

**FORMULATION AND EVALUATION OF MICROBEADS FOR COLON TARGETED
DRUG DELIVERY USING NATURAL POLYMER**Yashika Uniyal^{1*}, Manoj Kumar Sarangi² and Kriti Dabral³^{1,3}Dev Bhoomi Institute of Pharmacy and Research, Dehradun 248007 India.²Sardar Bhagwan Singh University, Dehradun 248001 India.***Corresponding Author: Yashika Uniyal**

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ABSTRACT

Colon targeted delivery is one of the most challenging task to the researcher in the field of developing dosage form. Colonic diseases are common and hazardous causing severe pain to the patients and needed direct targeting of drug to the desired site. So Multiparticulate dosage form are one of the best approach for treating colonic disorder involving carrier which release the drug at the effected site. My present work includes formulation of multiparticulate dosage form using natural polymer Fenugreek seed mucilage (FSM) which binds the drug in combination with other drug and a carrier that delivers the drug at infected site in colon 5-. Fluoro uracil was selected which belongs to the category of Antineoplastic (anticancer) used for the treatment of colorectal cancer. Multiparticulate dosage. i.e. microbeads were prepared using chemical cross linking in combination of polymer reinforcement technique. with FSM and sodium alginate as a bead forming agent and 5-fluorouracil as a drug. 12 batches were prepared of microbeads using variable concentration of polymers and drug and out of them, best formulations were evaluated for evaluation parameter i.e. Percentage yield, entrapment efficiency, *In vitro* drug release and scanning electron microscopy. Scanning electron microscopy determines the shape and surface morphology structure of beads. The studies concluded that fenugreek act as a good binder to take the drug to the colon in combination with other polymers.

KEYWORDS: Colon targeted drug delivery, Multiparticulate Dosage form, fluoro uracil, fenugreek mucilage, SEM, FTIR.**INTRODUCTION**

The main aim of any drug distribution is to provide a required amount of drug to the effected site in the body, in order to attain a desired therapeutic response. Targeting of drug at exact location is one of the important approaches of any delivery system as the medicament only gets released in specific part or pH for the local treatment of disease in the body. Most of the drug reaches at the targeted site without suffering any degradation and metabolism and does not produce any effect to other organ.

The delivery of drug into the colon is advantageous for the local treatment of the diseases like colitis, inflammatory bowel disease, colorectal cancer of colon etc and for the general delivery of protein and peptide. The colonic delivery has gain importance for the delivery of those drugs which shows less absorption from upper part in stomach and degraded in upper GI fluid due to increased retention time. Drug directly reaches to the colon by using sustained release system or the system which shows pH dependency which dissolves at the specific pH and drug release at the preferential site.

Need for Colon Targetting^[1,2]

- Supplying of medicine in particular part of colon is required most important for the direct treatment of local disease.
- Colonic drug deliveries are also suitable for the drug which is highly affected by acidic fluid of upper GIT, hepatic metabolism and chemical degradation.
- The delivery of drug in colon directly needs for specific delivery of anticancer drug to the specific site of cancer in colon follows no effect on the other part of the body.
- Colon targeting systems prolong the residence time as well as reduce dosing frequency.
- Needed for the control and sustained delivery of the drug reducing side effects.
- Good for specific delivery of protein and peptides.

Advantage of CTDDS^[1,2]

- Adverse reaction can be reduced by targeting specified area in colon for treatment of disease i.e. Colitis, IBD, Crohn's disease.

- Cell destruction caused by anticancer drug can be avoided by delivery of drug in the specific location for treatment of colorectal cancer.
- Gastric disturbance because of NSAID's can be overcome.
- By designing colon targeted delivery system, the first metabolism of steroidal medication can be reduced.
- Reduction in dose frequency.
- Enhancement in bioavailability of poorly absorbable drug by extending retention time.
- Enhanced drug use.
- A longer transit time of colon is good for the absorption improve for drug delivery.

Multiparticulate Drug Delivery System

Multiparticulate drug deliveries are the mainly oral dosage form consist of minute different units each having specific pharmacological response with diameter ranges between 0.05- 2 mm. Thus multiparticulate drug deliveries can be defined as a dosage form having active agents divided into several subunits or microparticles. The microparticles can be compressed or filled in capsule for total drug delivery. Multiparticulate drug deliveries can be microspheres, microbeads, micropellets, microemulsions, micro suspensions etc.^[2]

Multiparticulate drug deliveries are one of the best approaches over other delivery system as a colon Targeted drug delivery. Because of their small size and better bioavailability, the delivery of multiparticulate in colon can be achieved by using pH dependent or control release polymer which degrades at the colonic pH. Many rate controlling or pH dependent polymer were used such as Eudragit S-100., Ethyl cellulose, HPMC etc.^[3,4]

Advantage of Multiparticulate Drug Delivery System.^[4]

- Due to small size, multiparticulate dispersed easily in the colon without causing irritation.
- Slower transit time and good drug release.
- Bioavailability of drug can be increased as they are targeted directly specially to the targeted site.
- No dose dumping.
- Improve patient compliance
- Controlled release of drug can be achieved by using suitable polymer.
- Stability of drug can be improved.

MATERIAL AND METHOD

5-Fluorouracil, Sodium Alginate and Eudragit S-100 were obtained from Yarrow chem. Ethyl cellulose, calcium Chloride, Ethanol and methanol were obtained from Loba chem. Potassium and sodium dihydrogen phosphate were obtained from Hi-media. All the chemicals used in research work are of analytical grade provided by SBSPGI laboratory.

Preformulation Studies of Drug

Before formulation of any dosage form, identification and characterization of drug and polymer is very necessary. Many Preformulation factors are used to determine its properties and purity, these are Melting point, calibration plot, Scanning of drug, and IR studies.^[6]

Fourier Transform Infrared Spectroscopy (FTIR) Studies

FTIR Spectroscopy was done using potassium bromide pellets. FTIR interpreted the function group present in the structure which gives peak at particular wavenumber which was then compared with the standard. Pure drug was crushed with KBr pellets. The mixture was then compressed with disc and then placed in instrument. The IR spectra of all samples were recorded within a required range of wave number. The FTIR spectra of drug, polymer and the mixture of drug and polymer was recorded and interpreted.^[5]

UV Spectrophotometric Analysis of Drug 5-Fluorouracil

The maximum wavelength for the drug can be measured through UV analysis. The suitable solution of drug was prepared in different media and then it was analyzed in UV spectrometry giving the maximum wavelength of the drug in that media which was then compared with standard.^[7]

Drug Excipients Compatibility Studies

Drug polymer compatibility studies were done using FTIR and Differential scanning calorimeter (DSC). The drug and different polymer mixture is kept in a vial and store for one month in room temperature and then the samples are analyzed and the results were interpreted.^[8,9]

Extraction Method of Fenugreek

Fenugreek seeds (100 mg) were crushed and the powder was dipped in 500ml of double distilled water and heated on water bath at 80⁰c using water bath for 4 hours followed by stirring. After the thick mass was obtained, it was placed for 4 hrs at room temperature shaking continuously and then leave it overnight below 20⁰c. Separation of aqueous mucilage was done by using muslin cloth. The precipitate of mucilage was obtained after washing with 300 ml of alcohol. This precipitated mucilage was again filtered by using muslin cloth. Dehydration of separated mucilage was done by using 200ml of acetone. This process also helps in removing any oil extract which may be present in aqueous mucilage. The filtrate precipitated mass was dried in hot air oven for 12 hours at 50⁰c. After drying it was crushed by using mortar pestle to form uniform powder and then passed through the sieve having the mess size of #60. The resultant dried mucilage was subjected to pharmacotechnical evaluation in order to assess and compare their potentiality as a controlled release polymer.^[10]

4.3 Preliminary Phytochemical Analysis of Fenugreek Seed Mucilage

These studies pertain to pharmaceutical and analytical investigations that are necessary for proceeding and supporting formulation development efforts of the dosage form of the drug substance. They provide basic knowledge necessary to develop appropriate formulation for toxicological use. They aid in collecting information needed to describe the nature of the drug substance and provide a frame work for the drug combination with pharmaceutical excipients in the dosage form.^[11,12]

Viscosity Determination of polymer

1gm of dried and finely powdered mucilage or polymer was added to 75ml of water and allowed to stand for 5 hours and distilled water is added to make up the volume 100ml. The mixture of polymer in water was shaken using mechanical stirrer for 2 hour and its viscosity was determined by using Brookfield and Ostwald's viscometer using following formula,^[11,12]

$$\eta_1 = \eta_2 (\rho_1 t_1 / \rho_2 t_2)$$

Where η is the coefficient of viscosity, ρ is the density and t is the time.^[13]

Swelling index determination

According to B.P method, 1gm of mucilage powder was taken in a graduated cylinder of 100ml. To this, 25ml of water is added and the solution was shaken for every 10 consecutive minute for 1 hour and allowed to stand for 24 hours. The volume covered mucilage was measured the swelling index was calculated from the mean of three determination.^[13]

Angle of Repose

Angle of repose is a measure of the flow properties of the powder. It is the maximum angle made between the surface of the heap of powder and the horizontal plane. The angle of repose was determined according to the formula. The finely powdered mucilage was passed through funnel to a graph paper and the height of the funnel should be maintaining constant. The angle of repose was then calculated according to the USP by measuring the height and the base of the heap of powder formed and using equation.^[14]

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

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

Where θ represents the angle of repose,
H is height in cm
R is radius/base in cm.

Bulk Density (BD)

This is the ratio of the total mass of powder to the bulk volume of powder. Accurately weigh a portion of powdered mucilage (50 g) and was taken in a to a 100 ml graduated cylinder. The mixture was carefully leveled which is the initial apparent volume (V_o) of mucilage.

Loose bulk density can be calculated by using formula and expressed in g/ml

$$\rho_b = M / V_b$$

Where ρ_b = bulk density, M= bulk weight of blend, V_b = bulk volume of the blend.^[14,15]

Tapped Density (TD)

This is the ratio of total mass of the powder to the tapped volume of the powder. Accurately weigh some quantity of powder mixture (40 g) and was taken in a 100 ml measuring cylinder. The cylinder containing the sample was manually tapped for three taps (1250, 750, 500) and the final tapped volume (V_f) was then measured. Tapped bulk density can be calculated by using formula and expressed in g/ml.

$$\rho_t = M / V_t$$

Where ρ_t = Tapped density, M= weight of blend, V_b = tapped volume of the blend.^[15]

Compressibility Index (Carr's Index)

Compressibility index is the ratio of difference of tapped density and bulk density to the tapped density. It measures the powder flowability and is expressed in percentage:

$$\text{Carr's Index (\%)} = (D_t - D_b / D_t) * 100$$

Where D_t = Tapped density of the powder, D_b = **bulk** density of the powder.^[15,16]

Hausner Ratio

The Hausner ratio is a number that is correlated to the flowability of a powder or granular material. It is an indirect index to measure the ease of powder flow.^[17]

$$\text{Hausner Ratio} = \rho_b / \rho_t$$

Where ρ_b = Tapped density of the powder, ρ_t = bulk density of the powder.

Phytochemical testing for mucilage

Mucilage was tested for presence of various phytochemicals like alkaloids, flavanoids, glycosides, tannins, carbohydrates and proteins.

Formulation Development

Method of Preparation of Beads

The beads were prepared by combination of chemical linking method of polymer reinforcements technique. In chemical cross linking method, two phases were made. First phase consist of the solution of all the polymers and drug and the second phase consist of the calcium chloride solution which was used as the cross linking agent. The first phase was added to the second phase with the application of syringe of size 22#. drop wise in a magnetic stirrer having RPM 250-300. The chemical cross linking occurs and the beads formation takes place which was spherical in shape. The curing times of beads

were few hours and then it was filtered and washed with water and air dried for 24 hours. The prepared dried beads were evaluated for various parameters required.^[18]

Formulation of final batches

Table 1: Formulation batches.

Formulation code	Drug (mg)	Fenugreek mucilage (mg)	Sodium alginate (mg)	Ethyl cellulose (mg)	Calcium chloride solution (%)
F1	100	100	100	100	3%
F2	100	200	100	100	3%
F3	100	300	100	100	3%
F4	100	400	100	100	3%
F5	100	600	100	100	3%
F6	100	800	100	100	3%
F7	100	600	200	100	3%
F8	100	600	300	100	3%
F9	100	600	400	100	3%
F10	200	600	400	100	3%
F11	300	600	400	100	3%
F12	400	600	400	100	3%



Fig. 1: Preparation of beads.

Evaluation of Microbeads

Percentage yield

The percentage yield of total formulation can be calculated by dividing the weight of total microbeads

$$\text{Percentage yield} = \frac{\text{Amount of microbeads obtained (g)} \times 100}{\text{Theoretical amount (g)}}$$

Drug entrapment efficiency

Appropriate amount of dried microbeads (50mg) were weighed and crushed in a mortar pestle. The powdered microbeads were dipped in 50ml of prepared pH 7.4 phosphate buffer. The solution was stirred for 24 hours and then filtered. The filtered solution was analyzed in

formed by the total ingredients added multiplied by 100. It gives the total yield of dosage form.^[19,20]

UV for drug concentration after making suitable dilution if necessary at λ_{max} 266.9nm The absorbance was taken in triplicate and drug concentration was calculated by equation of straight line in pH 7.4 phosphate buffer. The % drug entrapment efficiency was calculated from the following formula.^[21,22]

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Scanning electron microscopy

The magnified shape, size and surface morphology of the beads was done by scanning electron microscopy. The beads were paste on aluminum stubs with double side tape and then gold sputtering was done to capture the actual shape of the beads. The samples were set in the instrument and the shape of the beads and its surface were observed with higher magnification.^[23,24]

Invitro Drug Release

Invitro drug release of formulation was carried out in USP type I Dissolution apparatus i.e. basket type. 100mg of microbeads were accurately weighed and placed in basket. Three different media i.e. 0.1N HCl, pH 6.8 phosphate buffer, pH 7.4 phosphate buffer were prepared and 900ml was filled in dissolution jars. The temperature of dissolution was maintained at 37⁰c and RPM was set to 100. the study was carried out in 0.1N HCl for first two hours, pH 6.8 phosphate buffer for next 6 hours and then pH 7.4 phosphate buffer for 12 hours to simulated the gastrointestinal condition. 5ml sample was

withdrawn after regular time interval and after suitable dilution; it was analyzed in UV spectrophotometer at λ_{max} of the drug in different medium. After withdrawing the sample, 5ml of the media was added to the dissolution media to maintain the sink condition. The procedure was repeated up to 12 hours until a constant concentration was determined and Drug release was calculated.^[25,26]

RESULTS AND DISCUSSION

Identification of Drug

Determination of melting point

The melting point of drug was found to be 280⁰c. The reported melting point of drug 5- Fluorouracil is 280-282⁰c. Thus drug was identified as 5- Fluorouracil and found to be pure.

Fourier transform infrared spectroscopy analysis

Identification of Fluorouracil was done by the FTIR Spectroscopy. The FTIR spectra was shown in figure.

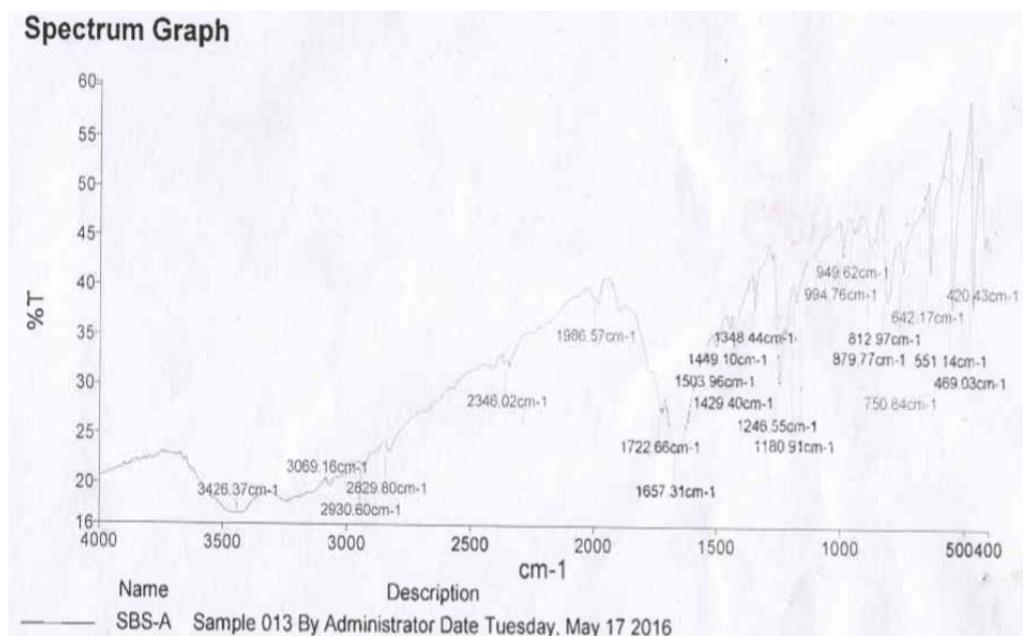


Fig. 2: ftir spectra of drug fluorouracil.

Analytical Studies

Table 2: Interpretation of FTIR spectra of drug.

Reported Wavenumber (cm ⁻¹)	Observed Wavenumber (cm ⁻¹)	Assignments
3000-2900	2930	C-H Stretching
1660	1657	C=N stretching
1429	1429.40	C=C Stretching
1348	1348.44	C-O
1246	1246	C-N
1180	1180.91	Pyrimidine

Table 3: Determination of λ_{max} of drug 5-fluorouracil in different media.

S. NO.	MEDIUM	OBSERVED λ_{max}	REPORTED
1	Distilled water	265.8 nm	266
2	0.1N HCl	265.9 nm	266
3	pH 6.8 phosphate buffer	266.5nm	266
4	pH 7.4 phosphate buffer	266.9nm	266

Determination of solubility of drug in different media

The solubility of fluorouracil was determined in different media i.e. distilled water, 0.1N HCl, pH 6.8 and pH 7.4 phosphate buffer. it is concluded that the drug is highly soluble in water, and sparingly soluble in other media.

Table 5: Solubility determination results of Drug 5-Fluorouracil in different media.

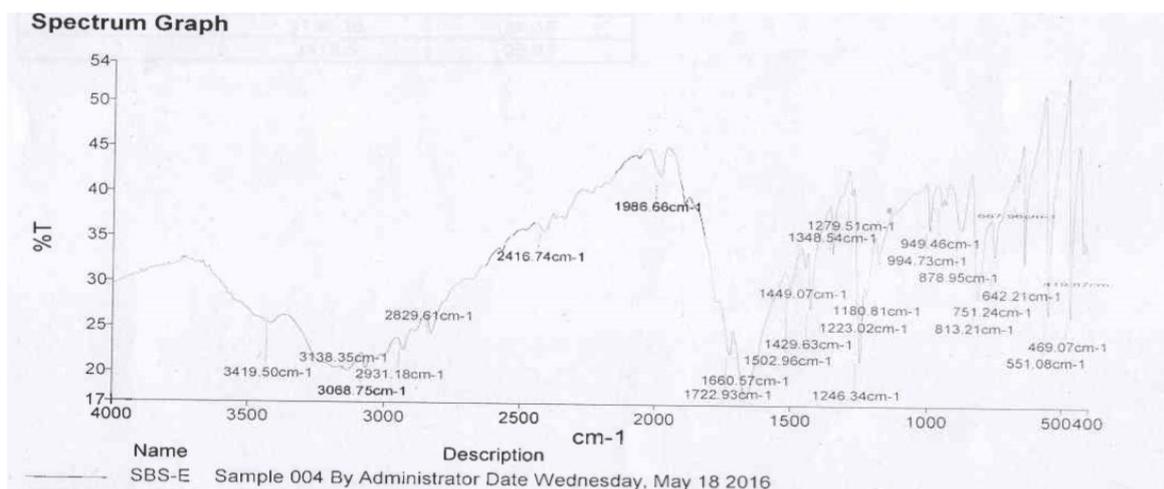
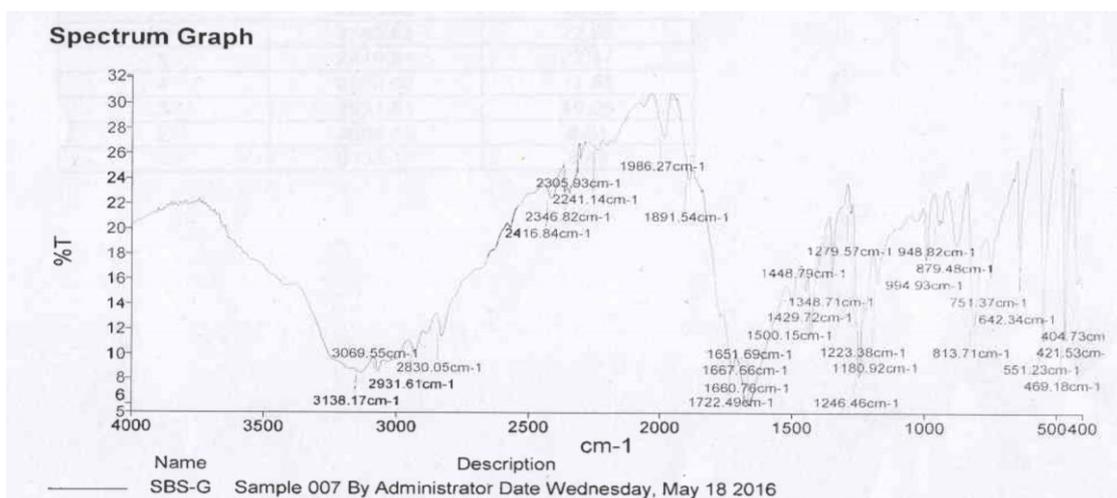
S. NO.	Media	Solubility
1	Distilled water	19.08mg/10ml
2	0.1N HCl	1.412mg/10ml
3	pH 6.8 phosphate buffer	5.57mg/10ml
4	pH 7.4 phosphate buffer	5.63mg/10ml

Determination of Partition Coefficient of Drug 5-Fluorouracil

The partition coefficient of drug 5-Fluorouracil was found to be 0.74. The reported partition coefficient of in 7.4 phosphate buffer is 0.76 this concluded that drug belongs to the BCS class III (high solubility and low permeability).

Drug Excipients Compatibility Studies

The drug excipients study was done by FTIR. The spectra show the interaction between the drug and polymer.

**Fig 3: Ftir spectra of drug and fenugreek mucilage.****Interaction study of drug, fenugreek mucilage and sodium alginate****Fig 4: Ftir spectra of drug, fsm and sodium alginate.**

Preliminary testing of fenugreek

Table 4: Preliminary testing of fenugreek.

S. No.	Physico-chemical properties	Values obtained
1.	Appearance	yellowish brown fine powder
2.	pH	5.5
3.	Viscosity	At 50rpm $C_p=12$ At 100rpm $C_p=18$ At 30rpm $C_p=20$
4.	Swelling index	150%
5.	Angle of repose	35°
6.	Bulk density	0.625g/cm^3
7.	Tapped density	0.760g/cm^3
8.	Hausner's ratio	1.23
9.	Car's index	18.73

Testing for phytoconstant

Table 5: Testing of fenugreek.

Carbohydrate	Molisch test	Positive
Alkaloids	Dragendoff's Test	Positive
	Mayer's reagent	Positive
	Hager's reagent	Positive
Protein	Ninhydrine test	Positive
Tannins	Ferric chloride test	Negative
Cardiac glycosides	Keller Killani test	Positive
	Legal test	Positive
	Brontrager's test	Positive
Saponins	Foam test	Positive
Flavanoids	Shinoda test	Negative

Evaluation Parameter of Microbeads

Percentage yield

Table 6: Percentage yield of microbeads.

Batches	Percentage Yield (%)
F7	61
F8	63
F9	65
F10	64
F11	66
F12	69

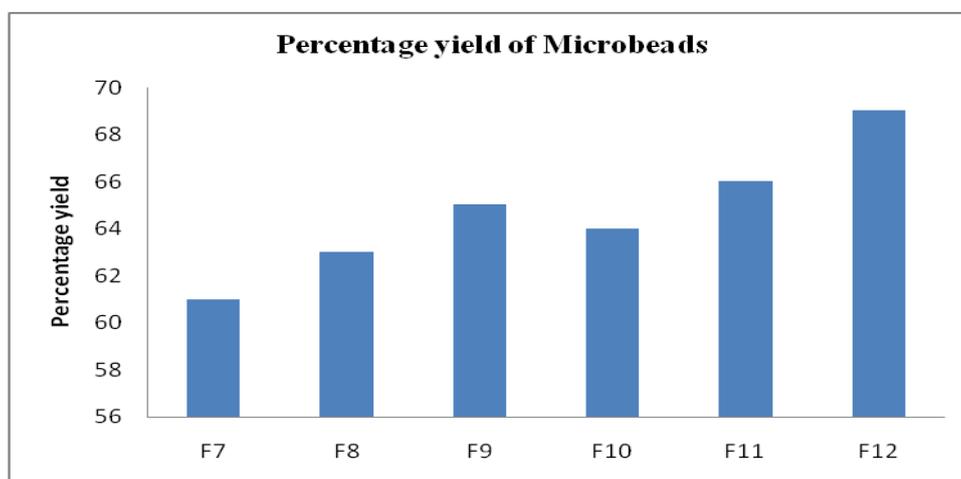


Fig. 5: Percentage yield of different batches of microbeads.

Drug Entrapment efficiency of microbeads
Table 7: Drug Entrapment Efficiency of Beads.

Batch	Drug entrapment efficiency (%)
F7	35.6%
F8	43.5%
F9	45.2%
F10	48.3%
F11	52.6%
F12	57.9%

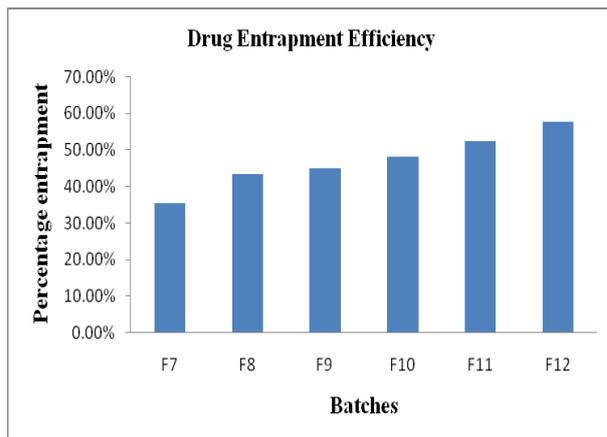


Fig. 6: Drug entrapment efficiency of microbeads.

Scanning electron microscopy of prepared Microbeads (9)

The surface morphology and the structure of microbeads were investigated through SEM. The best four formulations (F8-F12) were subjected to SEM analysis. The shapes of the Microbeads were clearly observed through SEM images which are spherical irregular and smooth due the entrapment of drug into it.

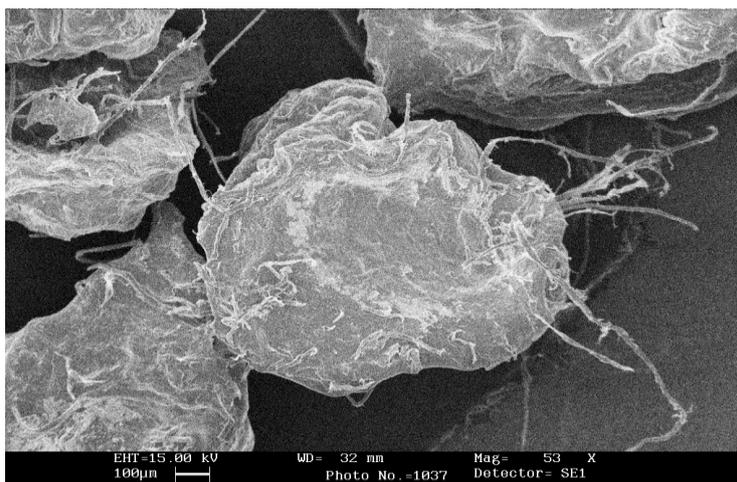


Fig. 7: SEM images of microbeads at magnification 53X.

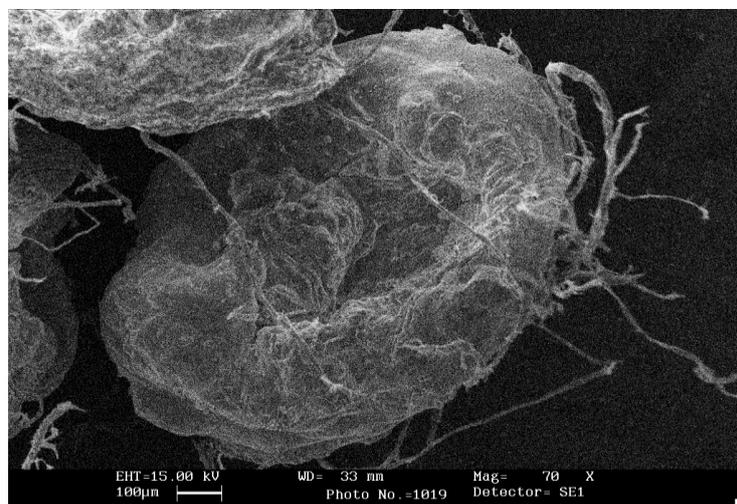


Fig. 8: SEM images of microbeads at magnification 70X.

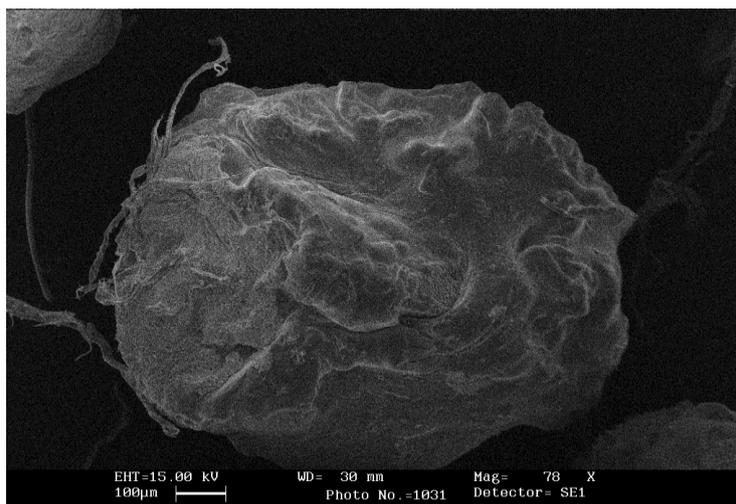


Fig. 9: SEM images of microbeads at magnification 78X.

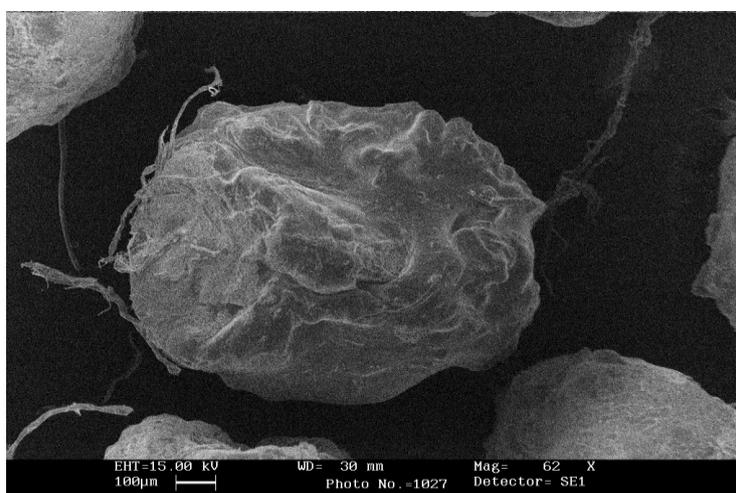


Fig. 10: SEM images of microbeads at magnification 62X.

In vitro drug release of beads

The in vitro drug release of the prepared microbeads were carried out in first in 0.1N HCl for two hours, in pH 6.8 phosphate buffer for 6 hour and in pH 7.4 phosphate buffer for 12 hours. The best four batches from the

prepared microbeads were selected and evaluated for drug release. At the end of 12 hour, the beads show maximum release of 80% in Ph 7.4 phosphate I.e. the pH of colon the best release of the drug was achieved.

Table 8: *INVITRO* drug release.

Dissolution media	Time (min)	%CDR			
		F9	F10	F11	F12
0.1N HCl	15	0.04	0.06	0.09	0.10
	30	0.08	0.11	0.21	1.5
	60	1.6	1.9	2.5	2.3
	120	2.2	2.8	5.3	5.9
pH 6.8 phosphate buffer	180	3.53	4.10	7.4	6.5
	240	6.53	7.79	10.54	8.4
	300	8.41	8.65	15.23	16.32
	360	9.14	9.38	19.22	26.89
pH 7.4 phosphate buffer	420	18.74	19.34	29.54	35.34
	480	25.60	27.33	43.21	45.66
	540	36.70	38.19	55.18	55.78
	600	59.45	49.55	67.85	65.75
	660	72.35	63.56	74.23.	76.82
	720	79.3	79.85	81.23	82.43

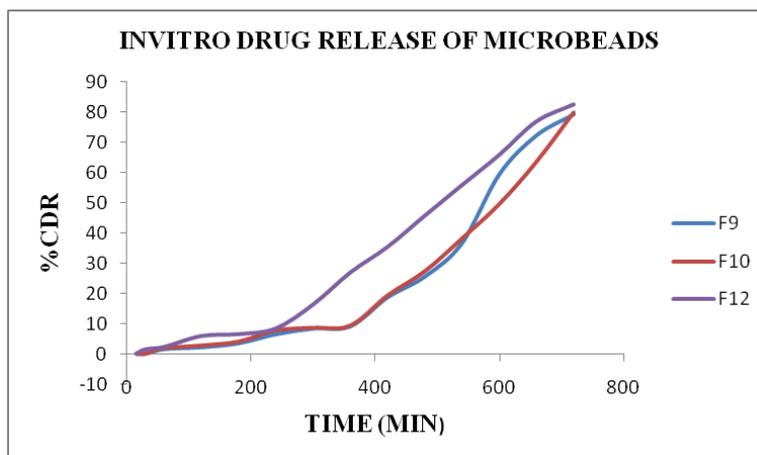


Fig. 11: *In vitro* drug release of microbeads.

CONCLUSION

5-Fluorouracil was chosen as a drug for the treatment of colorectal cancer. Melting point evaluation, FTIR scan and UV scan of 5-Fluorouracil was performed. From the result of above studies it may be concluded that the drug was pure with no impurities and can be used in preparing microbeads.

Fenugreek mucilage was taken which proves to be a good binder which is a new innovative step for the preparation of Microbeads formulation. The various physicochemical and phytochemical properties of fenugreek mucilage were evaluated.

The incompatibility study was performed for one month at the room temperature. The samples were analyzed and it was found that there was no interaction, physically or chemically between the drug and polymer.

The method used for the formulation of microbeads is chemical cross linking because it was a simple and effective method. Microbeads were evaluated for various parameters. The % drug release was found 80-90% at the end of 12 hours.

This formulation affords a platform to develop an oral as well as Parenteral controlled release drug delivery system of 5-FU that not only improves the patient condition but also reduced systematic toxicity and thus reduced adverse drug reaction of treatment.

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