RESEARCH ARTICLE

Formulation and Evaluation of the Microsphere of *Raupya Bhasma* for Colon-targeted Drug Delivery

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ABSTRACT

Ulcerative colitis and colon cancer are the major diseases associated with the colon. Numerous NSAIDs and steroids treat colitis but have serious side effects. Consider this aim of the study is a formulation of the microsphere of raupya bhasma and its evaluation for drug delivery to the colon. Microspheres were formulated using the emulsification polymerization method. Targeting of the colon by formulation was evaluated using suitable dissolution media.

Keywords: Colon targeting, Dissolution, Microsphere, *Raupya bhasma*, Ulcerative colitis.

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INTRODUCTION

The inflammation of the large intestine, known as ulcerative colitis (UC) affects about five lakh people annually, predominantly below the age of 30 years, and can ultimately enhance the chances of growing large bowel cancer. The complication of chronic UC may be the reason for colon cancer.[1,2] Surgery, chemotherapy, and radiation therapy are currently in practice for the treatment of cancers associated with the colon. The associated side-effect of chemotherapy and radiation therapy is directly related to the quality of life and life expectancy.

The anticancer property of cis-platin was explored in 1969, which encouraged the exploration of other metals having anticancer activity. Various *in-vitro* and *in-vivo* research has already proven the compatibility of raupya bhasma for its anti-inflammatory properties.

The antiangiogenic activity of silver nanoparticles (NPs) is proved.[3] Research for antiangiogenic activity indicates that most molecules possessing antiangiogenic activity are organic and have substantial toxicities like gastrointestinal tract perforations. Hence, opportunities exist to search for the potentials of antiangiogenic molecules, which may be inorganic compounds with minute or no severe side effects.[4-9]

Silver bhasma is selected for the treatment of colon cancer because bhasmas are claimed to be biologically produced nanoparticles. When metal is converted into metal bhasma, the toxicity of metal gets reduced while therapeutic activity increases. The cytotoxicity, antiproliferative and antiangiogenic activity of silver NPs are reported. It is reported that SNPs possess activity against inflammation, ulcerative colitis, and colon.[10-12]

MATERIALS AND METHODS

Formulation of Microsphere

Microspheres of raupya bhasma were formulated using the emulsification polymerization method.[4] The aqueous dispersion of guar gum and xanthan gum was dispersed in 100 mL of cold water containing the drug (0.5 g) and kept for swelling for 2 hours). After swelling, the mixture of drug and polymer were dispersed in castor oil (100 mL) containing 2 g of twin 80 and 0.1% silicon oil while continuously stirring with a mechanical stirrer with a speed of 4000 rpm. After thoroughly uniform mixing, 0.2 mL of concentrated H_2SO_4 followed by 3 mL of glutaraldehyde were added to the dispersion. The mixture was stirred at a constant speed for 4 hours at 50°C. The microspheres were collected by sedimentation followed by decantation of oil. After that obtained microsphere was washed with several fractions of isopropyl alcohol.[1] The formulation was optimized using a 22factorial design, as shown in Table 1

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Table1: Composition of formulation of microsphere

Formulation	*Composition*		
	Guar gum (g)	*Xanthan gum (g)*	*Raya bhasma (g)*
F1	1	0.5	0.5
F2	0.5	1	0.5
F3	0.5	0.5	0.5
F4	1	1	0.5

Characterization of Microsphere

Percentage Yield

To calculate the %yield prepared formulation was divided by the total amount of polymer and raupya bhasma taken in the preparation of the formulation. The percentage yield was calculated using the given formula

$$\text{Percentage yield} = \frac{\text{Actual yield of formulation}}{\text{Total weight of polymers and drug}} \times 100$$

Determination of Raupya Bhasma Included in the Formulation

The amount of raupya bhasma in the microsphere was determined by placing the microsphere in phosphate buffer saline (PBS, pH 7.4) for 48 hours at 37°C with vigorous stirring. The concentration of raupya bhasma was determined using ICP-MS. The percentage of loading efficiency and content was expressed with the following equation:

$$\text{Loading efficiency (\%)} = \frac{\text{Weight of loaded drug in microsphere}}{\text{Initial feeding weight of drug}} \times 100$$

$$\text{Loading content (\%)} = \frac{\text{Weight of loaded drug in microsphere}}{\text{Weight of microsphere}} \times 100$$

Surface-associated Drug Content

Microspheres of raupya bhasma were assessed for drug content which was associated with the surface of the formulation. Each batch of 100 mg of the formulation was shaken in 20 mL of 0.1N hydrochloric acid for 5 minutes and filtered using Whatman filter paper. The surface-associated drug content in the filtrate was determined using ICP-MS. All the investigations were conducted thrice (n = 3).

$$\text{Surface associated drug content} = \frac{\text{Amount of drug present in the filtrate}}{\text{Amount of drug used in the formulation}} \times 100.$$

Particle Size Analysis

A laser diffraction particle size analyzer analyzed the particle size of the microsphere. The microsphere of raupya bhasma was suspended in the cavity of the particle size analyzer having double distilled water, and the particle size was calculated by means of the software.

Morphology

The morphology of the microsphere of raupya bhasma was evaluated using scanning electron microscopy (SEM) (JEOL 100-CX USA Inc, Peabody, MA)

Scanning electron microscopy (SEM) was used to determine microspheres' shape and surface characteristics. The samples of the microsphere of raupya bhasma for evaluation using SEM were prepared by lightly sprinkling the microsphere on a double adhesive tape, which was fixed on an aluminum stub. The aluminium stubs were coated with gold using a gold sputter coater in a high vacuum evaporator, and SEM observed samples at 10 kV.

Equilibrium Swelling Studies of Formulation

Accurately weighed formulation (100 mg) were transferred in PBS (pH 7.4) and kept for swelling until weight became constant. The formulation was taken out and blotted with filter paper, then measured weight difference. The degree of swelling (α) of the formulation was calculated using the given formula:

$$\alpha = we - wo / wo$$

Where wo and we are the initial weight of the microspheres and the weight of the microspheres at equilibrium swelling in the medium, respectively.

Flow Properties

Angle of Repose

The angle of repose was determined by the funnel method. The formulation was poured through a funnel that could be elevated vertically until extreme cone height (h), was obtained. The diameter of the heap (D) was determined. The following formula was used to calculate the angle of repose.

$$\tan \theta = h / r,$$

$$\theta = \tan\text{-}1\ (h / r)$$

Where θ = angle of repose, h = height of the pile (cm), and r = radius of the pile (cm)[1].

In-vitro Release of Study of Microsphere

Preparation of Dissolution Media

Preparation of Fresh Human Fecal Content Medium

Human fecal slurries (freshly prepared) are usually used to evaluate the fermentation of polysaccharides (Non-starch). Human fecal slurries were prepared by homogenizing fresh feces in 0.1 M sodium phosphate buffer (pH 6.8) by supplying CO_2 to maintain anaerobic conditions. Freshly prepared human fecal slurries were added to the dissolution media to give a final dilution of 4% w/v. All the processes mentioned above were carried out in anaerobic conditions by the supply of carbon dioxide.[12]

Preparation of Goat Caecal Medium

Fresh caecal content of Goat was procured from the local market of Phagwara and kept in a desiccator under anaerobic conditions. Accurately weighed caecal content were suspended in the pH 6.8 buffer under anaerobic condition. Finally, 4% w/v cecal medium was prepared by adding dissolution media. All the above procedure was carried out anaerobically in the presence of carbon dioxide.[12]

In-vitro Drug Release using Human Fecal Slurries

Dissolution studies for colon-specific drug delivery were carried out using human fecal slurries (freshly prepared) for formulation F4. Basket type dissolution test apparatus

USP I)with minor alteration in process was used for evaluation of release of raupya bhasma in the presence of human fecal slurries (freshly prepared). Gradient pH dissolution method using human fecal contents was used to assess the release of raupya bhasma from formulations meant for drug delivery to the colon.

The experiments were conducted using a beaker (250 ml) submerged in water containing pots of dissolution apparatus. One capsule was transferred to each pot having the dissolution medium. The *In vitro* release of formulation was started in 150 ml media of pH 1.2 using 100 rpm at 37 ± 0.5°c for the first 2 hours. After 2 hours, the pH of the dissolution media was maintained to 6.8 by the addition of 50ml phosphate buffer and sodium hydroxide solution (q.s.). The study was continued for 4 hours. After 4 hours, the media was made anaerobic by degassing it with carbon dioxide for 15 min. Undissolved oxygen in the media was removed by the supply of carbon dioxide to the media. After that, 4% w/v fecal slurries (freshly prepared) was transferred to the dissolution media, and the experiment was continued for up to 24h under the continuous purging of carbon dioxide. Approximate 1.0 ml samples were withdrawn at the interval of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, and 24.0 hours from the dissolution medium and the same volume was substituted by the fresh medium which was previously kept up in anaerobic condition. The sample volume was adjusted to 10 ml, followed by filtration using 0.22-micron membrane filters, and subjected to ICP-MS analysis. All the studies were conducted six times, and the mean data was recorded.[12]

In-vitro Drug Release using Goat Caecal Content

Dissolution studies for colon-specific drug delivery were carried out using human Goat caecal content (freshly prepared) for formulation F4. Basket type dissolution test apparatus (USP I) with minor alteration in process was used for evaluation of release of raupya bhasma in the presence of Goat caecal content (freshly prepared). Gradient pH dissolution method using goat caecal content was used to assess the release of raupya bhasma from formulations meant for drug delivery to the colon.

The experiments were conducted using a beaker (250 mL) submerged in water containing pots of dissolution apparatus. One capsule was transferred to each pot having the dissolution medium. The *in-vitro* release of the formulation was started in 150 mL media of pH 1.2 using 100 rpm at 37 ± 0.5°C for the first 2 hours. After 2 hours, the pH of the dissolution media was maintained at 6.8 by adding 50 mL phosphate buffer and sodium hydroxide solution (q.s.). The study was continued for 4 hours. After 4 hours, the media was made anaerobic by degassing it with carbon dioxide for 15 minutes. Undissolved oxygen in the media was removed by the supply of carbon dioxide to the media. After that, 4% w/v goat caecal content (freshly prepared) was transferred to the dissolution media, and the experiment was continued up to 24 hours under the continuous purging of carbon dioxide. Approximate 1.0 mL samples were withdrawn at an interval of 1.0, 2.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, and 24.0 hours from the dissolution medium and the same volume was substituted by the fresh medium which was previously kept up in anaerobic condition. The sample volume was adjusted to 10 mL and followed by filtration using 0.22 micron membrane filters and was subjected to ICP-MS analysis. All the studies were conducted six times and the mean data was recorded.[12]

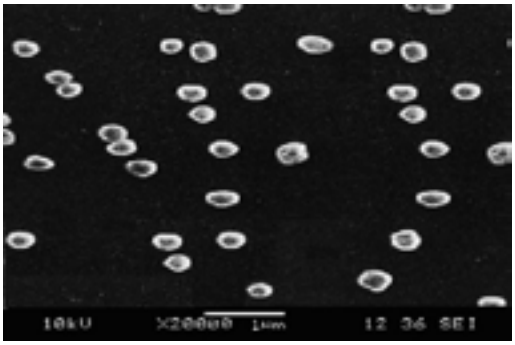

Figure 1: The SEM image of the microsphere distribution and size,

Note: The studies on the above-mentioned formulation were also carried out in the same way without adding probiotic culture, goat caecal content, and human fecal contents *i.e,* normal buffer media.

RESULTS AND DISCUSSION

Microsphere was prepared by emulsification polymerization technique using guar gum and xanthan gum as polymer. Xanthan gum and guar gum were chosen for encapsulation purposes to retard the release of the drug before reaching to the colon. Xanthan gum or guar gum has been used independently in colon-specific drug delivery, but when used in combination enhances gel properties, and drug release-retarding tendency drastically increases.

Cross-linking of microspheres using glutaraldehyde may lead to hardening, which is temperature-induced cross-linking in an acidic medium.

The determination of size, size distribution, and morphology are significant characteristics to be verified in the formulation development. The homogeneity of height in a formulation indicates stability and some system behavior. The morphology of drug-loaded raupya bhasma microspheres was analyzed using SEM. Figure 1 has shown the microsphere had a spherical shape with a relatively narrow size distribution; furthermore, no large aggregates were observed. Measurement of particle size by zeta sizer is based on the principle of dynamic light scattering (DLS) and found to be 599.6c nm (Figure 2). DLS (also known as PCS- Photo Correlation spectroscopy) determines Brownian motion and co-relates this to the particle biocompatibility of silver NPS has been confirmed by es size. It can be achieved by enlightening the particles with a laser and investigating the intensity of fluctuations in the scattered light. DLS uses particles' ability to scatter light and their natural Brownian motion when suspended in fluid, water in this case. Particle size is calculated based on an estimate of the particle diffusion coefficient while suspended in a medium.

Table 2: Characterization of microsphere (F1, F2, F3, F4)

Parameter	Value for F1	Value for F2	Value for F3	Value for F4
Percentage yield	83.25 ± 0.43	81.52 ± 0.37	89.47 ± 0.12	94 ± 0.16
Loading efficacy%	85.32 ± 0.75	83.48 ± 0.29	86.94 ± 0.27	97 ± 0.38
Loading content%	20.28 ± 0.63	19.92 ± 0.33	21.56 ± 0.14	23.12 ± 0.11
Surface-associated drug content %	0.77 ± 0.41	0.98 ± 0.32	0.68 ± 0.31	0.23 ± 0
Degree of swelling%	34.84 ± 0.41	35.55 ± 0.32	33.25 ± 0.31	31 ± 0.01
Angle of repose	25.32 ± 19	25.13 ± 17	25.62 ± 22	25.56 ± 37

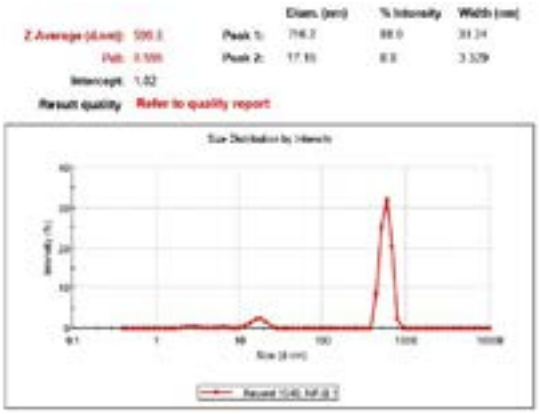

Figure2: Particle size determination by zeta sizer.

The relationship between the size of a particle and its speed due to Brownian motion can be understood by the Stokes-Einstein equation. Particle diffusion rates are inversely proportional to particle size.

Percentage yield, loading efficacy, loading content, surface-associated drug content, degree of swelling, and angle of repose of microsphere is reported in Table 2. The swelling index of guar gum and xanthan gum is very high, drastically decreasing due to cross-linking. Cross-linking restricts the free access of water to the polymer hydroxyl group, which reduces the cross-linked polymer's swelling properties. The ideal fate of microspheres *in-vivo* is to release their contents to the surrounding biological fluid. *In-vitro* dissolution testing provides a valuable tool for investigating drug release mechanisms. Also, drug release testing is a fundamental part of drug product development, and manufacturing is also employed as a quality control tool to monitor the batch-to-batch consistency of the drug release. *In-vitro* drug release study of microspheres in fecal content is shown in Table 3. The fecal content was collected from the healthy human volunteer. As the condition of GIT is anaerobic so anaerobic condition is maintained by the supply of nitrogen gas which prevents the death of microorganisms in aerobic conditions. The cumulative percentage of microspheres in different release media during the different periods is shown in Table 3. In the polysaccharide-based drug delivery system, polysaccharide polymer protects the drug from the stomach and small intestine and can deliver the drug to the colon. As guar gum and xanthan gum are prebiotics, the microflora of the colon feed them, and the drug gets released into the colon.

Based on the characterization of formulations F1, F2, F3, and F4, it was concluded that F4 is the best among all microspheres. Further studies were conducted only for formulation F4.

Table3: *In-vitro* drug release profile of microsphere in PBS

Time in hours	%Release in PBS	%Release in 4% fecal content	%Release in 4% caecal content
0	0	0	0
1	3.8 ± 0.16	3.81 ± 0.12	3.809 ± 0.11
2	8.75 ± 0.19	8.74 ± 0.17	8.75 ± 0.14
4	11.90 ± 0.18	11.74 ± 0.21	11.74 ± 0.76
5	24.87 ± 0.13	24.85 ± 0.26	24.76 ± 0.28
6	26.23 ± 0.22	38.71 ± 0.34	39.33 ± 0.33
8	27.91 ± 0.27	59.93 ± 0.56	60.18 ± 0.59
10	29.22 ± 0.28	80.29 ± 0.38	81.36 ± 0.34
12	30.83 ± 0.32	93.73 ± 0.76	93.89 ± 0.77
16	31.87 ± 0.33	94.56 ± 0.51	94.51 ± 0.54
20	33.18 ± 0.42	94.39 ± 0.40	95.50 ± 0.42
24	34.96 ± 0.37	94.28 ± 0.79	95.50 ± 0.78

CONCLUSION

The study is vital because the formulation of the microsphere of raupya bhasma was prepared for the first time for targeting to the colon. It enhances efficacy at a lower dose.

In-vitro drug release was carried out using PBS, human fecal content, and goat caecal content. The novel approach development of formulation release drug for colon-specific drug delivery.

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CONFLICT OF INTEREST

Nil

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