by Wallace et.al outlined in Table 2. [41]. The edema intensity was estimated by calculating the ratio of wet tissue weight to the length of the colon.

Table 1. Criteria for scoring of gross morphologic damage [38].

Score	Gross morphology
0	No damage
1	Localized hyperemia, but no ulcers or erosions
2	Ulcers or erosions with no significant inflammation
3	Ulcers or erosions with inflammation at one site
4	Two or more sites of ulceration and/or inflammation
5	Two or more major sites of inflammation and ulceration or one major site of inflammation and ulceration extending >1 cm along the length of the colon

Table 2. Criteria for histological scoring of damage.

Appearance	Score
Loss of mucosal architecture	0, 1, 2, or 3 (absent, mild, or severe)
Cellular infiltration	0, 1, 2, or 3 (absent, mild, or extensive)
Muscle thickening	0, 1, 2, or 3 (absent, mild, or extensive)
Crypt abscess formation	0 or 1 (absent or present)
Goblet cell depletion	0 or 1 (absent or present)
Total	Summation

4.2.6.12 Stability study

Stability studies of the optimized coated microsphere formulations were conceded according to the guidelines of ICH. The optimized drug loaded microparticles/pellets, were conducted at 25˚C±2˚C and 40˚C±2˚C,60% RH ±5%and 75±5% RH respectively. In the coated microparticles/pellets, 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 release was evaluated [42].

4.2.6.13 Statistical analysis

All the results are the mean ± SD of three independent experiments. The significance of differences ($p < 0.05$) between experimental variables was determined by the use of two-tailed Student's test as well as by one way ANOVA (Analysis of Variance) followed by *post hoc* Turkey's test (GraphPad Software Package). The statistical significance was indicated by $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Drug and excipients compatibility study

Based on the individual FTIR spectrum of pectin, Resveratrol and CAP and its comparison to the spectrum of Pectin-Resveratrol-CAP physical mixture, it is evident that major as well as the characteristic peaks of the drug and the excipients were found to be retained in the spectra, indicating that there is no

incompatibility between the excipients, suggesting that they are perfectly stable.

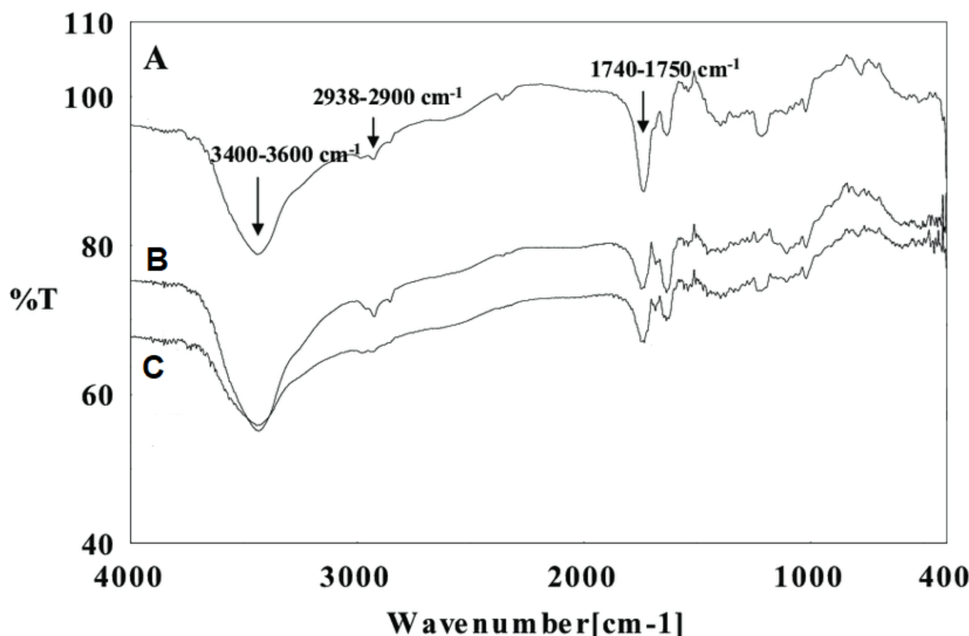

Figure 1. A. Shows FTIR spectra of pure Resveratrol. B. Shows FTIR spectra of pure Pectin. C. Shows FTIR spectra of physical m mixture.

5.1.2 Preparation of Resveratrol loaded microspheres

5.1.4.1 Preparation of uncoated microspheres

The pectin microspheres were prepared using the method reported by Esposito et al. [15] with slight modifications [15, 16].

5.1.4.2 Optimization of uncoated microsphere: Box-Behnken design (BBD) matrix

Systematic optimization of uncoated microsphere was done by Box-Behnken design (BBD) employing three critical factors, pectin concentration (A); Stirring speed (B) and Span 80 concentration (C). Particle size (µm), entrapment efficiency (EE %) and recovery (%) were the key CQAs. Table 3. shows design matrix, composition of seventeen formulations in coded form and level of critical factors. A total seventeen uncoated microsphere were prepared and evaluated for response variable such as particle size (µm), EE (%) and recovery (%). The second order quadratic models were applied to identify significant immediate interaction between studied critical factors. One-way analysis of variance (ANOVA) was employed to determine the significance of the model ($P<0.05$) and individual response. The residual plot and other parameters such as F-value, P-value, regression coefficient (R^2) and lack of fit were used to determine the good fit of the model. Response surface analysis was carried out with the help of 3D-plots for thorough understanding of the effect of each critical factors on the CQAs. Finally, the optimized formulation was selected from the numerical optimization based on the desirability function and the design space was also determined by graphical optimization.

Table 3. Box-Behnken design (BBD) matrix displaying seventeen formulations with varying levels of factors and result showing impact on CQAs.

Code	Run	A	B	C	Y1	Y2	Y3
MS1	1	0	1	-1	130.80 ±2.23	88.35±1.39	88.83±5.62
MS2	2	0	1	1	91.05±1.83	72.76±1.63	78.27±3.26
MS3	3	-1	0	-1	105.72±2.93	62.34±1.92	57.64±2.43
MS4	4	0	0	0	125.34±1.38	79.19±2.65	84.25±4.61
MS5	5	0	0	0	123.87±3.82	77.32±1.35	84.28±3.24
MS6	6	1	-1	0	219.58±1.57	89.23±1.79	69.83±5.73
MS7	7	0	0	0	126.80±3.82	77.24±1.83	84.21±1.53
MS8	8	-1	0	1	69.42±1.58	55.59±1.62	46.82±2.83
MS9	9	1	0	-1	201.33±2.72	87.32±1.02	69.62±2.46
MS10	10	1	1	0	174.57±1.96	92.13±1.11	80.43±3.09
MS11	11	0	0	0	126.43±0.93	78.28±1.86	85.21±4.32
MS12	12	-1	-1	0	125.12±2.06	63.24±2.19	57.05±1.09
MS13	13	-1	1	0	81.21±1.53	75.32±1.56	68.43±3.25
MS14	14	0	0	0	126.42±2.42	79.51±1.21	84.69±2.46
MS15	15	0	-1	1	135.61±1.28	65.93±1.10	66.67±4.05
MS16	16	1	0	1	160.57±1.62	72.48±2.17	58.86±3.28
MS17	17	0	-1	-1	174.51±2.24	70.95±1.63	77.47±4.82
Factors					**-1**	**0**	**+1**
A = Pectin concentration (%)					3.0	4.0	5.0
B = Stirring speed (RPM)					500	1000	1500
C = Span 80 concentration (%)					0.5	1.0	1.5

Y1= Particle size(μm)); Y2 = Entrapment efficiency (EE %); Y3=Recovery (%)

The responses were analyzed for ANOVA and result was presented in table 4. The table 5 shows coefficients of the polynomial equations generated using multiple linear regression analysis for all the CQAs. The significant p value (>0.05) and high F value of model for all three responses revealed that responses well fitted in selected model. The insignificant lack of fit test and closeness between adjusted R^2 and predicated R^2 also verified good fitting of selected models.

Table 4. Summary of ANOVA and other statistical parameters for the CQAs of uncoated microspheres.

Source	SS	Df	MSS	F	P value	Adj R^2	Pred R^2	Aedq preci
Response Y1 (Particle size)								
Model	25840.79	7	3691.54	4817.48	< 0.0001	0.9995	0.9994	249.70
Residual	6.90	9	0.77		-	-	-	-
Lack of fit	0.94	5	0.19	0.13	0.9781	-	-	-

Pure error	5.95	4	1.49	-	-	-	-	-
Cor total	25847.69	16		-	-	-	-	-
Response Y2 (Entrapment efficiency)								
Model	1548.26	7	221.18	71.65	< 0.0001	0.9687	0.9228	30.345
Residual	27.78	9	3.09	-	-	-	-	-
Lack of fit	23.44	5	4.69	4.32	0.0906	-	-	-
Pure error	4.34	4	1.09	-	-	-	-	-
Cor total	1576.04	16	-	-	-	-	-	-
Response Y3 ((Recovery)								
Model	2475.77	9	275.09	2071.47	< 0.0001	0.9991	0.9983	150.21
Residual	0.93	7	0.13	-	-	-	-	-
Lack of fit	0.20	3	0.066	0.36	0.7852	-	-	-
Pure error	0.73	4	0.18	-	-	-	-	-
Cor total	2476.70	16	-	-	-	-	-	-

Table 5. Coefficient and p value of terms for the CQAs of uncoated microspheres.

Factor	**Response Y1**		**Response Y2**		**Response Y3**	
	Coefficient	**P value**	**Coefficient**	**P value**	**Coefficient**	**P value**
A	46.81	<0.0001	10.58	<0.0001	6.10	<0.0001
B	-22.14	<0.0001	4.39	<0.0001	5.62	<0.0001
C	-19.47	<0.0001	-4.78	<0.0001	-5.37	<0.0001
AB	-0.26	**0.6082**	-2.30	0.0078	-0.19	**0.32**
AC	-1.13	0.05	-2.02	**0.0141**	0.015	**0.9367**
BC	-0.21	**0.6713**	-1.66	0.0323	0.060	**0.7516**
A^2	12.80	<0.0001	-1.19	**0.0908**	-17.58	<0.0001
B^2	11.56	<0.0001	2.86	**0.0022**	1.99	<0.0001
C^2	-4.31	<0.0001	-7.69	**<0.0001**	-8.71	<0.0001

Term indicated with Bold were insignificant (p> 0.05)

The mean particle size for all formulations was in the range 69.42±1.58 - 219.58±1.57 µm. The reduced polynomial equation for mean particle size in coded value was given below

$$Y_1 = +125.74 + 46.81A - 22.14B - 19.47C - 1.13AC + 12.80A^2 + 11.56B^2 - 4.31C^2$$

The sign of coeffecient (table 5.4.) revealed that A has positive effect whereas B and C have negative effect on the particle size.Interaction terms AC has negative effect on mean particle size. The quadratic terms A^2 and B^2 were favourable for increasing particle size while interaction term C^2 was unfavourable. The 3-D response surface graph (Figure 2.) shows effect of critical factors on particle size. The value of the coeffecient revealed that the pectin concentration has the highest effect over the particle size. The particle size increased as concentration of pectin increased. The increase in size of microsphere could be explained on basis of

the viscosity of dispersing media which was increased due to higher concentration of the pectin and causes larger droplets in the emulsion resulting in increased size[43].The particle size decreases with an increase in Span 80 concentration. This may be due to decrease in interfacial tension between dispersed droplets of pectin and oil leads reduction in droplets size and results decrease in particle size. With the increase of steering speed, the shear stress increases due to which the particle size decreases.

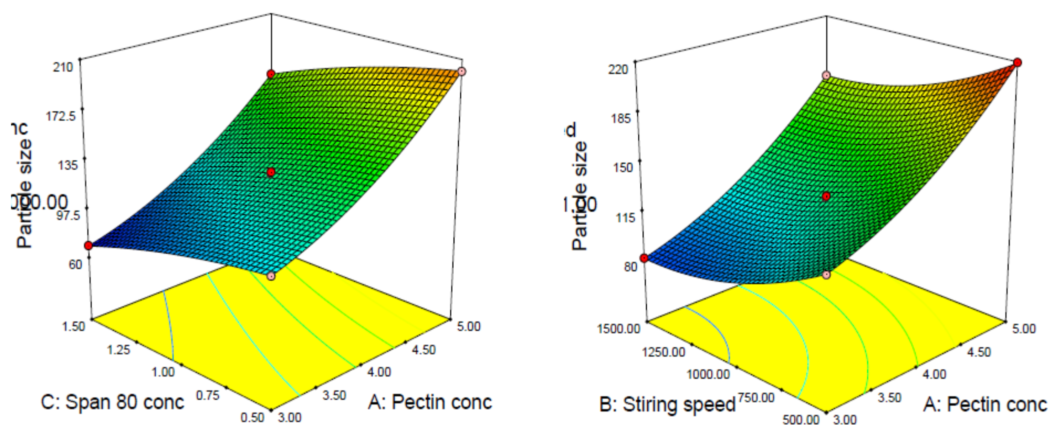

Figure 2: 3D response graph showing the effect of critical factors on particle size.

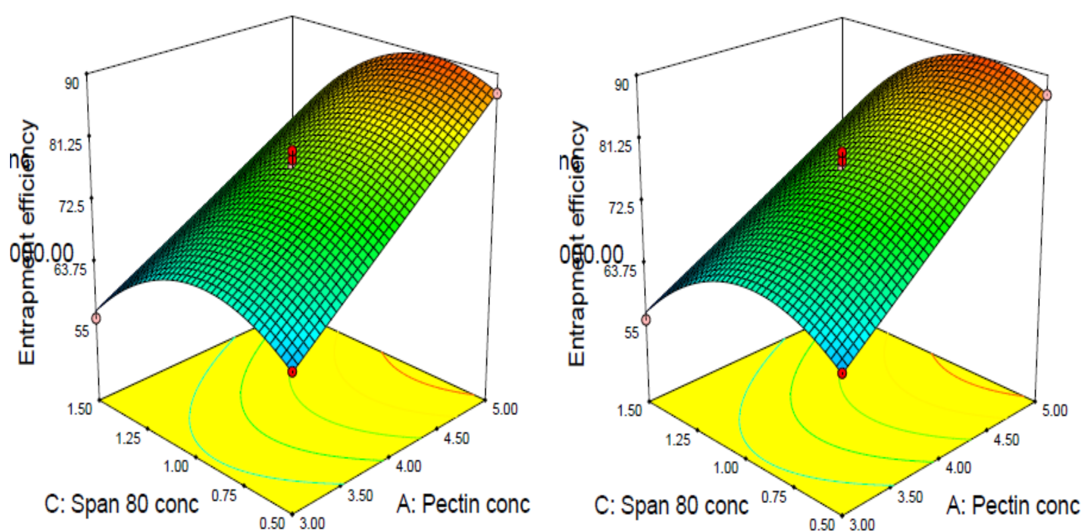

Figure 3: 3D response graph showing the effect of critical factors on EE.

The EE (%) of various microspheres was ranged from 55.59±1.62 % - 92.13±1.11 %. The effect of critical factors on EE is presented by reduced polynomial equation.

$$Y_2 = +77.81 + 10.58A + 4.39B - 4.78C - 2.30AB - 2.02AC + 2.80B^2 - 7.75C^2$$

The sign of coefficient displayed that pectin concentration and steering speed have positive effect on EE. With an increase in span 80 concentration EE decreases. The influence of critical variables on EE is depicted by response 3D plot (Figure 3.). The pectin concentration has highest effect on EE followed by span 80 concentration. The EE increased with increase in pectin concentration and speed indicating an enhancement in the loading efficiency of drug.

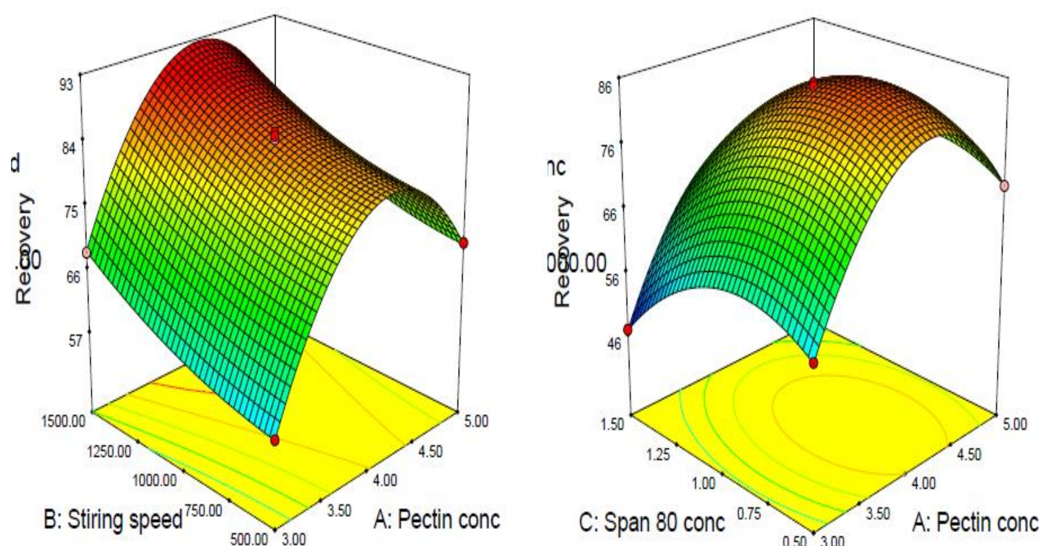

Figure 4: 3D response graph showing the effect of critical factors on recovery.

The recovery of all formulations varied from 57.05±1.09 - 88.83±5.62 %. The effect of critical factors on $T_{60\%}$ was presented as the reduced quadratic model equation as below.

$$Y_3 = +84.53 + 6.10A + 5.62B - 5.37C - 17.58A^2 + 1.99B^2 - 8.71C^2 \quad (6)$$

Pectin concentraion and steering speed have positive effect whereas span 80 concentration has negative effect on recovery(%). This is illustrated in 3D plot (Figure 4.). From the plot it is revealed that the recovery increases with increase in the pectin concentration and steering speed.

Selection of optimum formulation

The CQAs acceptance value was set within a desired range to select optimum microsphere formulation and to create a design space for formulation and process parameters. The value of adequate precision greater than 4 revealed that applied model can employed to navigate design space [44] (Table 5). The mean particle size was set to NMT 150 µm, EE was set to NLT 70% whereas recovery was set to NLT 70%. Figure 4 depicts an overlay plot comprising of two regions, the yellow region referring the optimal region and the gray area where the response was not in the acceptance criterion [45].

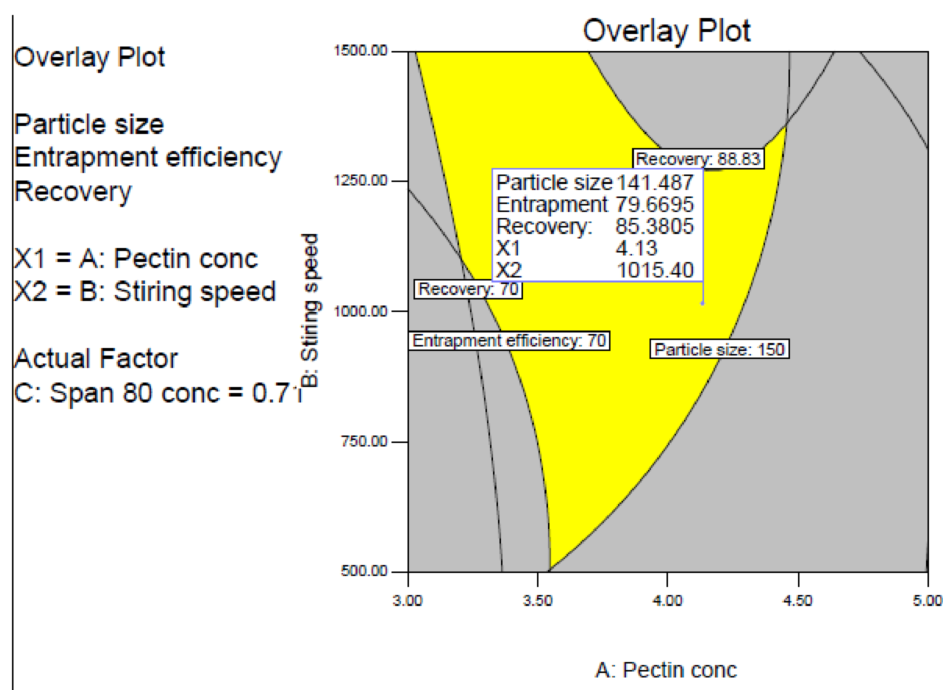

Figure 5. Design space showing optimize region for uncoated microsphere.

To select optimum formulation, numerical optimization approach was employed and setting for optimum microsphere formulation is shown in table 6. Lower value of biases and relative biases indicated close agreement with small differences between observed and targeted value of responses.

Table 6. Level of parameters, predicate value and experimental result for optimise formulation

Optimal setting	CQA	Predicated	Observed	Absolute biases	Relative biases (%)	Desirability
A = 4.13 %	Y_1 (µm)	141.49	143.45	1.38	2.24	100
B = 1015 RPM	Y_2 (%)	79.67	80.32	0.65	0.81	
C = 0.71 %	Y_3 (%)	85.35	82.81	2.54	2.97	

5.1.4.3 Characterization of the optimum uncoated microspheres

Recovery, entrapment efficiency

The entrapment efficiency and recovery of the optimum uncoated microsphere was found 80.32 ± 2.91 %, and 82.81 ± 3.14 % respectively.

Particle size

The mean particle size and polydispersibility index (PDI) of optimum uncoated microsphere were found 143.45 ± 4.82 µm and 0.65 respectively.

In-vitro dissolution and drug release kinetic study

Dissolution study was performed in different pH. *In-vitro* drug release profiles of resveratrol from optimum uncoated microsphere has been presented in figure 6. Table 7. shows the results of fitting of the drug release data for resveratrol from microsphere into various kinetic models. The result shows that resveratrol release found to be best fitted in the Higuchi kinetic

model (R^2 = 0.9891) and diffusional release exponent (n) of Korsmeyer Peppas model was found 0.7243 (< 0.5) indicating grossly non fickian diffusion [46].

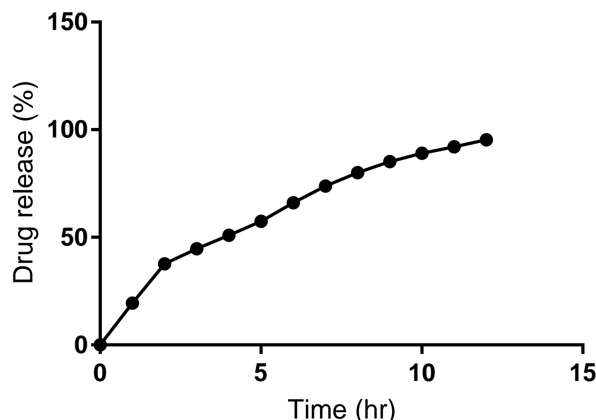

Figure 6. ***In vitro*** **drug release of resveratrol from optimised uncoated microsphere**

Table 7. Drug release kinetic analysis for optimized microsphere

Zero order		**Higuchi Kinetic**		**First order**		**Hixson Crowel**		**Korsmeyer Peppas**	
R^2	Ko	R^2	K_h	R^2	K_1	R^2	K_{hc}	R^2	n
0.9364	0.1271	0.9891	3.804	0.9874	0.0034	0.962	0.0008	0.973	0.7243

5.1.4.4 Coating of optimised Pectin Microparticles

Pectin microparticles coating was done using the oil-in-oil solvent evaporation method as reported by Lorenzo-Lamosa et al., (1998) with little changes [18]..

5.1.4.5 Characterization of optimised Resveratrol loaded coated microspheres

Percentage Yield of microspheres

The percentage yield of the prepared five formulations were as follows in table 8.

Table 8. Percentage Yield of optimised coated microspheres

Formulation Code	**Yield (%) (Mean ± SD) ***
MF1	77.43±0.35
MF2	77.67±0.79
MF3	76.89±0.52
MF4	77.98±0.97
MF5	77.63±0.89

Micromeritics properties of microspheres

The flow properties of prepared microspheres were measured by using various factors like angle of repose, bulk density, tapped density, Carr's Index and Hausner's, ratio. These were measured by using following equations [20].

Drug Loading and Entrapment Efficiency Determination

The average drug loading capacity for Resveratrol in coated microparticles was found to be in the range of 66.58±1.32 to 69.37±1.63 respectively. The % average drug entrapment of Resveratrol in coated microparticles was found to be in the range of 80.58±1.86 to 83.77±1.75.

Table 9. Micromeritics properties of optimised coated microspheres

Formulation codes	Angle of repose	Hausner's ratio	Compressibility index	Tapped density	Bulk density
MF1	18.46 ± 1.46	1.19 ± 0.11	15.62 ± 1.32	0.32 ± 0.02	0.27 ± 0.01
MF2	22.53 ± 1.95	1.15 ± 0.32	12.90 ± 2.42	0.31 ± 0.01	0.27 ± 0.01
MF3	24.81 ± 2.36	1.18 ± 0.15	15.15 ± 1.61	0.33 ± 0.01	0.28 ± 0.01
MF4	24.67 ± 2.56	1.12 ± 0.59	10.81 ± 2.41	0.37 ± 0.08	0.33 ± 0.01
MF5	26.38 ± 2.45	1.18 ± 0.29	15.79 ± 2.37	0.38 ± 0.04	0.32 ± 0.01

Table 10. Characteristics of Resveratrol Loaded Polymeric microspheres

Formulation code	Average Particle size (µm)	Polydispersity index (PDI)	Drug loading (%)	Entrapment efficiency (%)
			(Mean ± SD) *	
MF1	12.43 ± 1.63	0.705	66.58±1.32	80.58±1.86
MF2	22.41 ± 1.55	0.673	67.34±1.61	81.39±1.24
MF3	36.13 ± 1.27	0.641	69.37±1.63	82.56±1.91
MF4	30.566 ± 1.21	0.711	68.83±1.33	81.81±1.85
MF5	33.14 ± 1.16	0.609	67.28±1.61	83.77±1.75

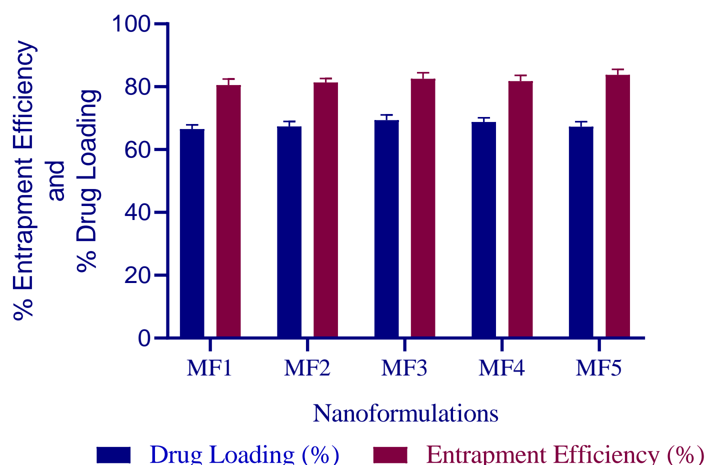

Figure 7. Entrapment efficiency and drug loading of MF1-MF5.

Particle Size Analysis

The particle size and size distribution of the microspheres were measured by using Malvern NANO ZS90 [23, 24].

Scanning Electron Microscopy (SEM)

As shown in the SEM and TEM images, the microparticles were found to be spherical and had smooth surfaces as shown in the figures 8.

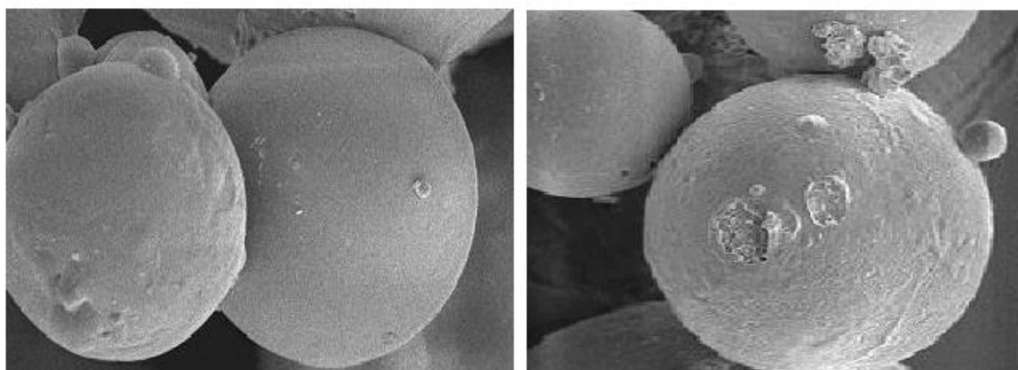

Figure 8. SEM images of prepared Resveratrol loaded microspheres

In-vitro drug release study

The release of Resveratrol from the formulated microparticles in simulated gastric, intestinal, and colonic media was investigated. Figure 9. reveals that the release of Resveratrol in gastric media for the first two hours was very less ranging from 0.9-5.59%. The release pattern also showed that drug release was lesser in formulation containing higher proportion of polymer. Drug release was comparatively higher in SIF ranging from 23.72%-32.41% as shown in table 11. However, release of drug from MF4 and MF5 was 31.96% and 37.44% respectively, this result clearly indicates that higher the proportion of coating higher is the ability to prevent the drug release in upper GIT. 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. Release of drug increased significantly in SCF from 44.66% to 60.12%, release rate at this environment was more for formulation MF4 and MF5. These release patterns clearly indicate the potential of this formulation to be a suitable colonic drug delivery carrier.

In vitro drug release study with 4% w/v rat caecal matter

Based on *in vitro* release pattern in SGF, SIF and SCF, formulation MF4 and MF5 was selected for further *in vitro* studies in SCF containing 4% w/v rat caecal matter as it predominantly renders anaerobic environment. The release pattern was similar during the first 5 h in SGF and SIF, but release rate increased significantly in SCF containing 4% w/v rat caecal matter in the 6 hrs (73.45% and 74.37%) as shown in the figure 10. The release rate increases to 77.38% and 79.46% in SCF containing 4% w/v rat caecal matter at the end of 10h as shown in table 12. This clearly reveals the susceptibility of the pectin-based system for biodegradation in anaerobic environment of colon which is present *in vivo.*

The colonic bacteria are predominately anaerobic in nature and secrete enzymes that are capable of metabolizing substrates such as carbohydrates and proteins that escape the digestion in the upper GI tract. Most common mechanisms of microbial activation in the colon are azo-reduction and glycosidic-bond hydrolysis.[47] Pectin consists of the methyl esters rhamnose, galacturonic acid, galactose, glucose and glucuronic acid. The galacturonic acid in the pectin could be in the L-configuration.

The main structural elements of pectin as reported earlier concluded that it contained a repeating unit of galacturonic acid residues.[48] The findings as shown in Figure 10 that drug release is less in upper gastrointestinal tract and comparatively more in colonic environment containing 4% w/v rat caecal matter can be clearly related to the above mentioned facts.

Table 11. Shows *in vitro* % cumulative release of formulations MF1-MF5 in SGF, SIF and SCF for ten hours.

Time (Hours)	% CR (Cumulative Release)				
	MF1	MF2	MF3	MF4	MF5
0	0	0	0	0	0
1	3.19±0.42	2.98±0.46	1.3±0.56	1.3±0.43	0.9±0.51
2	5.59±0.63	4.86±0.54	2.6±0.65	2.03±0.49	1.7±0.47
3	16.78±0.55	22.78±0.45	24.85±0.47	26.78±0.35	28.91±0.47
4	23.72±0.46	28.87±0.48	30.67±0.44	31.56±0.19	32.41±0.67
5	28.66±0.38	34.67±0.55	35.52±0.46	36.96±0.64	37.44±0.49
6	44.66±0.43	49.78±0.52	50.09±0.52	52.15±0.42	53.46±0.58
7	49.33±0.57	54.65±0.72	56.19±0.48	56.39±0.46	56.56±0.49
8	51.44±0.43	56.76±0.48	57.13±0.50	57.85±0.38	57.41±0.44
9	53.49±0.47	57.80±0.39	58.24±0.61	58.67±0.56	58.75±0.48
10	55.55±0.71	59.13±0.47	59.61±0.42	59.65±0.51	60.12±0.54

Table 12. Shows *in vitro* % cumulative release of formulations MF4 and MF5 in SGF, SIF and SCF containg 4% w/v Rat caecal matter for ten hours.

Time (Hours)	% Cumulative Release in SCF containing 4% w/v Rat caecal matter from sixth hours	
	MF4	MF5
0	0	0
1	1.3±0.37	0.98±0.48
2	2.15±0.51	1.97±0.61
3	26.78±0.53	28.75±0.53
4	31.56±0.38	32.41±0.44
5	36.76±0.39	37.34±0.47
6	73.45±0.58	74.37±0.51
7	74.67±0.63	75.51±0.54
8	75.45±0.58	76.62±0.48
9	76.15±0.49	77.51±0.36
10	77.38±0.47	79.46±0.46

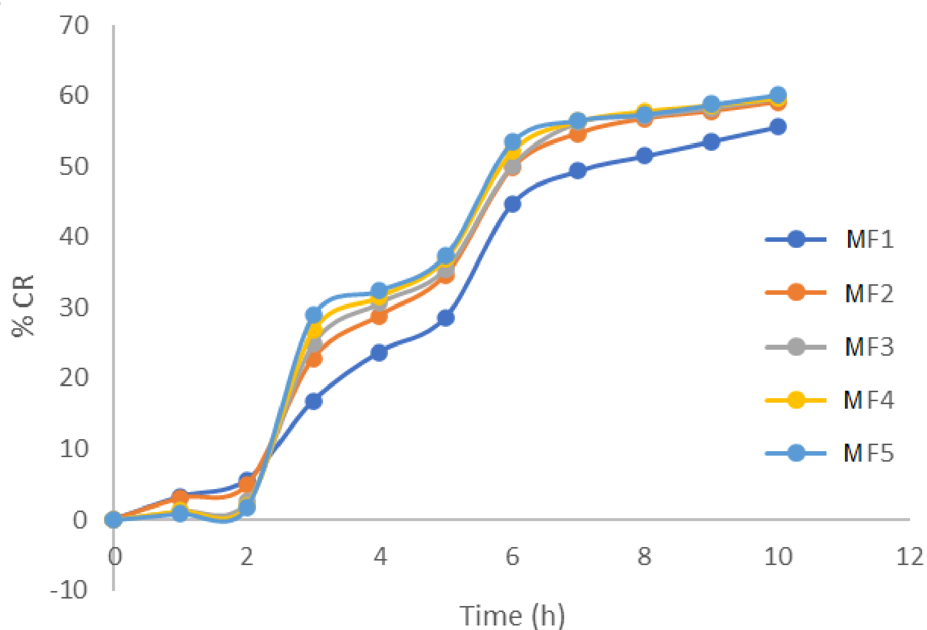

Figure 9. Shows *in vitro* cumulative drug release for ten hours in SGF (pH 1.2), SIF (pH 7.4) and SCF (pH 6.8)

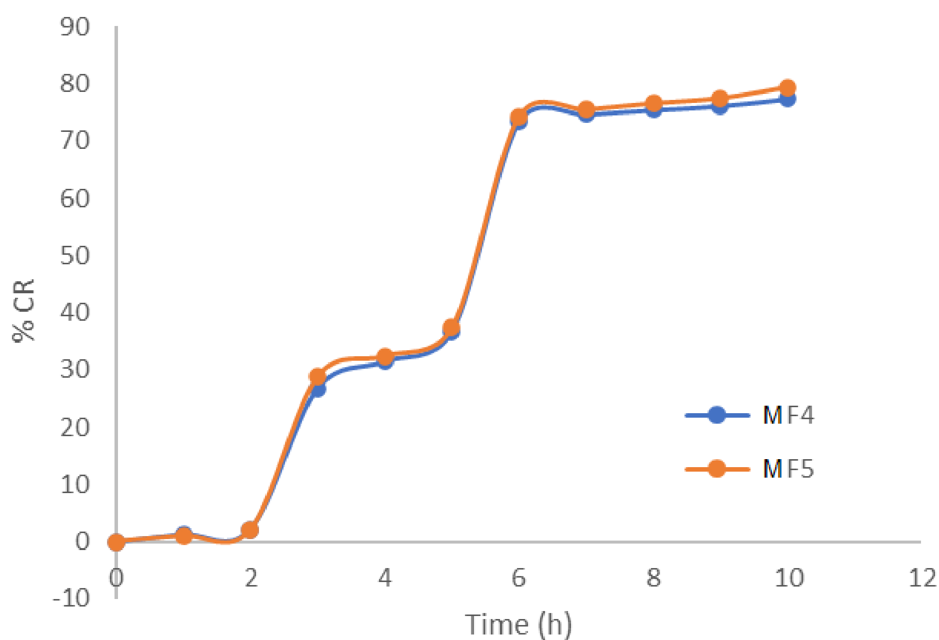

Figure 10. Shows in vitro cumulative release of formulations MF4 and MF5 in 4% w/v Rat caecal matter.

Table 13. Shows data for r^2 values of formulations MF4 and MF5 for different mathematical models.

Formulation	r^2 value	Mathematical Model
MF4	0.901	Zero order
	0.884	First order
	0.830	Higuchi
	0.910	Peppas
MF5	0.902	Zero order
	0.901	First order
	0.830	Higuchi
	0.917	Peppas

Mathematical treatment of the *in vitro* release kinetics

In vitro release kinetics of the two best formulations was subjected to mathematical treatment for zero order, 1st order, and Higuchi and Peppas model. Based upon the evaluated r^2 value, release pattern of both the formulations was best fitted to Korsmeyer-Peppas model as shown in table 13. according to equation:

$M_t/M_\infty = at^n$

For formulation MF4 and MF5 (n= 1.771 and n=2.013) respectively in case of Peppas model indicating the drug transport mechanism as Super Case II transport. This model describes the release of those polymeric dosage forms when more than one type of release phenomenon could be involved.[49] Therefore in the present case the release of drugs from the microparticles is by swelling and erosion which is also in accordance to *in vitro* drug release data.

Statistical analysis of the *in vitro* release data

Statistical analysis by *student's 't'* test clearly signifies that release rate significantly increased in (SCF) containing 4%w/v rat caecal matter ($p<0.0001$) as compared to release rate in (SCF) without 4% w/v rat caecal matter.

5.1.5 *In vivo* pharmacokinetic study

In vivo pharmacokinetic studies of plain Resveratrol and optimized coated microspheres were performed in rats. The pharmacokinetic parameters evaluated are listed in table 14. Plasma drug concentrations following administration of the plain drug and optimized coated microspheres were plotted against time (Figure 11). The study showed longer Tmax value for optimized coated microspheres than plain Resveratrol. However, Cmax and AUC0–48h values were higher in case of plain drug than optimized coated microspheres. In group 1, Resveratrol was detectable in plasma at 5 min. Drug concentration was rapidly increased and reached to maximum concentration at 3 h, and then quickly decreased. Drug was not detectable in plasma at 12 h. In case of group 2, the drug was not detectable in plasma upto 12 h following administration of optimized coated microspheres. Thereafter, maximum concentration of Resveratrol was detected in plasma at 22 h, since enzymatic degradation of polysaccharides/pectin by colonic microflora is a slow process that requires several hours for completion [50, 51]. Drug concentration in plasma gradually decreased with time such that the drug was not detectable in plasma at 48 h.

Measurement of drug concentration in blood was performed to determine drug release in the GI tract following administration of the formulation. Many researchers used pharmacokinetic study to predict in vivo colon specific drug release [52-56]. It is well established that drug in any formulation needs about 5–6 h for its arrival to the colon [53, 57]. Hence, plain Resveratrol cannot be considered as colon specific since the drug starts appearing in 5 min and the concentration was maximum at 3 h. On the other hand, no drug was detected in plasma till 12 h after administration of optimized coated microspheres/pellets. Absorption was delayed in case of optimized coated microspheres, indicated by its longer Tmax, indicating that the optimized coated microspheres prevented drug release in the upper part of the GI tract and releases it in the colon. The higher Cmax and AUC0–48 with plain Resveratrol is due to the absorption of maximum amount of drug from the upper part of GI tract, which has a large surface area available for absorption. The lower value of Cmax and AUC0–48 in case of optimized coated microspheres/pellets would account for the low systemic bioavailability. Thus, minimal amount of drug is available systemically for interaction with non-target site such that most of the drug resides in the colonic tissue for longer duration for its desired local action at colonic sites. This signifies that CAP coated microspheres loaded with Resveratrol showed in vivo colon specific release.

Table 14. Pharmacokinetic parameters of plain Resveratrol and optimized coated microspheres (Mean ± SD, n = 3).

Optimized coated microspheres		
Pharmacokinetic parameters	**Group 1 (Plain Resveratrol)**	**Group 2 (Optimized coated microspheres)**
Tmax (h)	3.1	23
Cmax (mg/ml)	1.78 ± 0.62	0.98 ± 1.32
AUC0–48h (mg h ml_1)	6.37 ± 1.24	4.24 ± 2.16

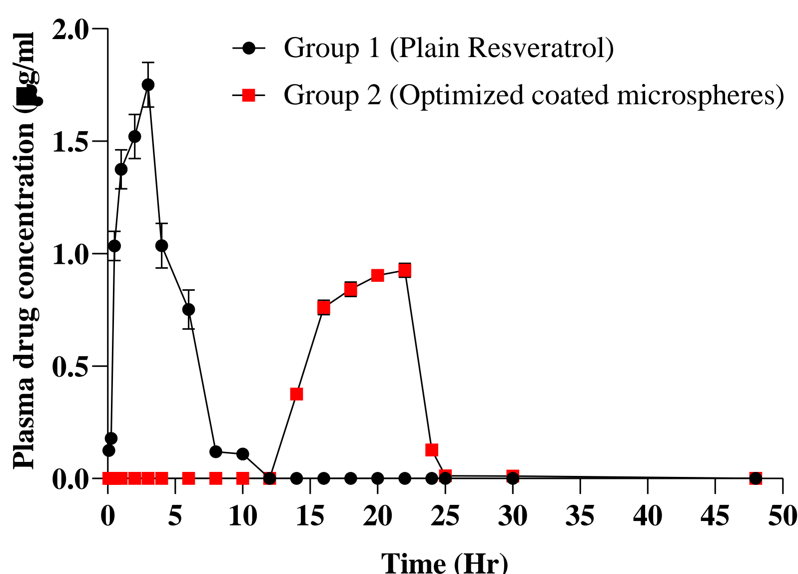

Figure 11. Plasma concentrations time profile of Resveratrol after a single oral dose of plain Resveratrol (Group 1) and optimized coated microspheres (Group 2) ($p > 0.05$).

5.1.6 *In vivo* Pharmacodynamic studies: TNBS induced colitis

Effects on General Condition, Body Weight, and Colon Weight/Length

TNBS-induced rats had hypomotility, frequent loose, purulent, and bloody stools with significant weight loss compared to the normal control group. The rats treated with microsphere formulation in different doses (10 mg or 20 mg/kg) via oral route and sulfasalazine (500 mg/kg) showed remarkable dose-dependent improvement of mentioned symptoms after 7 days of treatment. The body weight changes and improved colon weight/length ratios are demonstrated in figure 12. on the 7th day of research.

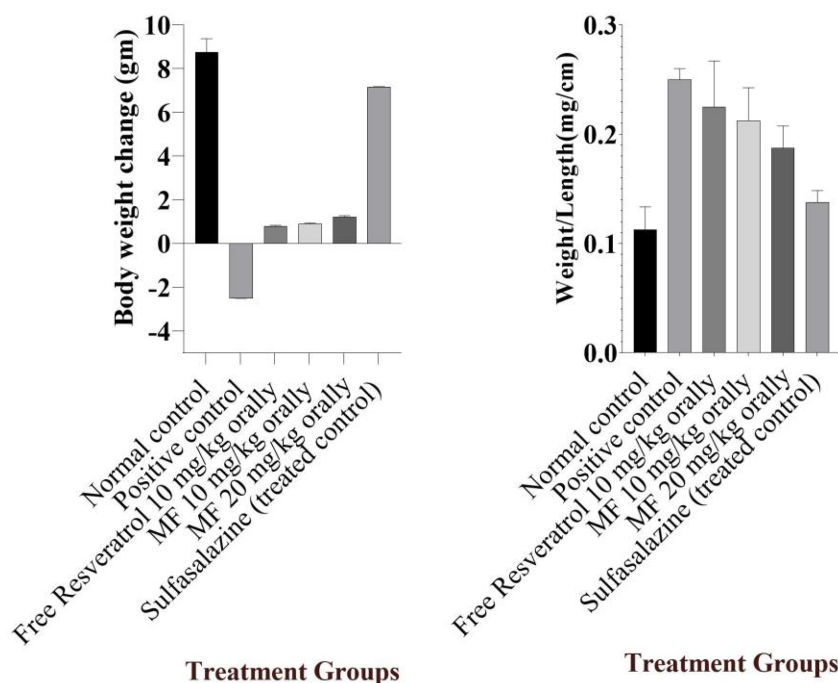

Figure 12. Effects of trinitrobenzenesulfonic acid- (TNBS-) induced colitis and administration of optimised formulation or sulfasalazine on body weight change in comparison to the initial body weight before the start of the study (a) and the ratio of colon weight to colon length (b) at the 7th day after induction of colitis. Data are expressed as mean ± SD. N=8 in each experimental group.

Effects on Macroscopic Changes in TNBS Induced Colitis

The gross resemblance of the untreated TNBS-induced colon model was severe edema, inflammation, and hyperaemia compared with the healthy group that had no inflammation. As shown in table 15., the administration of formulations and sulfasalazine significantly improved the macroscopic scores of colitis with decreasing hyperaemia and inflammation of the colon in TNBS-induced groups. These improvements were dose-dependent.

Effects on Histologic Changes in TNBS-Induced Colitis

The histologic appearance of untreated TNBS induced colitis mucosa was edema, ulceration, acute inflammatory cell infiltration, loss of cells, distorted mucosal architecture, and thickening of lamina propria (Table 15. and Figure 13.). The extent and severity of inflammatory cell infiltration and cellular damage were significantly attenuated by treatment with formulations orally (10 and 20 mg/kg) and sulfasalazine (500 mg/kg). These

treatments also improved edema of the colon mucosa and crypt architecture. The changes were dose-dependent, so more improvement was seen with 20 mg/kg formulation and sulfasalazine.

Table 15. The effects of optimised formulations (microspheres) and sulfasalazine administration.

Optimised coated microsphere formulation		
Group	**Group A: Macroscopic score**	**B: Histological score**
Normal control	0	0.72 ± 0.08
Positive control	5	10.20 ± 0.74
Free Resveratrol 10 mg/kg orally	3	8.41 ± 0.41
MF 10 mg/kg orally	2	6.92 ± 0.21
MF 20 mg/kg orally	1	6.74 ± 0.42
Sulfasalazine (treated control)	1	6.56 ± 0.13

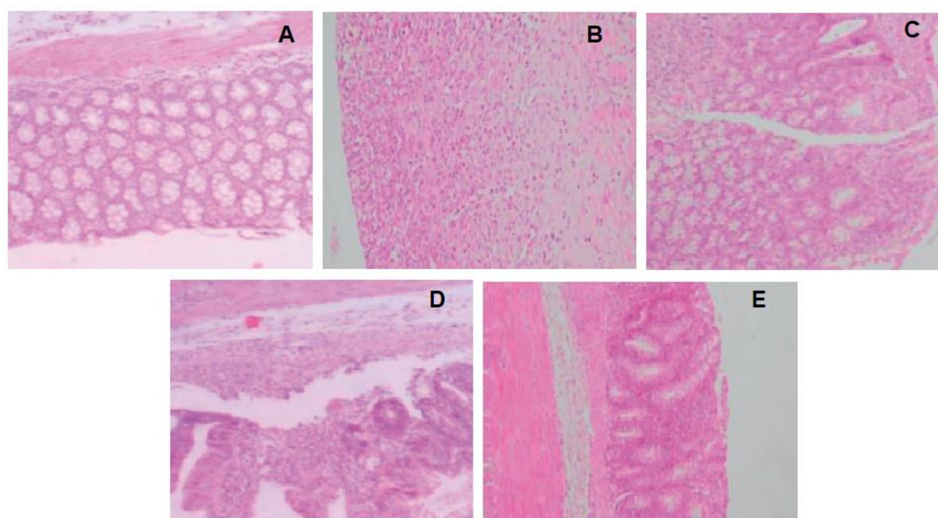

Figure 13. Histopathological colonic mucosal sections at different groups contain 8 rats (n = 8): Normal control mucosa with the intact epithelial surface and crypts (A), TNBS-induced colitis with severe mucosal ulceration, and totally glandular destruction, as well as crypt abscess formation and loss of mucosal architecture (B, X100), TNBS colitis treated with optimised microsphere formulation 10 mg/kg intragastrically shows moderate loss of mucosal architecture, cryptitis, goblet cell depletion, and muscle thickening (C, X100), TNBS colitis treated with optimised microsphere formulation 20 mg/kg intragastrically shows mild loss of mucosal architecture, cryptitis, goblet cell depletion, and muscle thickening (D, X100) and TNBS colitis treated with sulfasalazine (E, X100) with mild glandular destruction, cryptitis, and goblet cell depletion.

5.1.8 Stability study

The stability study of optimised coated microparticle best formulation 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 16. There was no significant decrease in % drug entrapment, moisture content and % cumulative drug release of formulations indicating high stability of the coated microparticles formulations as shown in table 16.

Table 16. Stability of coated microparticle formulations (MF5) loaded with drug after 6 months.

MF5								
Coated microparticles	**Stability at 25±2 ^{0}C**				**Stability at 40±2 ^{0}C**			
	Day 0	**Day 60**	**Day 120**	**Day 180**	**Day 0**	**Day 60**	**Day 120**	**Day 180**
% Drug entrapment	81.49±3.14	81.21±2.55	80.87±4.52	80.38±3.64	81.49±3.16	82.01±3.65	79.94±3.54	80.12±4.48
% Cumulative drug release	79.12±1.6	79.54±1.8	79.29±2.17	79.11±3.8	79.12±1.4	78.97±2.7	78.97±2.5	80.86±3.5

4. CONCLUSION

The study clearly demonstrated a successful fabrication and optimisation of a colon targeted microsphere formulation. The *in vitro* and *in-vivo* study indicated that the formulation MF5 could protect the drug in upper GI tract while releasing significant ($p<0.0001$) of Resveratrol in SCF containing caecal content at the end of 10 h under anaerobic conditions. Thus, based on the above-mentioned findings it could be concluded that Pectin based enteric coated system could be successfully used in colon specific delivery systems. In this study, 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.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest in the manuscript.

FUNDING

None

REFERENCES

1. Omar S, Aldosari B, Refai H andAl Gohary O. Colon-specific drug delivery for mebeverine hydrochloride. Journal of Drug Targeting. 2007;15(10):691-700.
2. Chourasia MK andJain SK. Pharmaceutical approaches to colon targated drug delivery systems. Journal of Pharmacy and Pharmaceutical Sciences. 2003;6(1):33-6.
3. Yang L. Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. Journal of Controlled Release. 2008;125(2):77-86.
4. Krishnaiah YSR andSrinivas BP. Effect of 5-Fluorouracil Pretreatment on the In Vitro Drug Release from Colon-Targeted Guar Gum Matrix Tablets. The Open Drug Delivery Journal. 2008;2:71-6.
5. Coviello T, Matricardi P, Marianecci C andAlhaique F. Polysaccharide hydrogels for modified release

formulations. Journal of Controlled Release. 2007;119(1):5-24.

6. Sinha VR andKumria R. Polysaccharides in colon-specific drug delivery. International Journal of Pharmaceutics. 2001;224(1):19-38.
7. Chopra RN, Nayar SL andChopra IC. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific & Industrial Research; 1956.
8. Jha PK, Choudhary RS andChoudhary SK. Studies of medicinal plants of Palamau (Bihar). Biojournal. 1997;9:21-38.
9. Pal S, Chakrborty SK, Banerjee A andMukharji B. Search of anticancer drugs from Indian medicinal plants (Ayurvedic, Unani etc.). Indian Journal of Medical Research. 1968;56:445-55.
10. Nykänen P, Lempää S, Aaltonen ML, Jürjenson H, Veski P andMarvola M. Citric acid as excipient in multiple-unit enteric-coated tablets for targeting drugs on the colon. International Journal of Pharmaceutics. 2001;229(1-2):155-62.
11. Smith ML, Hawcroft G andHull MA. The effect of non-steroidal anti-inflammatory drugs on human colorectal cancer cells: evidence of different mechanisms of action. European Journal of Cancer. 2000;36(5):664-74.
12. Samsami-Kor M, Daryani NE, Asl PR andHekmatdoost A. Anti-Inflammatory Effects of Resveratrol in Patients with Ulcerative Colitis: A Randomized, Double-Blind, Placebo-controlled Pilot Study. Archives of medical research. 2015;46(4):280-5.
13. Cui X, Jin Y, Hofseth AB, Pena E, Habiger J, Chumanevich A, Poudyal D, Nagarkatti M, Nagarkatti PS, Singh UP andHofseth LJ. Resveratrol suppresses colitis and colon cancer associated with colitis. Cancer prevention research (Philadelphia, Pa). 2010;3(4):549-59.
14. Ameena K, Dilip C, Saraswathi R, Krishnan PN, Sankar C andSimi SP. Isolation of the mucilages from Hibiscus rosasinensis linn. and Okra (Abelmoschus esculentus linn.) and studies of the binding effects of the mucilages. Asian Pacific Journal of Tropical Medicine. 2010;3(7):539-43.
15. Esposito E, Cortesi R, Luca G andNastruzzi C. Pectin-Based Microspheres. 2001;944(1):160-79.
16. Vaidya A, Jain A, Khare P, Agrawal RK andJain SK. Metronidazole loaded pectin microspheres for colon targeting. Journal of pharmaceutical sciences. 2009;98(11):4229-36.
17. Peng X, Yang G, Shi Y, Zhou Y, Zhang M andLi S. Box–Behnken design based statistical modeling for the extraction and physicochemical properties of pectin from sunflower heads and the comparison with commercial low-methoxyl pectin. Scientific reports. 2020;10(1):3595.
18. Lorenzo-Lamosa ML, Remuñán-López C, Vila-Jato JL andAlonso MJ. Design of microencapsulated chitosan microspheres for colonic drug delivery. Journal of controlled release : official journal of the Controlled Release Society. 1998;52(1-2):109-18.
19. Mukerjee A andVishwanatha JK. Formulation, characterization and evaluation of curcumin-loaded PLGA nanospheres for cancer therapy. Anticancer research. 2009;29(10):3867-75.
20. Jain SK, Awasthi AM, Jain NK andAgrawal GP. Calcium silicate based microspheres of repaglinide for gastroretentive floating drug delivery: preparation and in vitro characterization. Journal of controlled release : official journal of the Controlled Release Society. 2005;107(2):300-9.

21. Maji R, Dey NS, Satapathy BS, Mukherjee B andMondal S. Preparation and characterization of Tamoxifen citrate loaded nanoparticles for breast cancer therapy. International journal of nanomedicine. 2014;9:3107.
22. Cetin M, Atila A andKadioglu Y. Formulation and in vitro characterization of Eudragit® L100 and Eudragit® L100-PLGA nanoparticles containing diclofenac sodium. AAPS PharmSciTech. 2010;11(3):1250-6.
23. Averineni RK, Shavi GV, Gurram AK, Deshpande PB, Arumugam K, Maliyakkal N andMeka SRJBoMS. PLGA 50: 50 nanoparticles of paclitaxel: development, in vitro anti-tumor activity in BT-549 cells and in vivo evaluation. 2012;35(3):319-26.
24. Chen J, Li S, Shen Q, He H andZhang Y. Enhanced cellular uptake of folic acid–conjugated PLGA–PEG nanoparticles loaded with vincristine sulfate in human breast cancer. Drug development industrial pharmacy. 2011;37(11):1339-46.
25. Dora CP, Singh SK, Kumar S, Datusalia AK andDeep A. Development and characterization of nanoparticles of glibenclamide by solvent displacement method. Acta pol pharm. 2010;67(3):283-90.
26. Shen J andBurgess DJ. In vitro dissolution testing strategies for nanoparticulate drug delivery systems: recent developments and challenges. Drug delivery and translational research. 2013;3(5):409-15.
27. D'Souza S. A review of in vitro drug release test methods for nano-sized dosage forms. Advances in Pharmaceutics. 2014;2014.
28. Kumar P andMishra B. Colon targated drug delivery-An Over view. Current Drug Delivery. 2008;5(3):186-98.
29. Prasad YVR, Krishnaiah YSR andSatyanarayana S. In vitro evaluation of guar gum as a carrier for colon-specific drug delivery. Journal of Controlled Release. 1998;51(2-3):281-7.
30. Chaurasia M, Chourasia MK, Jain MK, Jain A, Soni V, Gupta Y andJain SK. Methotrexate bearing calcium pectinate microspheres; a platform to achieve colon specific drug release. Current Drug Delivery. 2008;5(3):215-9.
31. Al-Saidan SM, Krishnaiah YSR, Satyanarayana V andRao GS. In vitro and in vivo evaluation of guar gum based metrix tablets of rofecoxib for colonic drug delivery. Current Drug Delivery. 2005;2(2):155-63.
32. Krishnaiah YSR, Satyanarayana S, Rama Prasad YV andNarasimha Rao S. Evaluation of guar gum as a compression coat for drug targeting to colon. International Journal of Pharmaceutics. 1998;171(2):137-46.
33. Jana U, Mohanty AK, Pal SL, Manna PK andMohanta GP. Felodipine loaded PLGA nanoparticles: preparation, physicochemical characterization and in vivo toxicity study. Nano Convergence. 2014;1(1):31.
34. Siepmann F, Siepmann J, Walther M, MacRae RJ andBodmeier R. Polymer blends for controlled release coatings. Journal of Controlled Release. 2008;125(1):1-15.
35. Krishnaiah YSR, Veer Raju P, Dinesh Kumar B, Bhaskar P andSatyanarayana V. Development of colon targeted drug delivery systems for mebendazole. Journal of Controlled Release. 2001;77(1-2):87-95.
36. Tanideh N, Bahrani M, Khoshnood-Mansoorkhani M, Mehrabani D, Firoozi D, Koohi-Hosseinabadi O andIraji A. Evaluating the Effect of

Melillotus officinalis L. Aqueous Extracts on Healing of Acetic Acid-Induced Ulcerative Colitis in Male Rats. Annals of Colorectal Research. 2016;In press.

37. Tozaki H, Fujita T, Odoriba T, Terabe A, Suzuki T, Tanaka C, Okabe S, Muranishi S andYamamoto A. Colon-specific delivery of R68070, a new thromboxane synthase inhibitor, using chitosan capsules: therapeutic effects against 2,4,6-trinitrobenzene sulfonic acid-induced ulcerative colitis in rats. Life sciences. 1999;64(13):1155-62.
38. Naini MA, Zargari-Samadnejad A, Mehrvarz S, Tanideh R, Ghorbani M, Dehghanian A, Hasanzarrini M, Banaee F, Koohi-Hosseinabadi O, Tanideh N andIraji A. Anti-Inflammatory, Antioxidant, and Healing-Promoting Effects of Aloe vera Extract in the Experimental Colitis in Rats. Evidence-based complementary and alternative medicine : eCAM. 2021;2021:9945244.
39. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR andWallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology. 1989;96(3):795-803.
40. Chen Y, Si JM, Liu WL, Cai JT, Du Q, Wang LJ andGao M. Induction of experimental acute ulcerative colitis in rats by administration of dextran sulfate sodium at low concentration followed by intracolonic administration of 30% ethanol. Journal of Zhejiang University Science B. 2007;8(9):632-7.
41. Appleyard CB andWallace JL. Reactivation of hapten-induced colitis and its prevention by anti-inflammatory drugs. The American journal of physiology. 1995;269(1 Pt 1):G119-25.
42. Kumar A, Gulati M, Singh SK, Gowthamarajan K, Prashar R, Mankotia D, Gupta JP, Banerjee M, Sinha S, Awasthi A, Corrie L, Kumar R, Patni P, Kumar B, Pandey NK, Sadotra M, Kumar P, Kumar R, Wadhwa S andKhursheed R. Effect of co-administration of probiotics with guar gum, pectin and eudragit S100 based colon targeted mini tablets containing 5-Fluorouracil for site specific release. Journal of Drug Delivery Science and Technology. 2020;60:102004.
43. Jain A, Khare P, Agrawal RK andJain SK. Metronidazole loaded pectin microspheres for colon targeting. Journal of pharmaceutical sciences. 2009;98(11):4229-36.
44. Bhowmick A, Saha T, Karmoker JR andReza MS. Design of experiment (doe) approach to prepare, characterize and optimize the gastroretentive mucoadhesive microspheres of repaglinide. Bangladesh Pharmaceutical Journal. 2019;22(2):135-45.
45. Parikh K, Mundada P andSawant K. Design and Optimization of Controlled Release Felbamate Tablets by D-optimal Mixture Design: In vitro-in vivo Evaluation. Indian J Pharm Sci. 2019;81(1):71-81.
46. Costa P andLobo JMS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001;13(2):123-33.
47. Singh BN. Modified release formulations for colonic delivery. Recent Patents on Drug Delivery and Formulation. 2007;1:53-63.
48. Sengkhamparn N, Verhoef R, Schols HA, Sajjaanantakul T andVoragen AGJ. Characterisation of cell wall polysaccharides from okra (Abelmoschus esculentus (L.) Moench). Carbohydrate Research. 2009;344(14):1824-32.

49. Costa P andSousa Lobo JM. Modeling and comparison of dissolution profiles. European Journal of Pharmaceutical Sciences. 2001;13(2):123-33.
50. Rahman Z, Kohli K, Zhang S-Q, Khar RK, Ali M, Charoo NA, Tauseef M, Shamsher AAA, Mohammed NN andRepka MA. In-vivo evaluation in rats of colon-specific microspheres containing 5-fluorouracil. Journal of Pharmacy and Pharmacology. 2010;60(5):615-23.
51. Yang L, Chu JS andFix JA. Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. International Journal of Pharmaceutics. 2002;235(1):1-15.
52. Bourgeois S, Tsapis N, Honnas H, Andremont A, Shakweh M, Besnard M andFattal E. Colonic Delivery of β‐Lactamases Does not Affect Amoxicillin Pharmacokinetics in Rats. Journal of pharmaceutical sciences. 2008;97(5):1853-63.
53. Das S, Chaudhury A andNg K-Y. Preparation and evaluation of zinc–pectin–chitosan composite particles for drug delivery to the colon: Role of chitosan in modifying in vitro and in vivo drug release. International Journal of Pharmaceutics. 2011;406(1):11-20.
54. Krishnaiah YSR, Satyanarayana V, Dinesh Kumar B, Karthikeyan RS andBhaskar P. In vivo pharmacokinetics in human volunteers: oral administered guar gum-based colon-targeted 5-fluorouracil tablets. European Journal of Pharmaceutical Sciences. 2003;19(5):355-62.
55. Munjeri O, Hodza P, Osim EE andMusabayane CT. An Investigation into the Suitability of Amidated Pectin Hydrogel Beads as a Delivery Matrix for Chloroquine. Journal of pharmaceutical sciences. 1998;87(8):905-8.
56. Zhao X-L, Li K-X, Zhao X-F, Pang D-H andChen D-W. Study on Colon-Specific 5-Fu pH-Enzyme Di-Dependent Chitosan Microspheres. Chemical and Pharmaceutical Bulletin. 2008;56(7):963-8.
57. Liu L, Fishman ML, Kost J andHicks KB. Pectin-based systems for colon-specific drug delivery via oral route. Biomaterials. 2003;24(19):3333-43.