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Design and Evaluation of Linseed Mucilage Based Mucoadhesive Microspheres of Capacitabine: *In Vitro* Characterization

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Abstract: Nanotechnology mediated drug delivery is being utilized nowadays for effective drug delivery at desired cell target. Many scientific investigators have utilized nanocarrier mediated drug delivery of antitumor drug for active targeting of encapsulated drug at desired target cells. Capacitabine is approved anticancer drug especially used for management of breast and colon cancer. Various conventional drug delivery systems of capacitabine are available for clinical use. However, limited oral bioavailability is major hurdle associated with conventional delivery of capacitabine. Thus, present study aimed to improve oral bioavailability of capacitabine through gastroretentive drug delivery approach. The capacitabine loaded mucilage-alginate microspheres were formulated using ionic gelation technique and characterized with respect to physicochemical characteristics. The drug loaded microspheres showed acceptable physicochemical characteristics. Thus, natural mucilage could be promising excipient for formulation of gastroretentive microspheres of capacitabine.

Keywords: Capacitabine, Natural mucilage, Linseed mucilage, Microspheres, Gastro retention

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Introduction

Cancer is uncontrolled cell proliferation that aggressively invades other parts of the body. According to the International Agency for Research on Cancer (IARC), 14.1 million new cases of cancer were estimated to occur in 2012, with almost 8.2 million mortality cases. Based on IARC estimates, cancer is the 2nd most common cause of death in both economically developing and developed countries. About 13% of global cancer

cases are estimated to have occurred in southwestern of Asia. The main cancer treatment modality, chemotherapy, has limitations including various side effects (Nazari-Vanani *et al.*, 2019).

The nanotechnology mediated drug delivery is widely used nowadays. Various nanocarriers like liposomes (Sansare *et al.*, 2021), niosomes, ethosomes, transfersomes, glycosomes, nanoparticles (Gupta *et al.*, 2021), solid lipid

nanoparticles (Sansare *et al.*, 2020), nanostructured lipid carriers (Gupta *et al.*, 2022), microspheres (Abrar, 2020) etc. are utilized for effective drug delivery. The drug loaded in nanocarriers is released in controlled manner. In addition to this, the cell specific targeting of loaded drug is one more advantage of nanocarrier based drug delivery system. Capecitabine is used nowadays in the management of colon and breast cancer. It is used alone or in combination with other medicine (Nazari-Vanani *et al.*, 2019). Inside the body, capecitabine gets converted into 5-fluorouracil. 5-fluorouracil hampers the formation of RNA and DNA in the cancer cells which eventually minimize the growth of cells. The major drawback of capecitabine is limited oral bioavailability.

The use of natural excipients as carriers in drug delivery systems is recent trend of oral drug delivery. At present, socio-economic condition of the modern world has elevated the interest of natural polymers. Environmental concerns are also playing considerable role and contributing to the growing interest in natural polymers due to their biocompatibility, biodegradability and low processing cost (Reddy *et al.*, 2021). Naturally obtaining polymers are diverse class of macromolecules with a wide range of pharmaceutical applications. Various natural polymers can be classified as proteins-based natural polymers like collagen, gelatin, silk fibroin, fibrin and natural polysaccharides like chitosan, starch, alginate, gellan gum, pectin, gum acacia, gum tragacanth and guar gum (Bahadur, 2017). These polysaccharides have some excellent water solubility as well as swelling potential, which eventually are useful for oral controlled drug delivery. The term mucilage indicates substances which have high water absorbing and swelling capability on contact with water. Several species of mucilaginous species of plants have been used in traditional system of medicine in the world since last 4000 year. Mucilage is metabolic product of the plant formed by various cells. Chemically these are high molecular weight (approx. 200,000 Da) compounds consisting of

sugar and uronic acid units. These are generally sulphuric acid esters and have a complex structure of polysaccharide. The high-water absorbing capability of mucilage is due to presence of hydroxyl groups in sugar structure of mucilage. The promising application of mucilage is drug delivery. Mucilages are widely investigated for development of drug delivery systems (Cherian *et al.*, 2019). The less toxicity, biocompatibility and biodegradability are ideal properties of mucilage which are useful in development of drug delivery systems. Many scientific investigators have utilized plant derived mucilage for development of nano and microcarrier based systems.

Thus, present study aimed to formulate capecitabine loaded linseed mucilage based microspheres for controlled drug delivery. The mucilage from linseed was isolated with water and utilized for formulation of drug loaded microspheres. The drug loaded microspheres were formulated using ionic gelation technique in presence of calcium ions and characterized with respect to particle size, entrapment efficiency, *in vitro* drug release, mucoadhesion capability to goat intestinal mucosa and swelling index. The microspheres showed acceptable physicochemical properties and mucoadhesion behavior. Thus, mucilage based microspheres could be promising alternative for controlled drug delivery.

Materials and Methods

Capecitabine was purchased from Naprod Life Sciences Pvt. Ltd. (India). *Linum usitatissimum* seeds were purchased locally. Calcium carbonate and sodium alginate were purchased from S. D. Fine Chemicals Ltd. (India). Dialysis membrane was purchased from Himedia (India). All other reagents, chemicals and solvents were laboratory grade and purchased locally.

Isolation and characterization of mucilage from Linum usitatissimum seeds:

The hot water treatment was used for isolation of mucilage from linseed. Briefly, 100 g of linseed seeds were soaked in 500 ml of water for 10 h for hydration of seeds. The hydrated mucilage along

Table 1: Formulation batches of capacitabine loaded mucilage-alginate microspheres

Formulation code	Polymer concentration (% w/v)	Polymer to drug ratio	Calcium chloride concentration (% w/v)
F1	2	2.9	2.9
F2	2.2	2.9	2.9
F3	2.5	3	3
F4	3	3	3

with seeds were dried in hot air oven at 50°C. The dried mucilages were sieved through sieve 18 for separation from seeds (Saquib Hasnain *et al.*, 2018). The isolated mucilage from seeds was characterized with respect to colour, odour and taste.

Design of capacitabine loaded mucilage-alginate microspheres:

The ionic gelation technique was used for fabrication of capacitabine loaded microspheres (Hou *et al.*, 2014). Briefly appropriate quantities of linseed mucilage and sodium alginate were dissolved in distilled water with continuous stirring to polymeric solution. The weighed quantity of capacitabine was dissolved in polymeric solution with continuous stirring. The resulting medicated polymeric solution was injected in 100 ml of 7% w/v calcium chloride solution using 24-G needle with continuous stirring at 500 rpm using magnetic stirrer. The resulting polymeric dispersion was stirred for 30 min for crosslinking of alginate in presence of calcium ions. After stirring continuous stirring for specified time, the dispersion was kept in standing for 1 h for complete crosslinking of polymer. After 1 h the microspheres were collected by filtration, washed with double distilled water and finally dried in hot air oven at 40°C for 10 h. Four batches of microspheres were formulated as highlighted in Table 1.

Evaluation of linseed mucilage-alginate microspheres:

Assessment of particle size:

Particle size of capacitabine loaded mucilage-

alginate microspheres was assessed by optical microscopy using calibrated eyepiece micrometer (Akin-Ajani *et al.*, 2022). Briefly, 50 mg of drug loaded microspheres were spread over the clean glass slide and observed under compound microscope under the 10X scale. The diameter of 100 particle was measured randomly and arithmetic mean diameter was calculated.

Measurement of entrapment efficiency:

The entrapment of capacitabine in mucilage-alginate microspheres was quantitatively measured in percentage using UV spectrometric measurement. The dried drug loaded microspheres were finely ground using mortar pestle to obtain fine powder. The 40 mg of powder was weighed and dispersed in phosphate buffer (pH 6.8). The resulting dispersion was stirred after 12 h and filtered. The filtrate was diluted ten times using phosphate buffer and subjected to spectrometric measurement at 240 nm. The entrapment efficiency of capacitabine in microspheres was then calculated using below equation.

$$\text{Per cent entrapment of capacitabine} = \frac{W_p}{W_t} \times 100$$

Where, W_p is practical content of capacitabine in dispersion and W_t is theoretical content of capacitabine in microspheres (20 mg).

Assessment of capacitabine release behavior:

The dialysis membrane drug diffusion method was used for assessment of capacitabine release (Boddupalli *et al.*, 2012). Dialysis membrane (Mol. weight: 12–14 kDa) was soaked in distilled water

overnight. The capacitabine encapsulated microspheres were dispersed in 5 ml of distilled water. The resulting dispersion was filled in membrane and closed at both ends using dialysis bag locks. The microspheres equivalent to 10 mg of capacitabine was taken for drug release study. The weight of dried microspheres required was calculated based on entrapment efficiency study. The resulting dialysis membrane was fixed on USP type II dissolution apparatus. The drug release study was carried out in 500 ml of 0.1N HCl for first 2 h. After 2 h, the release medium was changed to phosphate buffer (pH 6.8) for next 8 h. The temperature of both release media was adjusted to $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The rotational speed of the paddle was fixed at 50 rpm. At fixed time intervals from start of study, the 2 ml of release medium was withdrawn and subjected to UV-spectrophotometry at 240 nm for assessment of extent of capacitabine release in medium.

In vitro mucoadhesive behavior:

The mucoadhesion potential of formulated microspheres was assessed on goat intestinal mucosa (Kaur *et al.*, 2021). The intestine was obtained from a local slaughterhouse. The isotonic saline solution was used to wash the intestine and pieces of dimension 2×4 cm were made. The piece of intestine was mounted on a glass slide separately and slide was fixed at an angle of 45° .

50 mg of dried microspheres were accurately weighed and sprinkled over the surface of each piece of intestinal mucosa. The isotonic solution was sprinkled over the microspheres and kept for 15 min for hydration and swelling of microspheres. After hydration, the 50 ml of isotonic saline (37°C) was passed through the mucosa at a flow rate of 5 ml/min and collected in pre-weighed petri plate. Finally, the collected saline solution was subjected to evaporation and weight of petri plate was recorded after complete evaporation of saline solution. Based on initial weight of microspheres applied on mucosa and weight of dried microspheres collected in petri plate, the weight of microspheres adhere to mucosa was calculated. The percentage

mucoadhesion was calculated using following formula:

$$\% \text{ Mucoadhesion} = \frac{\text{Wt. of microspheres adhere to mucosa}}{\text{Wt. of microspheres initially added}} \times 100$$

Assessment of micromeritic properties:

The dried microspheres were evaluated for micromeritics properties like bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose.

Bulk density: Bulk density was determined by placing 5 g of microspheres into a graduated cylinder and by measuring the volume.

Tapped density: Tapped density was calculated by placing 5 g of the microspheres in a graduated cylinder and tapping it for 100 times.

Angle of repose: Angle of repose is the most common method used for assessing the flow property of material. It is angle between the horizontal surface and the slope of cone of solid dropped from some elevation. Angle of repose of microspheres blend was determined using funnel method. Briefly 5 g of microspheres were allowed to pass through a funnel that was raised vertically until a maximum cone height, 'h' was obtained. The radius of heap, 'r', was measured and angle of repose ' θ ' was calculated using the following formula;

$$\text{Angle of repose} = \tan^{-1} \frac{h}{r}$$

Where, 'h' is height of microsphere pile and 'r' is radius.

Carr's Index and Hausner's ratio:

The Carr's Index provides information regarding ease with which a powder material can be induced to flow. It is a one-point determination. The Carr's Index and Hausners ratio were calculated using the following formula:

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Results and Discussion

Isolation and characterization of linseed mucilage:

The mucilage from the seeds was isolated with hot water treatment. The isolated mucilage was dried in hot air oven and pass through sieve to obtain powder. Initially colour, odour, taste and texture of mucilage was checked. The results are illustrated in Table 2.

Design of capacitabine loaded mucilage-alginate microspheres:

Capacitabine loaded microspheres were formulated using ionic gelation technique. The matrix of microsphere was prepared by combination of sodium alginate and *linseed* mucilage. The microspheres were spherical in shape as represented in Figure 1. The slow injection of polymeric solution with continuous stirring maintain spherical shape of microspheres.

Evaluation of linseed mucilage-alginate microspheres:

Assessment of particle size:

The particle size of formulated mucilage-alginate based microspheres was measured using optical microscopy. The dried microspheres were spread on the clean glass slide and subjected to particle size measurement using compound microscope and calibrated eyepiece micrometer. The particle diameter of 100 particle was randomly measured and mean particle size was calculated. The particle size distribution was assessed by plotting particle size distribution curve as shown in Figure 2. The mean particle size was found to be in the range of 702.14 to 758.39 micrometer. The formulation F4 showed maximum mean particle size of 758.39 micrometer whereas formulation F1 showed minimum particle size of 702.14 micrometer.

Measurement of entrapment efficiency:

Per cent entrapment of capacitabine in dried microspheres was assessed using UV spectrometric measurement. All batches of formulated microspheres showed per cent entrapment in the range of 75.17 to 80.83 %.

Assessment of capacitabine release behavior:

In vitro capacitabine release behavior from formulated mucilage-alginate microspheres was

assessed using dialysis diffusion technique. The release study was performed in both acidic as well as basic buffers. The 0.1 N HCl was selected as an acidic medium and Phosphate buffer pH 6.8 was selected as basic medium for assessment of drug release behavior. The drug release profile is illustrated in Figure 3. The initial burst release of capacitabine was observed in first 2 h for all four batches of microspheres, with maximum 50 % of drug release in case of formulation F1. The initial burst release of drug could be due initial release of drug loaded at the surface of microsphere matrix. After 2 h, the sustained drug release was observed for next 14 h. The sustained drug could be due to slow penetration of drug across mucilage-alginate microsphere matrix. The formulation F4 showed controlled release of drug compared to other three batches which could be due to more concentration of mucilage in batch F4.

In vitro mucoadhesive behavior:

Assessment of mucoadhesion potential and swelling ability of microspheres is essential evaluation parameter governing *in vivo* performance of microspheres based systems. The swelling behavior of microsphere in presence of phosphate buffer (pH 6.8) is depicted in Figure 4. The microspheres showed increased swelling capability up to 8 h with maximum 72.17 % swelling index. After the 8 h swelling behavior of microspheres was progressively declined up to 12 h. The reduction in swelling of microspheres after 8 h could be due to slow erosion of polymer. The formulation F4 showed maximum swelling index of $72 \pm 1.7\%$ whereas formulation F1 showed minimum swelling of $59.42 \pm 2.1\%$. The % swelling of microspheres was found to increase from formulation F1 to F4. The F4 showed maximum swelling whereas F1 showed minimum swelling ability. The increase in swelling potential of microspheres could be due to increase in concentration of mucilage in microspheres. The *linseed* mucilage has swelling and mucoadhesive capability. The per cent mucoadhesion of mucilage-alginate microspheres (F4) on goat intestinal mucosa was found to be $64.47 \pm 2.7\%$ as

Table 2: Organoleptic properties of linseed mucilage

Colour	Odour	Taste	Texture
Brownish	None	Mucilaginous	Smooth



Fig. 1: Capacitabine loaded mucilage-alginate microspheres.

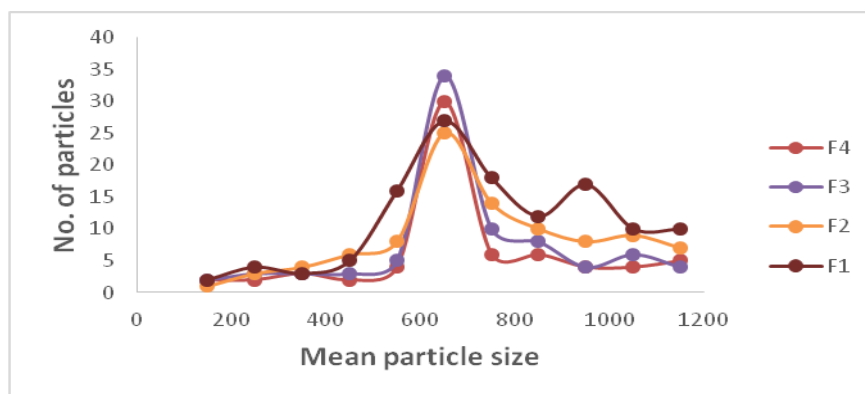


Fig. 2: Particle size distribution of mucilage-alginate microspheres.

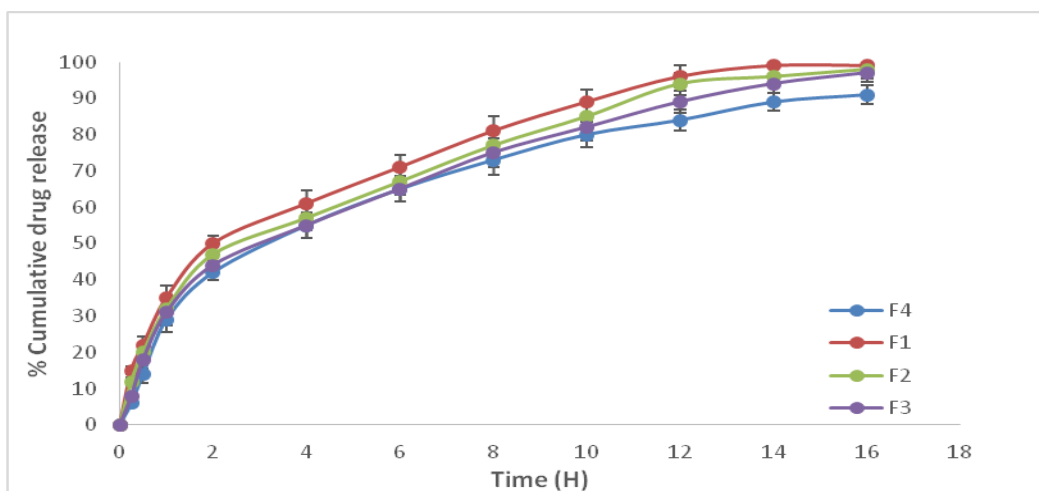


Fig. 3: *In vitro* capacitabine release profile of mucilage-alginate microspheres (n=3).

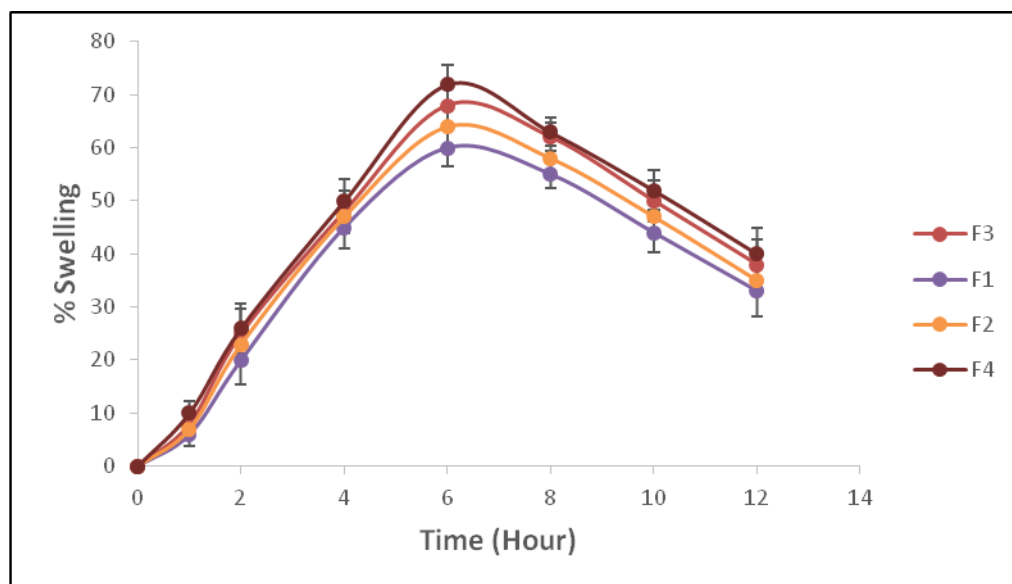


Fig. 4: Swelling behavior of mucilage-alginate microspheres.

Table 3: Mucoadhesive potential of mucilage alginate microspheres

Formulation code	Per cent mucoadhesion (%)
F1	41.47 ± 2.17
F2	45.61 ± 2.73
F3	57.19 ± 2.61
F4	64.47 ± 2.7

Table 4: Micromeritics properties of mucilage based microspheres (n=3)

Formulation	Bulk density (g/cm ³)	Tapped density (g/cm ³)	Carr's Index (%)	Hausner's ratio	Angle of repose
F1	0.64±0.02	0.71±0.01	9.85±0.04	1.10±0.02	26.56±1.34
F2	0.58±0.05	0.64±0.03	9.37±0.02	1.10±0.01	22.34±1.74
F3	0.46±0.07	0.51±0.04	9.80±0.06	1.10±0.03	21.28±1.81
F4	0.39±0.06	0.45±0.03	13.33±0.05	1.15±0.04	19.22±1.67

highlighted in Table 3. The formulated microspheres showed acceptable swelling and mucoadhesion capabilities.

Assessment of micromeritic properties:

The formulated microspheres was free flowing as indicated by the values of bulk density (0.39 to 0.64 gm/cm³), tapped density (0.45 to 0.71 gm/cm³), compressibility index (9.85 to 13.33%) and Hausner's ratio (1.10 to 1.15). Angle of repose

ranged from 19.22 to 26.56 °. The values are given in Table 4.

Conclusion

The aim for present study was to fabricate linseed mucilage based microspheres of capacitabine for controlled gastroreintive drug delivery. The capacitabine loaded microspheres was formulated using ionic gelation technique and evaluated. All four batches of microspheres exhibited acceptable

particle size and entrapment efficiency. In addition to this, the sustained release of drug was observed for the period of 16 h. The mucoadhesion capability of microspheres on goat intestinal mucosa was found to be good with acceptable swelling index. Thus, natural mucilage could be promising alternative for gastroretentive drug delivery.

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