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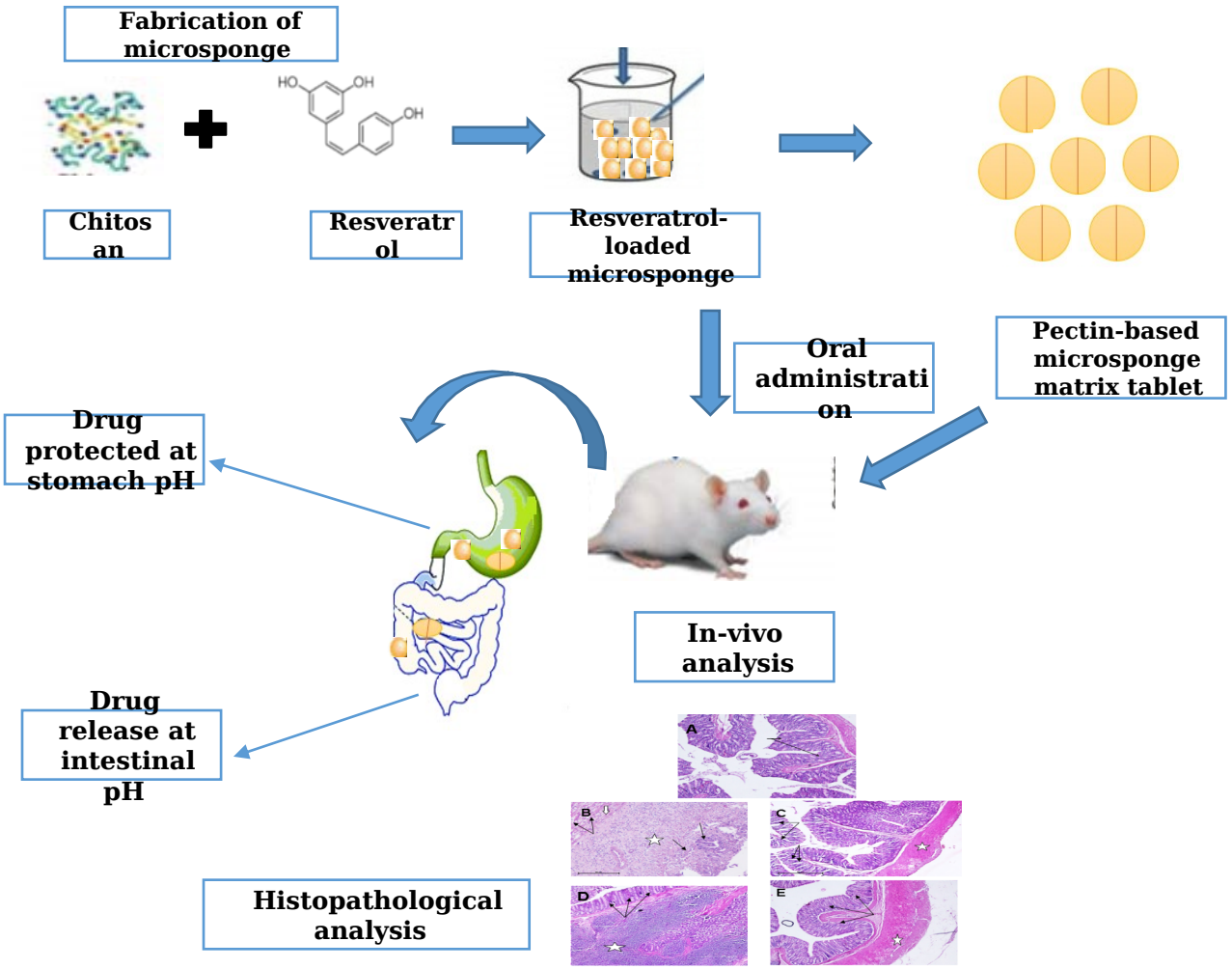
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Efficacy of resveratrol encapsulated microsponges delivered by pectin based matrix tablets in rats with acetic acid-induced ulcerative colitis

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Abstract

Objectives: The objective of the present work to encapsulate the resveratrol (RES) inside the chitosan-based microsponges, employing the systematic optimization by 3^3 Box-Behnken design for the colonic targeting.

Significance: Enhanced therapeutic efficacy of RES-loaded microsponges and matrix tablets, *vis-a-vis* pureRES for ulcerative colitis.

Methods: RES-loaded microsponges were prepared employing the systematic optimization by 3^3 Box-Behnken design for the colonic targeting. The best-optimizedRES-loaded microsponge was compressed in the form of a tablet, employing pectin as a matrix-forming material. The encapsulation of RES inside microsponge was confirmed by XRD, DSC and FT-IR. Further, both RES-loaded microsponges and matrix tablets were evaluated for *in vitro* release kinetics and further evaluated for *in vivo* ulcerative colitis animal model.

Results: Optimization experiments was obtained as the high value of r^2 (particle size = 0.9999; %EE= 0.9652; %CDR = 0.9469) inferred excellent goodness of fit. SEM revealed nearly spherical and porous nature of RES-loaded microsponges. The *in vitro* release kinetic showed zero-order release for RES-loaded microsponges and Korsmeyer-Peppas model for matrix tablets.The pharmacodynamic studies, in ulcerative colitis rat model, indicated better therapeutic efficacy of drug-loaded microsponges and matrix tablets, *vis-a-vis* pure RES. Thus,the present study advocates the potential of RES based microsponges delivered by pectin based matrix tablet, in the treatment of various colonic disorders.

Conclusion: The present study proved that RES-loaded microsponges and matrix tablets based on chitosan and pectin, can be the ideal delivery system for colonic delivery of RES.

Keywords: Chitosan;Box-behnkendesign;quasi emulsion solvent diffusion method; pectin; ulcerative colitis; release kinetics.

Introduction

Resveratrol (RES) (3,5,4'-trihydroxystilbene), is a non-flavonoid polyphenolic phytoalexin molecule, synthesized by various plant species like grapes, berries, and peanuts, in response to stress and microbial infections[1, 2]. RES acts as a strong antioxidant by inhibiting reactive oxygen species (ROS) primarily by activating protein kinase and suppresses cyclooxygenase (COX-2), and lipid peroxidation. It has demonstrated its therapeutic roles as anti-inflammatory, analgesic, cardio-protective, neuroprotective, chemo-preventive, and anti-aging agents [3]. There are many reports available for its decent therapeutic efficacy for lower gastrointestinal (GI) diseases like ulcerative colitis, peptic ulcer, Crohn's diseases and colon cancer [4, 5]. Despite of high oral absorption (~ 75%), the oral bioavailability of RES is less than 1%, due to its extensive intestine and liver metabolism [6, 7]. Moreover, rapid absorption in the upper GI tract and pre-systemic metabolism subsequently results in the lower amount of drug reaching to the colon [8, 9]. Thus, there is a necessity to develop an efficient drug delivery system for RES, which would be able to target the drug directly to the colon and prevent its release in the upper GI tract.

Novel drug delivery carrier systems *viz*. nanoparticles, nano-spheres, micro-particles, microspheres, beads and micro/nano-sponges based on various polymers, have been consumedfor colon-specific drug delivery[10-13]. These systems are, controlled by GI transit time, GI pressure differences, GI pH differences, and colonic bacterial enzymes [14]. In the recent past, polysaccharides that are particularly metabolised by the colonic flora have increased acceptance as a colon-specific drug delivery systems. Pectin, a linear polysaccharide is extensively used as a colon-specific matrix carrier due to some attractive features, *viz.* hydrophobicity, and ability to form gel, biodegradability, and persistence to intestinal enzymes. The other polymer in the polysaccharide class is chitosan, which is pH-sensitive and is used as a colon-specific carrier owing to its ability to restrict the drug release in the gastric pH, and significantly release the drug at higher pH [15]. Based on the above considerations, the advantages of both chitosan and pectin for colon-specific delivery of RES were combined into a unit dosage form, wherein chitosan was employed in the form of microsponges, and pectin as a matrix-forming material. Microsponges have the competency to encapsulate and adsorb a high degree of active ingredients onto its surface owing to numerous interconnected pores. Apart from high drug entrapment, and site-specificity,

microsponges have a specific property of retaining on the surface of the colon, and thereby, increase the absorption of the drug in the colon [16, 17]. Further, it can prevent the drug from early absorption, as the drug is enclosed inside the micro-sponge, and also the frequency of dosing may be decreased *via* controlled delivery of drug over a longer period of time, and hence the patient compliance [18].

Polymeric erosion based matrix systems comprising of hydrophilic polymer is highly popular in tablet manufacturing for controlled release application. Such matrix system, retard the drug release, owing to the formation of the gelatinous surface layer due to swelling in the aqueous medium, which controls the diffusion of water when placed in an aqueous medium[19, 20]. The present work was designed to systematically optimize the RES-loaded chitosan microsponges employing Box-behnken design with respect to particle size, entrapment efficiency, and percent cumulative drug release. The best-optimized RES loaded microsponge formulation was developed into the erosion based matrix tablet employing pectin. Finally, all the RES formulations were evaluated employing an acetic-acid induced ulcerative colitis model in rats.

Experiment

Materials

RES was received *ex gratis* from Tirupati Medicare Ltd., PaontaSahib, Sirmaur, Himachal Pradesh, India. Acetone, sodium chloride, polyvinyl alcohol (PVA), sodium dihydrogen phosphate, potassium dihydrogen phosphate, sodium hydroxide (NaOH), and pectin, were procured from Nice Chemical Pvt. Ltd., Cochin, India. Ethanol, and HCl were purchased from Merck Specialties Pvt. Ltd., Mumbai, India, while Span 80, was purchased from Qualikems Laboratory Reagents, New Delhi, India. Chitosan was purchased from Hi-Media Laboratory Pvt. Ltd., Mumbai, India, while, PVP K30, and microcrystalline cellulose (MCC), were obtained from Loba Chemical Pvt. Ltd., Mumbai, India.

Methods

Fabrication and evaluation of microsponges

Microsponge preparation was done by quassi-emulsion technique as previously reported by authors [21]. Methanol was employed as a solvent to dissolved RES. 0.1 mL of internal aqueous phase was added into the organic phase to form w/o primary emulsion. Herein, the

internal phase comprised of 1% (w/v) aqueous solution of NaCl (as porogen) and Span 80. The organic phase was prepared in the DCM. Separately, chitosan was dissolved in minimum amount of 1% w/v aqueous solution of the glacial acetic acid solution, and RES was dissolved in methanol. Both the solution was then added to the required volume of DCM to form the organic phase. Finally, primary w/o emulsion was added into 5% w/v aqueous PVA (external phase), to form w/o/w double emulsion. The prepared emulsion was then, continuously stirred for 2h on a mechanical stirrer. Microsponges so prepared were filtered, dried at 60˚C, and stored in the desiccator until further use[22].

Systematic optimization of microsponges as per the experimental design

RES-loaded microsponges were optimized employing three factor three-level, Box–Behnken design (BBD). The dependent variables were the amount of RES (X1), polymer (X2), and solvent (X3), used at three different levels of each variable, *viz.* low (-1), intermediate (0), and high (+1). Various microsponge formulations (Table 1) prepared as per the design were investigated for the response variables like particle size, percent cumulative drug release (% CDR), and percent entrapment efficiency (%EE) as the response variables.

Characterization of microsponges

Particle size determination

The mean particle size of microsponge was analysed by Malvern Master Sizer (Scinrocco, 2000), installed at the University Institute of Pharmaceutical Sciences (UIPS), Chandigarh, India. All the samples were diluted 50 times before analysis. The samples were then placed into cuvettes and the intensity of fluctuation of the laser beam was recorded and interrelated with the particle size of the dispersed phase[23].

Entrapment efficiency (%EE), percentage yield (%Y) and percentage drug loading(%DL)

For %EE, microsponge equivalent to 10 mg of the drug were crushed and extracted employing methanol by ultra-sonication. To separate the insoluble residue, centrifugation was then carried out at 2000 rpm for 10 min. The supernatant was analysed by the U.V spectrophotometer (Systronics-Model-2202) atλ_{max}302 nm after appropriate dilution [24]. To determine the concentration of RES, the value of absorptivity used was 1437. The amount of% EE, % Y and %DL was calculated employing the following Equations (1),(2) and (3) respectively.

$$\%E = \frac{Mass\ of\ the\ drug\ in\ microsponge}{Initial\ mass\ of\ the\ drug} \times 100 (1)$$

$$\%Y = \frac{Mass\ of\ the\ drug\ in\ microsponge}{Initial\ mass\ of\ the\ drug + Initial\ mass\ of polymer} \times 100 (2)$$

$$\%DL = \frac{Mass\ of\ the\ drug\ in\ microsponge}{Mass\ of\ microsponge} \times 100 (3)$$

In vitro drug release

In vitro dissolution study of RES for a period of 12 h, from all the prepared microsponge formulations, was carried out employing the USP–Type-II dissolution apparatus(ElectrolabETC 11LX) [25]. Microsponges equivalent to 10 mg of the RES, was placed in the jar, and the study was performed at different pH, i.e. pH 1.2 (200mL), for 2 h, pH 6.8 for 2-6 h and pH 7.4 (700 mL) for subsequent hours i.e., 6-12 h to simulate the same conditions of GIT. The stirring was maintained at 100 rpm at 37±5˚Ctemperature. Samples (5 mL) were withdrawn periodically at regular time intervals (0, 0.5 1.5, 2, 3, 4, 6, 8,10, 12h) while an equal volume of fresh medium was added to maintain the sink conditions. The samples were diluted with methanol and analysed spectrophotometrically at λ_{max}302 nm to calculate the percent cumulative drug release (%CDR) values [26].

Optimisation data analysis and validation

The optimization and validation of data obtained for various response variables *viz.,* particle size, %EE, and %CDR were performed employing mathematical modelling. The second-order quadratic polynomial model was selected using multiple linear regression analysis (MLRA) to study the probability of a significant interaction(s) among the response variables[27, 28]. The response surface analysis was studied employing three dimensional (3D) response surface plots, and two-dimensional (2D) contour plots, constructed using Design Expert® ver.10.0.1 (Stat-Ease Inc., Minnepolis, MN). The numerical optimization using desirability function by 'trading off' of the response variables was employed to select the optimum microsponge formulation (RES:340 mg; polymer; 455 mg). A total of ten check-point microsponge formulations were selected and evaluated. The observed and predicted values for the studied responses, i.e. particle size, %CDRand %EE, were critically compared. Percent bias (percent error) was determined with respect to the observed responses and the residual plots were also generated.

Characterization of the optimized microsponge formulation

Scanning electron microscopy

To observe the surface of microsponge, dried samples were mounted on a metal stub using double-sided adhesive tape and sputter-coated with gold for 1 min. under vacuum and then observed under a scanning electron microscope (SEM) at 10 kV (QUANTA 250, FEI Makers, Singapore), installed at IIT, Mandi, H.P, India[27].

Differential scanning calorimetric (DSC) analysis

DSC (STA 449 F1 Jupiter) thermal analysis was carried out, on the optimized RES-loaded microsponge formulation, pure RES, and chitosan. Approximately 5mg sample was weighed, and sealed into aluminium pans. All the samples were heated at the rate of 10°C/min in a temperature range of 25-300°C, in nitrogen atmosphere [29].

Thermal gravimetric analysis (TGA)

TGA (STA449F1 Netzsch) thermal analysis was carried out, on the optimized RES-loaded microsponge formulation, pure RES, and chitosan. Approximately 5mg sample was weighed, and sealed into aluminum pans. The experiment was conducted in temperature range of 25-300°C at a heating rate of 10°C/min in a temperature range of 25-300°C, in a nitrogen atmosphere (20 ml/min) [29, 38].

X-ray powder diffraction (XRD) study

XRD was recorded to characterize the crystal and physical state of microsponge, pure RES and chitosan. The instrument (SmartLab 9kW rotating anode x-ray diffractometer) was operated at a voltage of 45mV and current 20A, and the diffraction patterns over a range of 5-10°C/min in terms of 2Ø [29].

Formulation and evaluation of colon-targeted matrix tablet

Colon targeted matrix tablets of optimized RES-loaded microsponges were prepared by direct compression method. Pectin (150 mg, matrix diluent), optimized microsponge formulation (150 mg),polyvinyl pyrollidine (PVPk30) (binder, 150 mg), and microcrystalline cellulose (MCC, 50 mg) were accurately weighed and mixed uniformly to form a homogenous powder-mixture. The final mixture was then passed through sieve no. 22, and was directly compressed into tablets, employing Rotatory tablet punching machine (Cadmech. Pvt.

Ltd.)[30].Tablets were evaluated for various pharmacopoeial (weight variation, friability and *in vitro* dissolution) and non- pharmacopoeial (hardness) aspects.

The *in vitro* dissolution of the matrix tablets was done as describes in section 2.4.3. The weight variation of the matrix tablets was determined as per Indian Pharmacopoeia [31]. Briefly, twenty tablets were weighed and the average weight was calculated. The percentage of weight variation was also determined by using the following formula as shown in the Equation. (4)

$$\%\ \text{Weight Variation} = \frac{\text{Individual weight} - \text{Average Weight}}{\text{Average Weight}} \text{x}100 \quad (4)$$

The friability of prepared matrix tablets was determined with Roche type friabilator. 10 tablets were weighed and tested at a speed of 25 rpm for 4 min. After the process was stopped, tables were removed out of the friabilator then after, dust was wiped-off, and tablets were weighed again. The difference between the weight before, and after, the process, was determined. The percentage friability (%) was calculated using the following Equation (5)

$$\%\text{Friability} = \frac{\text{Tablet weight before} - \text{Tablet weight after}}{\text{Tablet weight after}} \text{x}100 \quad (5)$$

The hardness of the prepared matrix tablets was determined to employ Monsento hardness tester. The hardness was measured in terms of force (Kg/cm^2), required to break the tablet. The tablet was placed between two anvils, the force was applied, until the tablet breaks and this force was recorded. The hardness test was performed on twenty tablets, and the average hardness was recorded [31].

In vitro drug release kinetics

Release data from the best-optimized microsponge formulation and its matrix tablet were fitted to various mathematical models to study the drug release mechanisms. The various models employed were zero-order (% cumulative drug release vs. time) Equation(6), first-order (log % drug release vs. time) Equation (7), Higuchi model (% cumulative drug release vs. square root of time) Equation(8), and Peppas model (log % drug release vs. log time) Equation(9). The kinetic model was selected based on best fit with the highest value of the regression coefficient (r^2) [7, 21].

$$Q_t = k0t \quad (6)$$

$$\ln Q_t = \ln Q_\alpha + k_1 t \quad (7)$$

$$Q_t = k\sqrt{t} \quad (8)$$

$$Q_t = k_k t \quad (9)$$

Here Q_t is the amount of drug released at time t, Q_α is the initial amount of drug, whereas, k_0, k_1, k and k_k are the corresponding release rate constants for zero-order, first-order, Higuchi and Korsmeyer-Peppas model respectively.

In vivo pharmacodynamic study

The animal study was carried out in prior approval of the Animal Ethical Committee, of Shoolini University Animal Ethics Committee, duly approved for the purpose of control and supervision of experiments on animals by the Government of India, (IAEC No/SU-PHARM/7/10).

Acetic acid-induced experimental ulcerative colitis in the colon

Fifteen wistar albino rats (body weight = 160–200 g), were taken, and caged individually with food and water *ad libitum)*. The rats were distributed randomly into five groups with each group comprising of three animals. Except for the negative control group, colitis was induced in all the groups by intrarectal administration of 1 mL of (4%) (v/v) acetic acid, which resembles with the inflammatory bowel disease (IBD). The catheter was introduced into the anus up to a length of 6 cm, and then acetic acid was administered [32]. The full IBD model was developed by keeping animals untreated for about three days [30]. After three days, each group received the treatment orally in 0.5% carboxymethyl cellulose (w/v) solution. Group 1 served as a negative control, group 2 served as colitis group without any treatment, group 3 received pure RES (25mg/kg), group 4 received RES -loaded microsponges (equivalent to 25mg/kg), and group 5 received RES-loaded microsponge matrix tablets (equivalent to 25mg/kg) [33].

Pharmacological Assessments

After seven days of treatment, the animals were sacrificed and colon was removed, and based on inflammatory scales, and ulcer projections were visualized. The inflammatory scales were categorised as; 0 = normal coloured colon, 0.5 = red coloration, 1 = spot ulcer, 1.5 = haemorrhagic streaks, and 2 = haemorrhagic ulcer.

Histopathology assessment

Histopathological analysis was performed by preserving the part of the colon in a 10% formalin solution. These colonic sections were stained with hematoxylin and eosin (H&E) and examined using a light microscope with a fitted Nikon camera for the presence of any necrosis, ulceration, haemorrhage, and inflammatory cell infiltration [34].

Results and discussion

Formulation and optimization of microsponges

In the present research, quassi emulsion technique was used to fabricate the various RES-loaded microsponge formulations. Chitosan was employed as a polymer for the preparation of microsponge due to its ability to release the drug, particularly at the colonic site. The prompt mixing of w/o primary emulsion and water at the interface resulted in the precipitation of RES-loaded structures of chitosan. For the optimization RES-loaded microsponges, a three-factor, three-level, the Box-Behnken design was employed. Table 1 summarises an account of the 17 experimental runs studied, along with the coded values and actual values for the studied factors. Various microsponge formulations fabricated as per the design were investigated for %EE, %CDR and particle size as the response variables.

[Space for Table 1]

Response surface mapping and data analysis

The data analysis of the response variables employing second-order quadratic polynomial models [27, 28], suggested that the quadratic model was highly significant ($p<0.05$) along with the model terms ($p<0.0001$).The special polynomial mathematical model encompassing ten coefficients (β0- β33) represent quadratic and interaction terms, as shown in Equation(10).

$$Y=\beta 0 + \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 12X1X2 + \beta 13X1X3 + \beta 23X2X3 + \beta 11X12 + \beta 22X22 + \beta 33X32 \quad (10)$$

A very high degree of predictive ability of the optimization experiments was obtained as the value of overall bias was 0.1109 ± 0.2253%. Further the high value of r^2 (particle size = 0.9999; %EE= 0.9652; %CDR = 0.9469) inferred excellent goodness of fit. The residual plots were found to be uniform, comparatively narrow and random scatter around the zero-axis [27, 35].

[Space for Table 2]

Figure 1, depicts 2D-contour plots and corresponding 3D-response surface plots for % EE (A), %CDR (B), and particle size (C). In the current studies, polymer and drug concentration had a greater effect on all the studied response variables, out of all the studied input variables. A curvilinear dip in the values of % EE followed by an increasing trend was observed with an increase in the drug concentration, and a decrease in polymer concentration as shown in Figure 1A. In case of %CDR, a twisted shape curve was observed with an increase in both drug as well as polymer concentration as given in Figure 1B. An increase in polymer concentration negatively influenced the %CDR, while, an increase in drug concentration enhanced the former. This can be attributed to the fact that the drug release from the polymer matrix occurs after complete swelling of the polymer, and as the quantity of polymer in the formulation increases, so the time required to swell also increases, and hence, slower the drug release. In the case of particle size shown in Figure 1C, a linear relationship was obtained for polymer and drug concentration. With the increase in polymer or drug concentration, particle size was increased, however, the effect was more pronounced in case of polymer concentration. The increase in the polymer concentration results in an increase in the viscosity of the internal phase, which subsequently gives rise to the generation of more viscous forces resisting droplet breakdown, and thus bigger sized particles. The search for optimum microsponge formulation was carried out using numerical optimisation and desirability function to get the required goals for the response variables. Table 3 presents the constraint set for numerical optimisation. In model validation, a total of ten check-point formulations were selected from the RSM. Hence, based on these parameters the best formulation was selected and further used for characterization.

[Space for Figure 1]

[Space for Table 3]

Percentage yield (%Y) and Drug loading (DL)

The %Y of all the prepared microsponges ranged between 69.45±0.52-86.68±0.67%, while %DL values were in the range of 40.1±0.34-71.45±0.76%. With an increase in the drug concentration, %Y and %DL were found to be increased. This might be due to the high drug and polymer concentration, which led to increase in viscosity of the dispersed phase, and reduced the diffusion rate of DCM from viscous solutions into the aqueous phase, thus improving the yield and loading [36].

Characterization of optimized formulation

Surface morphology

The SEM image revealed formulation to be of nearly spherical shape having sufficient surface porosity as shown in Figure 2a. The presence of numerous interlinked pores all over the particle was also present imitating the spongy structure.

X-ray powder diffraction (XRD) study

The XRD pattern of pure RES, polymer, and optimized microsponge formulation were recorded, and the overlay-XRD spectrum is shown in Figure 2b.RES showed numerous sharp and intense diffraction peaks at 13.3, 16.4, 19.2, 22.4, 25.3, 28.3 and 45.3°, which indicates crystalline nature at the respective 2Ø positions. The polymer chitosan did not show any peaks, suggesting its amorphous nature as depicted in literature. The microsponge formulation also did not reveal any peaks of polymer and drug, indicating the entrapment of drug inside the microsponges [29].

FT-IR spectra

The overlay FTIR spectra of RES, Chitosan and microsponge formulation is depicted in Figure 2c.RES exhibited three strong absorption bands at 1611 cm^{-1}, 1588 cm^{-1}, and 1387.90 cm^{-1}, analogous to C-C aromatic double bond stretching, C-C olefinic stretching, and C-C stretching, respectively. The peaks from 3167 to 3201 cm^{-1}depicts the O-H stretching and the peak at 1561cm^{-1}correspond to aromatic C=C bending. The peak at 1447 cm^{-1} corresponds to C-C ring stretching [37]. The characteristic chitosan peaks belonging to its saccharide structure at 1055 and 898 cm^{-1} and at 1655 cm^{-1} (amide I) were close to the literature value. FTIR spectra of RES-loaded microsponges displayed all peaks analogous to pure RES and chitosan, however, with a decreased intensity of peaks. Further, the RES-loaded microsponges did not show any major peaks corresponding to the bioactive incorporated. This indicates the encapsulation of the RES within the microsponge formulation.

Differential scanning calorimetric (DSC) analysis

DSC thermograms of the drug, chitosan, and RES-loaded microsponge formulation are shown in Figure 2d. The drug RES demonstrated an endothermic peak around 260°C, which is very close to the melting point of the drug, i.e., 261°C to 263°C. Chitosan revealed an endothermic peak, at around 220°C, which is close to its reported value. In the case of microsponge formulation, there was no peak of RES and chitosan, which indicates the

complete entrapment of drug inside the microsponge formulation and the amorphous state of microsponge formulation [29, 38].

Thermal gravimetric analysis (TGA)

TGA thermograms of the RES-loaded microsponge formulation, pure RES, and chitosan, are shown in Figure 2e. TGA thermograms of chitosan indicated the % weight loss of approximately 90% of the polymer ranges between 260 -290°C as shown in figure 2e (A). whereas the % weight loss of drug found to be approximately 80% ranges between 220-270°C has shown in figure 2e (B). Drug-loaded Optimized microsponge formulation showed TGA thermogram at a temperature range between 110°C - 190°C signifying that the drug was either completely or partially changed into amorphous form [38].

[Space for Figure2]

Evaluation of RES-loaded microsponge matrix tablets

All the evaluation parameters of matrix tables are shown in Table 4 and suggest its satisfactory characteristics. The formulations exhibited a hardness of 4.13±0.13kg/cm^2 and friability below 0.69±0.23% which showed satisfactory mechanical strength of the tablets. The average weight of the twenty tablets was found to be 499.65 ±1.35 which is well within the IP limit of weight variation i.e.(±5%).

[Space for Table4]

In vitro release of RES-loaded microsponges matrix tablets

The *in vitro* release pattern of RES, from plain microsponges, and microsponge-matrix tablet is shown in the Figure 3. The rate of drug release in 12 h was gradually increased, with an increase in time, and then became constant or attained equilibrium, in optimized microsponge formulation. Drug release mechanisms from microsponge could be linked to its porous surface. The latter permits easy penetration of the release media, and its approachability to the entrapped drug. It was witnessed that the microsponge system was able to control the drug release in gastric pH i.e., only 10% of the drug was released during an initial 2h. However, the drug release from matrix tablets at all the pH conditions was on the lower side *vis-à-vis* microsponge formulation. This could be due to the slow swelling property of the pectin, followed by gradual erosion, and release of the drug from the matrix tablets [17, 21]. It is well reported that the drug release from a swellable hydrophilic polymer like pectin can

be controlled and relating the liquid penetration within the polymer matrix, the swelling of the hydrated polymer, drug diffusion throughout the swollen matrix and, erosion. PVP due to its water-soluble characteristic could help in the solubilization in the aqueous phase and helps in the permeation of dissolution media through the matrix causing its erosion [21].

[Space for Figure3]

Kinetic release analysis of drug

The fitting of drug release data by suitable mathematical models is a powerful tool, which not only enables the better interpretation, and comprehension of the mechanisms involved in the drug release process but also helps in controlling the release features according to specific therapeutic needs. Different mathematical models as shown in Table 5 were applied to *in vitro* drug dissolution profiles and their respective coefficients were estimated.

According to r^2 values, as shown in Table 5, it can be noted that the microsponge fitted better with the zero-order kinetic model, while the microsponge tablets fitted best with the Peppas model. In the Korsmeyer-Peppas model, the value of n specifies the release mechanism of the drug as defined. For the case of spherical matrix tablets, $0.43 \leq n$ corresponds to a Fickian diffusion mechanism, $0.43 < n < 0.85$ to non-Fickian transport, and $n > 0.85$ to super case II transport [35]. Here, the value of n was determined to be more than 0.85, thus mimicking the super case II transport kinetics.

[Space for Table5]

In vivo pharmacodynamic study

After the completion of *in vivo* pharmacodynamic study, rats were sacrificed, and colon was examined visually, on the basis of inflammatory scales as shown in Table 6 [17,40]. It is vivid from the results that there were less colonic lesions seen in the case of treated groups *vis-à-vis* colitis group, indicating positive therapeutic outcomes of RES, RES-loaded microsponge, and microsponge-matrix tablets.

[Space for Table 6]

Histopathological studies

All the histological pictures of various treated and untreated groups are depicted in Figure 4. While negative control group as shown in Figure 4A, revealed healthy looking mucosal or

sub-mucosal lining, and intact mucosal crypt, acetic acid-induced colitis group (Figure 4B) showed severe surface, and mucosal haemorrhage (white arrows), marked necrotic alterations, and leftovers of colonic crypts (black arrows). Besides, the sub-mucosal layer in the acetic acid-induced colitis group revealed polymorphic inflammatory cell infiltration (stars). Thus, it can be concluded that the colitis group revealed severe mucosal ulceration, inflammatory cell infiltration, submucosal edema, and goblet hyperplasia [40] Figure 4Cpertaining to the pure RES treated group showed colonic mucosa with no haemorrhage streak, mild preservation of crypts with slight dilations, and almost intact mucosal lining cells (arrows). Microsponge formulation treated group, i.e. (Figure 4D), and microsponge-matrix tablet treated group, (Figure 4E) revealed intact mucosal crypts, healthy mucosal and submucosal lines, suggesting both the formulations to preserve the normal colonic condition. However, RES-loaded microsponge treated group revealed much prominent results in comparison to the microsponge-matrix tablet treated group. Overall, all the RES treated groups exhibited the complete cure of ulcerative colitis after the 7th day [33].

[Space for Figure 4]

Conclusion

The present study successfully ratified that the chitosan microsponge were able to entrap the RES. The systemic optimization employing BBD aided in studying the most influential variables to select the best-optimized formulation. The *in vitro* release kinetics data revealed the sustained release nature of the developed systems. Finally, in the *in vivo* ulcerative colitis model, better therapeutic outcomes from drug-loaded microsponge, and microsponge loaded matrix tablets were achieved *vis-à-vis* pure RES. Overall, the present studies corroborated that the developed microsponges matrix system based on chitosan and pectin can be the ideal delivery system for colonic delivery of RES.

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Declaration of interest

The authors report no declarations of interest.

References

[1] KasiotisKM,Pratsinis H, KletsasD. Resveratrol and related stilbenes: their anti-aging and anti-angiogenic properties. Food ChemToxicol. 2011;61:112-20

[2] BaurJA, Sinclair DA.Therapeutic potential of resveratrol: the in vivo evidence. Drug Discovery. 2006;5(6):493–506.
[3] PangeniR,SahniJK,AliJ.Resveratrol: review on therapeutic potential and recent advances in drug delivery. Expert Opin Drug Deliv. 2014;11(8):1285-1298.
[4] Das S, Ng KY, Ho PC. Design of a pectin-based microparticle formulation using zinc ions as the cross-linking agent and gluteraldehyde as the hardening agent for colonic specific delivery of resveratrol: in vitro and in vivo evaluations. J Drug Target. 2011;19:446-57. doi.org/10.3109/1061186X.2010.504272
[5] Jeong JB, Lee J, Lee SH. TCF4 is a Molecular Target of Resveratrol in the Prevention of Colorectal Cancer. International journal of molecular sciences. 2015;10411-10425.
[6] Negi P, Aggarwal M, Sharma G, Rathore C. Niosome-based hydrogel of resveratrol for topical applications: An effective therapy for pain related disorder(s). Biomedicine & Pharmacotherapy. 2017;88:480–487.
[7] Zu Y, Zhang Y, Wang W.Preparation and in vitro/ in vivo evaluation of resveratrol-loaded carboxymethyl chitosan nanoparticles. Drug Delivery. 2014;1-11.
[8] Das S, Ng KY.Colon-specific delivery of Resveratrol: Optimization of multi-particulate calcium-pectinate carrier. International Journal of Pharmaceutics. 2010;35:20-28.
[9] Gabriel DP, McClements DJ. Resveratrol encapsulation: designing delivery Resveratrol encapsulation: designing delivery systems to overcome solubility, stability and bioavailability issues. Trends in food science and technology. 2014;38:88-103.
[10] Bonechi C, Martini S, Ciani L. Using liposomes as carriers for polyphenolic compounds: the case of trans-resveratrol. PLoS One. 2011;7:1-11. doi: org/10.1371/journal.pone.0041438.
[11] Zu Y, Zhang Y, Wang W, Zhao X, Han X, Wang K, et al. Preparation andin vitro/in vivoevaluation of resveratrol-loaded carboxymethyl chitosan nanoparticles. Drug Delivery. 2016;1–11. doi:10.3109/10717544.2014.924167
[12] LoftssonT, Brewster ME. Pharmaceutical application of cyclodextrins. Drug solubilization and stabilization. J Pharm Sci. 1996;85:1017-25.doi: org/10.1021/js950534b
[13] Peng X, Xiong H, Li J.Vanillin cross-linked chitosan microspheres for controlled release of resveratrol. J. food chem. 2010;121(1):23–28. doi: org/10.1016/j.foodchem.2009.11.085
[14] Pando D, Gutierrez G, Coca J.Preparation and characterization of niosomes containing resveratrol. J Food Eng.2013;117:227-34.
[15] Ahmed RZ, PatilG, ZaheerZ. Nanosponges- a completely new nano-horizon: pharmaceutical applications and recent advances.Drug Dev. Ind. Pharm. 2013;39(9):1263-72.
[16] Osmani RAM, Aloorkar NH. Microsponge based drug delivery system for augmented gastroparesis therapy: Formulation development and evaluation. Asian Journal Pharmaceutical Sci. 2015;10:442-451.
[17] Nief RA, Hussein AA. Preparation and Evaluation of Meloxicam Microsponges as Transdermal Delivery System. Iraqi Journal of Pharmaceutical Sci. 2014;23(2).
[18] Srivastava R, Kumar D, Pathak K. Colonic Luminal surface retention of meloxicam microsponges delivered by erosion based colon-targeted matrix tablet. International Journal of Pharm. 2012;427:153-62.
[19] MaheshwariR, SharmaP. Microsponge Embedded Tablets for Sustained Delivery of Nifedipine.PharmNanotechnol. 2017;5(3):192-202.
[20] Ashord M, Fell JT, Attwood D.An evaluation of pectin as a carrier for drug targeting to the colon. Journal of Control Release.1993;26:213- 220.

[21] Maestrelli F, Zerroukm N, Cirri M. Comparative evaluation of polymeric and waxy microspheres for combined colon delivery of ascorbic acid and ketoprofen. International Journal of Pharm. 2015;485:365–373.
[22] Kumar V, Soni GC, Prajapati SK. Sustained Release Hydrophilic Matrix Tablet of Ibuprofen: Influence of Polymers on *Invitro* Release and Bioavailability. International Journal of Pharmaceutical Research and Sci. 2012;1(4):69-83.
[23] RathoreC, Jain N, Garg N. Polysaccharide-microsponge based matrix tablet for colon targeting of ketoprofen:*In vitro* and *in vivo* evidence. IJPSR. 2017;8(10):4250-4260.
[24] Perge L, Robitzer M, Guillemot C. New solid lipid microparticles for controlled ibuprofen release: formulation and characterization study. International Journal of Pharm. 2012;422:59–67.
[25] Shinde AJ, Paithane MB, Sawant SS. Development and Evaluation of Fenoprofen Microsponges and its Colonic Delivery using Natural Polysaccharides. Asian Journal of Pharmaceutical Sciences and Nanotechnol.2014;1(27):42-30.
[26] DevrimB, CanefeK.Preparation and evaluation of modified release ibuprofenmicrospheres with acrylic polymers (eudragit®) by quasiemulsion solvent diffusion method: effect of variables. Acta Pol. Pharm. Drug Res. 2006;63:521–534.
[27] Anwer MK, Al-Shdefat R, Ezzeldin E, AlshahraniSM,Alshetaili AS, et al. Preparation, Evaluation and Bioavailability Studies of Eudragit Coated PLGA Nanoparticles for Sustained Release of Eluxadoline for the Treatment of Irritable Bowel Syndrome. Frontiers in Pharmacol.2017;8:844. doi:10.3389/fphar.2017.00844
[28] Negi P, Singh B, Sharma G. Phospholipid microemulsion-based hydrogel for enhanced topical delivery of lidocaine and prilocaine: QbD-based development and evaluation. Drug Deliv. 2016; 23(3);941-57. doi: 10.3109/10717544.2014.923067.
[29] Zu Y, Zhang Y, Wang W, Zhao X, Han X, Wang K and Ge Y. Preparation and *in vitro/ in vivo* evaluation of resveratrol-loaded carboxymethyl chitosan nanoparticles. Drug Delivery. 2014; 1-11.
[30] Negi P,Singh B, SharmaG..Enhanced Topical Delivery of Lidocaine*via.* Ethosomes-Based Hydrogel: *Ex-vivo* and *In-vivo* Evaluation**.** Journal of Nanopharmaceutics and Drug Deliv.2014;2(2):138-147
[31] Malipeddi VR, Awasthi R, Dua K. Formulation and evaluation of controlled-release matrix systems of ciprofloxacin.Polim Med. 2017;47(2):101-10628.
[32] Sareen R, Nath K, Jain N. Curcumin Loaded Microsponges for Colon Targeting in-flammatory Bowel Disease: Fabrication, Optimization, and *In vitro* and Pharmacodynamic Evaluation. BioMed Research Int.2014;2014.
[33] Indian Pharmacopoeia (IP) 2014. Government of India Ministry of health & Family Welfare, The Indian Pharmacopoeia commission, Ghaziabad. 2014: II: 1864-1866.
[34] TahanG, AytacE, AytekinH. Vitamin E has a dual effect of anti-infammatory and anti-oxidat activities in acetic-acid induced colitis in rats. Can J Surg. 2011;54(5):333-38.
[35] Murad HAS, Abdallah HM, Ali SS. Menthalongifolia protects against acetic acid induced colitis in rats. Journal of Ethnopharm. 2016;1-22.
[36] Singh B, Kapil R, Nandi M. Developing oral drug delivery systems using formulation by design: Vital precepts, retrospect and prospects. Expert OpinDrugDeliv. 2011;8:1341–60.
[37] Fernanda MC, Ana DS, Raul CE.Insights into the swelling process and drug release mechanisms from cross-linked pectin/high amylose starch matrices. Asian journal of pharmaceutical sci. 2014;9:27-34.

[38] Gupta A, Tiwari G, Tiwari R, Srivastava R, Rai AK. Enteric coated HPMC capsules plugged with 5-FU loaded microsponges: a potential approach for treatment of colon cancer. Brazilian Journal of Pharmaceutical Sciences. 2015;51(3):591-605.
[39] Esiringu F, Demiroz FT, AcarturkF.Investigation of the effect of intracolonic melatonin gel formulation on acetic acid-induce colitis. Drug Deliv. 2014;1-9.

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Figure 1. Two-dimensional contour plots and corresponding three-dimensional response surface plot depicting the effect of various input variables on; (A) Entrapment Efficiency, (B) %CDR(cumulative drug release), and (C) Particle Size.

Figure 2. a) SEM image of resveratrol-loaded microsponge formulation (Magnification 8000 x) **b)** XRD spectra of drug, polymer and microsponge formulation **c)** FTIR spectra for resveratrol, chitosan and resveratrol-loaded microsponge formulation **d)** Heating curves of differential scanning calorimetry (DSC) for polymer, drug and microsponge formulation **e)** Thermal gravimetric analysis (TGA) of polymer, drug and microsponge formulation.

Figure 3. Comparision of % CDR between optimized resveratrol-loaded microsponge formulation and resveratrol-loaded microsponge-matrix tablet. Each cross bar indicates average value±SD (n=3).

Figure 4. Histology of colonic section of (A) Normal control group, (B) Acetic acid-induced colitis group, (C) Resveratrol treated group, (D) Resveratrol-loaded microsponge treated group, (E) Resveratrol-loaded microsponge-matrix tablet treated group.

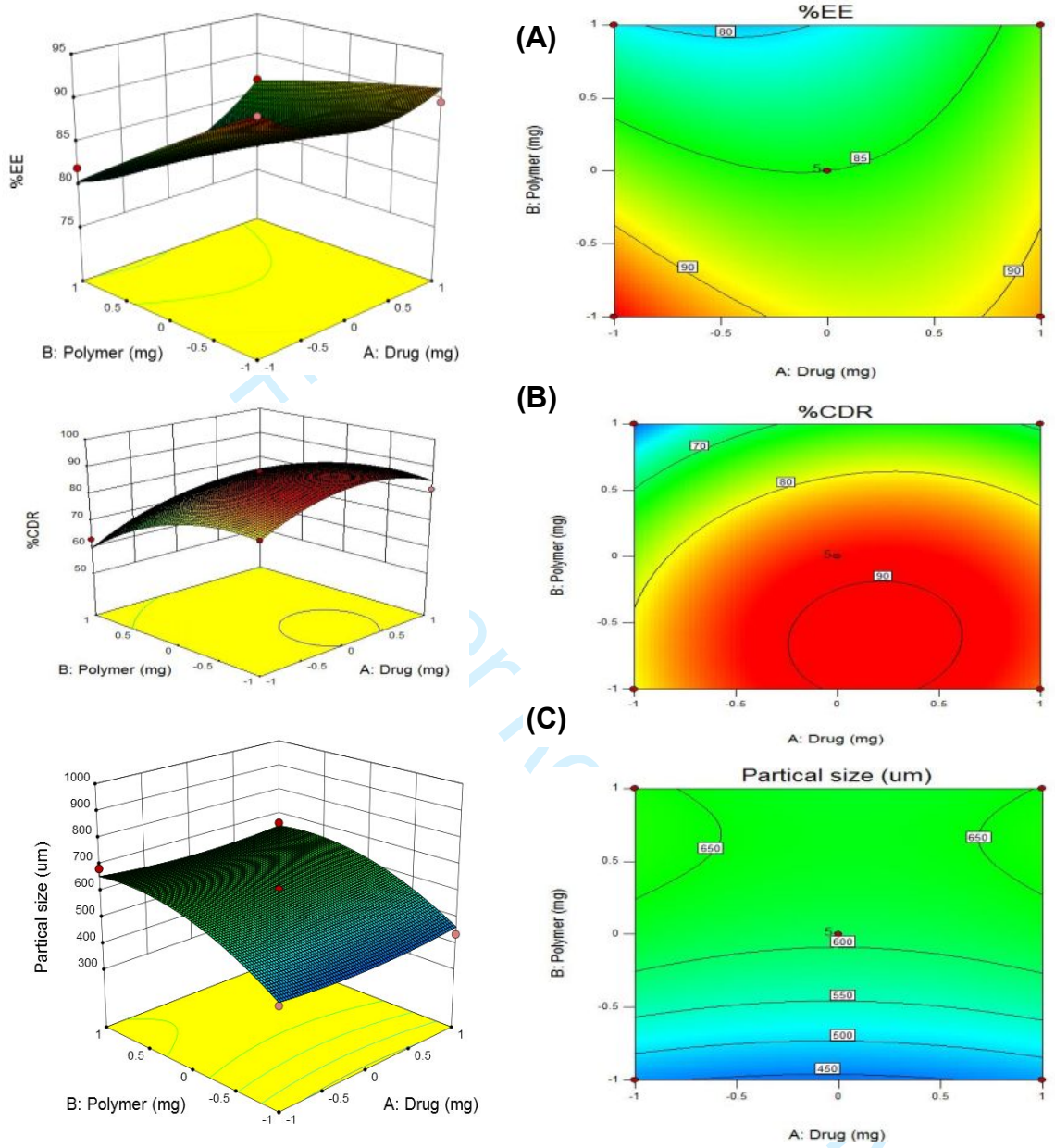
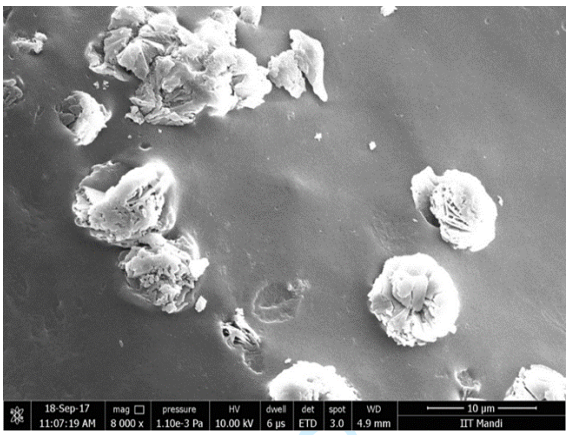
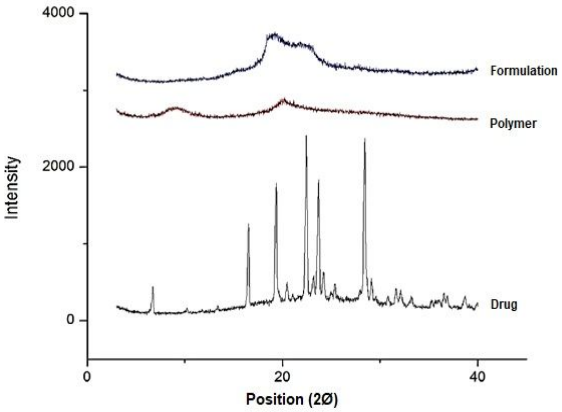


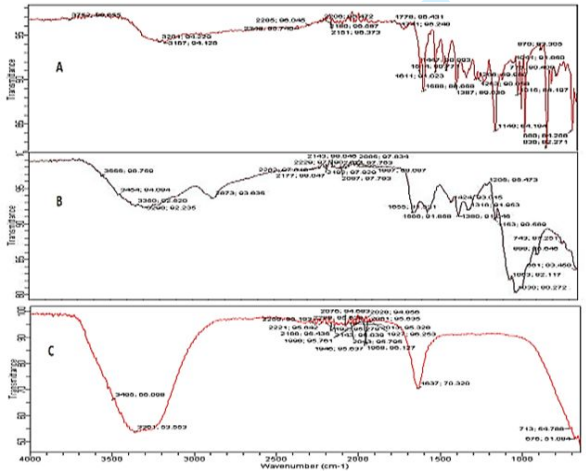
Fig. 1. Two-dimensional contour plots and corresponding three-dimensional response surface plot depicting the effect of various input variables on; **(A)** Entrapment Efficiency, **(B)** %CDR(cumulative drug release), and **(C)** Particle Size


18-Sep-17
11:07:19 AM
mag
8 000 x
pressure
1.10e-3 Pa
HV
10.00 kV
dwell
6 µs
det
ETD
spot
3.0
WD
4.9 mm
10 µm
IIT Mandi

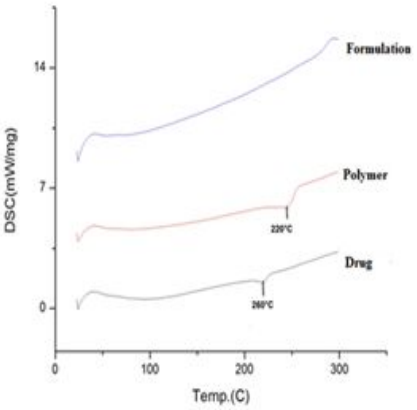
a)


4000
2000
0
Intensity
Formulation
Polymer
Drug
0
20
40
Position (2Ø)

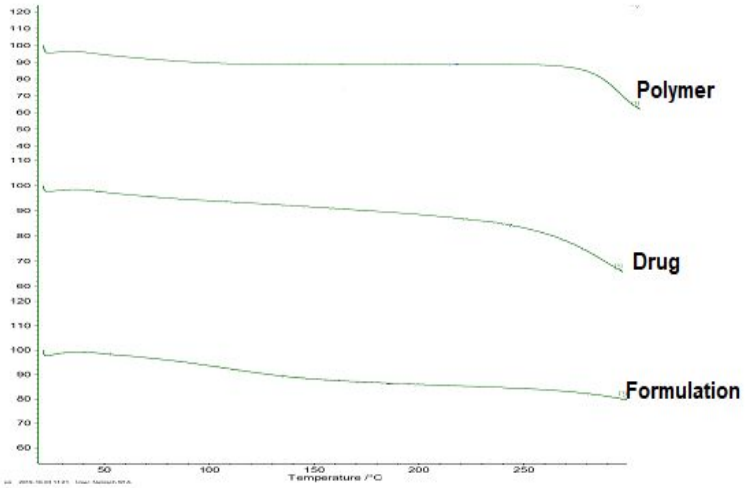
b)


A
B
C
Transmittance
Wavenumber (cm-1)
4000
3500
3000
2500
2000
1500
1000

c)


Formulation
Polymer
Drug
220°C
260°C
DSC(mW/mg)
14
7
0
0
100
200
300
Temp.(C)

d)


Polymer
Drug
Formulation
Temperature /°C
50
100
150
200
250

e)

Fig. 2. a) SEM image of RES-loaded microsponge formulation (Magnification 8000 x)**b)**XRD spectra of drug, polymer and microsponge formulation **c)**FTIR spectra for A) RESB) Chitosan and C) RES-loaded microsponge formulation **d)**Heating curves of differential scanning calorimetry (DSC) for polymer, drug and microsponge formulation **e)**Thermal gravimetric analysis (TGA) of polymer, drug and microsponge formulation..

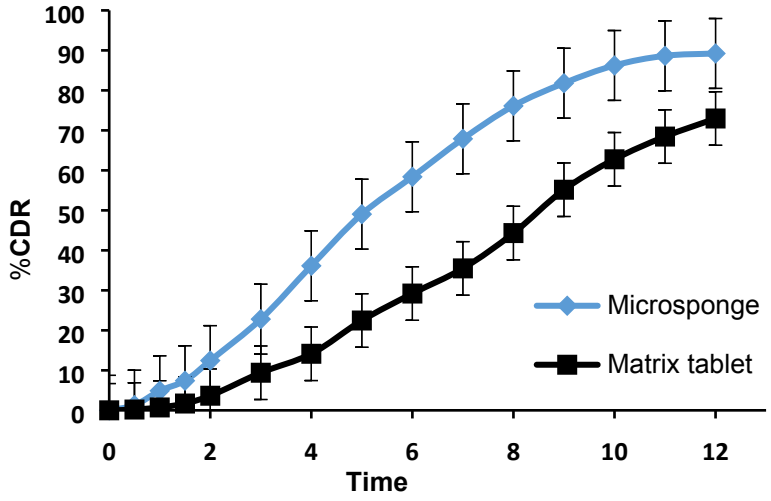


Fig. 3. Comparision of % CDR between optimized RES-loaded microsponge formulation and RES-loaded microsponge-matrix tablet. Each cross bar indicates average value±SD (n=3).

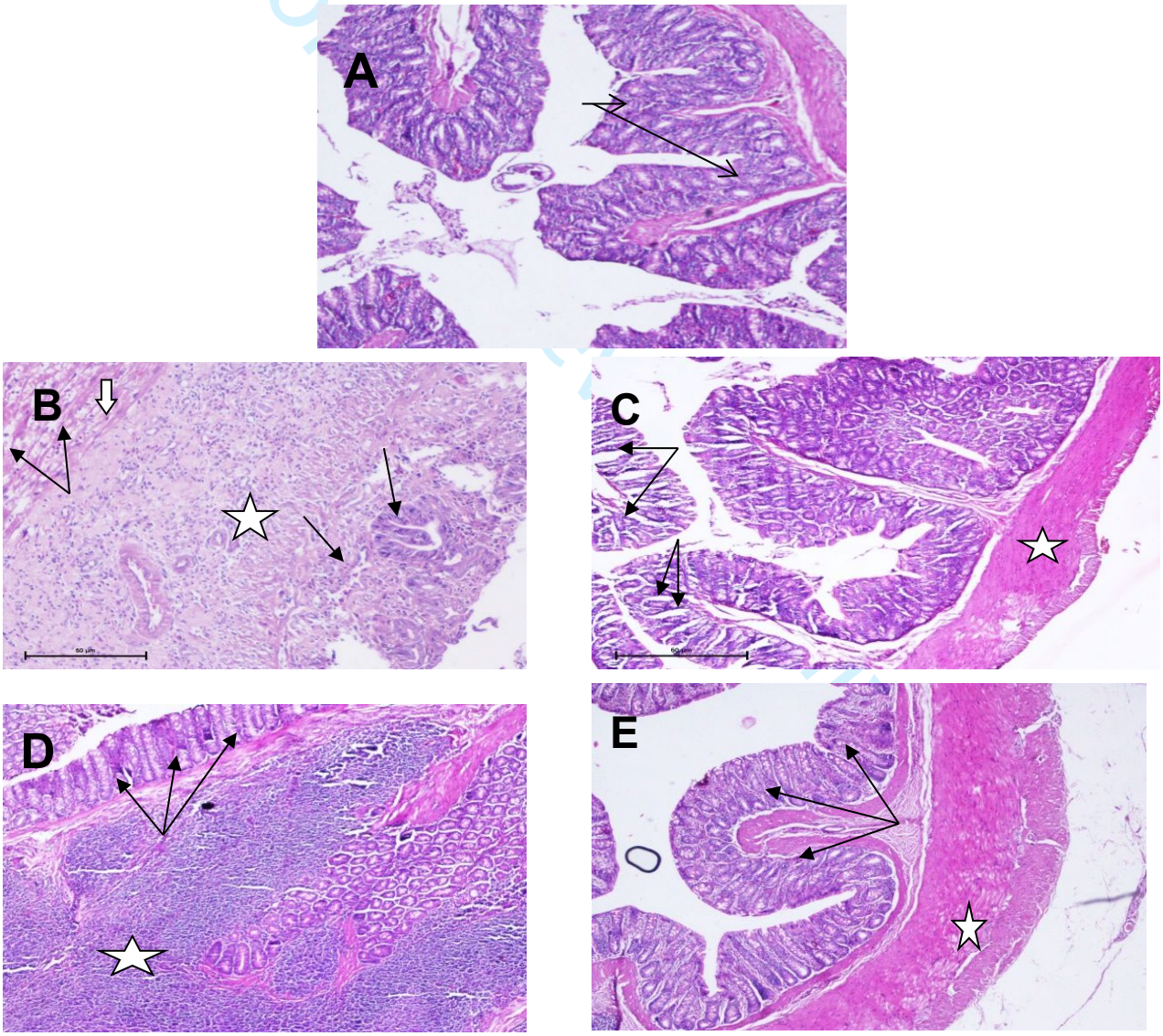


Fig. 4. Histology of colonic section of **(A)** Normal control group, **(B)** Acetic acid-induced colitis group, **(C)** RES treated group, **(D)** RES-loaded microsponge treated group, **(E)** RES-loaded microsponge-matrix

tablet treated group.**White arrow** indicates severe surface, and mucosal haemorrhage, **Black arrow** indicates marked necrotic changes, and remnants of colonic crypts and **Star** indicates the sub-mucosal layer revealing polymorphic inflammatory cell infiltration

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Table 1. Experimental runs of BBD design matrix and their responses

Runs	Drug (mg)	Polymer (mL)	Solvent (mL)	%EE	%CDR	Particle size (µm)
1	-1	0	-1	82.8	68.47	464.172
2	1	1	0	8 6.8	68.88	663.563
3	0	0	0	85	88.18	609.211
4	0	0	0	85	88.18	609.211
5	1	0	-1	83	81.74	471.391
6	0	1	-1	76	56.96	378.227
7	-1	-1	0	94	80.30	446.452
8	-1	0	1	87	70.71	853.771
9	1	-1	0	89.6	81.8	436.582
10	0	0	0	85	88.18	609.211
11	0	1	1	79	72.48	923.211
12	0	0	0	85	88.18	609.211
13	-1	1	0	82	62.97	686.98
14	1	0	1	93	78.52	869.236
15	0	-1	-1	84.6	87.61	395.606
16	0	0	0	85	88.18	609.211
17	0	-1	1	92	84.64	600.834

Independent variables	**Level used, actual (coded)**		
	Low (-1)	Medium (1)	High (+1)
Drug	250	375	500
Polymer	250	375	500
Solvent	2.5	5	7.2

Runs	Drug (mg)	Polymer (mL)	Solvent (mL)	%EE	%CDR	Particle size (μm)
1	-1	0	-1	82.8	68.47	464.172
2	1	1	0	8 6.8	68.88	663.563
3	0	0	0	85	88.18	609.211
4	0	0	0	85	88.18	609.211
5	1	0	-1	83	81.74	471.391
6	0	1	-1	76	56.96	378.227
7	-1	-1	0	94	80.30	446.452
8	-1	0	1	87	70.71	853.771
9	1	-1	0	89.6	81.8	436.582
10	0	0	0	85	88.18	609.211
11	0	1	1	79	72.48	923.211
12	0	0	0	85	88.18	609.211
13	-1	1	0	82	62.97	686.98
14	1	0	1	93	78.52	869.236
15	0	-1	-1	84.6	87.61	395.606
16	0	0	0	85	88.18	609.211
17	0	-1	1	92	84.64	600.834

Independent variables	**Level used, actual (coded)**		
	Low(-1)	Medium(1)	High(+1)
Drug	250	375	500
Polymer	250	375	500
Solvent	2.5	5	7.2

Table 2. Polynomial mathematical model data

Coefficient code	**Second-order Polynomial coefficients for response variables**

	%EE	%CDR	Particle size
β_0	85	88.18	609.211
β_1	0.825	3.5275	-0.07537
β_2	-4.55	-9.1325	96.58588
β_3	3.075	1.4125	193.4795
β_{11}	2.3	1.1025	-3.38675
β_{22}	1.45	-1.2975	4.5615
β_{33}	-1.1	4.6225	84.984
β_{12}	3.325	-7.59375	20.90563
β_{13}	-0.225	-7.09875	-71.7224
β_{23}	-1.875	-5.65875	37.02588
R^2	0.9234	0.9060	0.9784

Table 3. Constraints for numeric optimization and predicted solutions

Variable	Goal	Lower limit	Upper limit	Importance
Drug (A)	In range	-1	1	***
Polymer (B)	In range	-1	1	***
Solvent (C)	In range	-1	1	***
%EE	In range	85.00	94.00	****
%CDR	In range	56.96	88.18	****
Particle size	In range	378.22	923.39	****

A	B	C	%EE	%CDR	Particle size	Desirability

0.36	0.82	0.15	88.89	89.07	463.962	1.000	**Selected**

Table 4. Various evaluation parameters of matrix tablets

Parameter	Value
Average weight (n=20) mg	499.65 ±1.35
Friability test (%) (n=10)	0.69±0.23%
Hardness (n=5) (Kg/cm^2)	4.13±0.13

Table 5. Regression cofficient values of microsponge matrix tablet and microsponge formulation

Kinetic models	Regression cofficient (r^2) Matrix tablet	Regression cofficient (r^2) Microsponge formulation
Zero order kinetic	0.9547	0.9691
First order kinetic	0.8339	0.5933
Higuchi model	0.735	0.8631
Peppas model	0.9894	0.7661

Table 6. Macroscopic evaluation of colonic lesions of rat

Groups	0	0.5	1	1.5	2
Control	_	1	2	5	1
Resveratrol	_	1	_	2	_
Microsponge	_	2	1	-	_
Matrix Tablet	_	3	2	-	_