Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(12):19-27

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Formulation and *in-vitro* evaluation of 5-flourouracil nanoparticles incorporated in sucralfate suspension as drug delivery system

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ABSTRACT

The object of the present work was formulation and in vitro evaluation of 5-Fluorouracil nanoparticles incorporated in sucralfate suspension as drug delivery system. Sucralfate acts as the cytoproytective agent and carry the antineoplastic drug loaded nanoparticles to the carcinoma site in the g .i.t which will protect and alleviate the carcinoma. 5-Fluorouracil nanoparticles were prepared by nanoprecipitation method sodium alginate using as cross linking agent. Nanoparticulates were characterized in terms of drug entrapment efficiency, particle size, in vitro drug release were analyzed. Sucraalfate Suspension was prepared and evaluated for physical stability characteristics. An increase in the concentration of polymer increase in the entrapment efficiency and average particle size. FTIR and DSC studies exhibited there is no interaction between drug and excipients. The TEM analysis showed the particle size is ranges from170±3.2 to 560±2.3 and with optimum zeta potential. Fr om the above results the formulation FN12 was to be best formulation 85.33±1 of drug release at the end of 12 hr. Sucralfate suspension were prepared in order to select the optimum concentration of suspending agent and based on the physical stability parameters such as sedimentation volume, pH, and viscosity of these formulations FS5, FS10, FS15, were selected for incorporation of FN12 nanoparticles formulation. The Incorporated nanopparticulate formulation (FNS12) showed that the value "n" was greater than 1 indicating that super case-II mechanism on drug release behavior. The drug release follows zero order with regression value near to (0.859 to 0.965) with diffusion type of mechanism. Incorporation of nanoparticulates in to Sucralfate Suspension was a useful tool to carry the antineoplastic drug to carcinoma site.FSN12 showed outstanding characteristics of optimum Zetapotential and longer drug delivery. It could be a promising carrier for the oral administration of 5-Fluorouracil.

Key words: 5-Fluorouracil, Sucralfate, Nanoprecipitated method, Polaxomer407, Sodium alginate, Nanoparticles

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INRODUCTION

Sucralfate is a non-absorbable, basic aluminum salt of a Sulphated disaccharide which has proven effective in the treatment of gastric and duodenal ulcers. [1, 2, 3] Sucralfate forms polyvalent bridges to the positively charged proteins present in the mucosa and form pasta like, adhesive substances; a protective barrier is thus formed against further mucosal damage. [4] The binding of sucralfate is most effective at low pH but may still occur at higher pH 3 and 5.

Sucralfate suspension has gained increased importance not just for the delivery of anti ulcer drugs for the treatment of ulcerates, gastroenteritis but also cytoprotective action and also used in the treatment of cancer chemotherapy and Radiation induced toxicity. [5, 6] Oral administration of polymeric particle suspensions (nanoparticles or micron range microspheres made from non sweallable polymers) leads to mucoadhesion of a significant fraction of particles involves different steps i.e., step 1 administration of the colloidal system, step 2 adsorption of particles, step 3 Muco adhesion and luminal transit of colloidal suspension step 4 particle detachment [7]

5-Flourouracil (5-FU or 5-fluoro-2, 4-pyrimidinedione) is an antimetabolite of pyrimidine analogue type, with a broad spectrum activity against solid tumors (of gastrointestinal tract, pancreas, ovary, brain, breast, etc. [8, 9] To prolong the circulation time of 5-FU and increases its efficacy, its delivery has to be modified by incorporation in to nanoparticulate carriers to reduce the 5-FU associated side effects and there by improve its therapeutic index [10]

EXPERIMENTAL SECTION

5- Fluorouracil (5Fu) was obtained from Celone Pharmaceuticals Pvt. Ltd., India. Sodium alginate, PVP K30 and PVP K90 were purchased from S.D fine chemicals Mumbai India. Sodium carboxymethylcellulose, Xanthangum, Polaxomer407 were procured as gift samples from Cheminnova Remedies Pvt. Ltd, Methanol, was procured from Loba chemicals Mumbai, India. Sucralfate was purchased from Yarrow chem. Products (Mumbai), Ltd. Mohali. India . All other chemicals were of analytical grade and used without further purification.

Preparation of Sucralfate suspension:

Preparation of 5-Fluorouracil Nanoparticles:

Nanoparticles were prepared by Nanoprecipitation according to the method developed by Fessi and collegues [11]. Pvp was dissolved in methanol then 5-Fluorouracil was added and dissolved. The organic solution was injected at the rate of 48ml/min in distilled water containing Poloxamer 407 under magnetic stirring at room temperature. Methanol and some proportion of water were eliminated under reduced pressure. The final Nanosuspension was subjected for lyophilization to remove the moisture and subjected for further characterization with different polymer concentration levels to obtain higher encapsulation efficiency, desired particle size.

Table:1 Formulations of Nanoparticles by Nanonoprecipitation Method

FORMU LATIONS	5-Fu (mg)	PVPK30 (%)	PVPK90 (%)	Sodium alginate (mg)	Poloxamer 407(mg)	Water (ml)	Methanol (ml)
FN1	50	0.5	-	50	200	40	20
FN2	50	1	-	50	200	40	20
FN3	50	2	-	50	200	40	20
FN4	50	2.5	-	50	200	40	20
FN5	50	3	-	50	200	40	20
FN6	50	4	-	50	200	40	20
FN7	50	-	0.5	50	200	40	20
FN8	50	-	1	50	200	40	20
FN9	50	-	2	50	200	40	20
FN10	50	-	2.5	50	200	40	20
FN11	50	-	3	50	200	40	20
FN12	50	-	4	50	200	40	20

Preformulation Studies

Drug and Excipients Compatability studies

FTIR Studies

Drug identification was carried out by FTIR spectroscopy. The spectrum was recorded for pure drug (5-fluorouracil) using spectrum BX (Perkin Elmer) infrared spectrophotometer to study the characteristic absorption peaks for drug. Samples were prepared in KBr disk (2mg sample in 200 mg KBr) with a hydrostatic press at a force of 40psi for 4min.The scanning range was 400-4000$cm^{-1}$and the resolution was 4 cm^{-1} [12-13]. The FTIR spectra of pure drug was given in the Figure: 3

DSC Studies

Differential scanning calorimetric (DSC) analysis of the drug 5- Fluorouracil was carried out by using differential scanning calorimeter DSC 200F3 Maia equipped with computer analyzer. Samples (3-7 mg) were heated under nitrogen atmosphere on an aluminum pan at a heating rate of 10 °C/min over the temperature range of 0-500^{O}C [14].

Physical Stability studies of Sucralfate Suspension

All prepared Suspensions were kept 30 and 45 C and evaluated for their physical and chemical properties including pH, Density, Sedimentation volume, viscosity.

P^{H}: It is important parameter for the suspension stability. The pH determination study was carried out by using digital pH meter. The pH meter was calibrated and the sample of suspension was taken and pH was measured at room temperature.

Density: Density of the formulated suspension was determined by specific gravity bottle. The values are tabulated in table :3

Sedimentation volume: Sedimentation volume of suspension was determined by transferred the prepared suspension in to 50 ml measuring cylinder and make up to final volume. The cylinder was then inverted 10 times to ensure complete mixing and place in a constant temperature water bath at 25 to 0.1^{o}C.no wet ability problems were encountered in the preparation of the suspension. The sedimentation volume can be expressed as the ratio of the final volume of sediment to original volume of the suspension before settling [15].

Viscosity: The viscosity of the prepared formulations was determined at different angular velocities at 25^{0} c using a rotary viscometer (DV-III, Brookfield, USA).The rotation speed was 20rpm, with spin 18#. The average of two readings was used to calculate the viscosity.

Characterization of Nanoparticles:

Percentage yield

The nanoparticles are prepared by Nanoprecipitation method from the above references, and percentage yield was calculated by dividing the weight of obtained nanoparticles by the weight of calculated ingredients of nanoparticles and expressed in terms of percentage.

$$\text{Percentage yield} = \frac{\text{Practical yield of Nanoparticles}}{\text{Total Theoretical yield of Nanoparticles}} \times 100$$

Entrapment efficiency

The entrapment efficiency was determined by using the following formula

$$\text{Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Amount of free drug}}{\text{Total amount of drug added}} \times 100$$

Transmission Electron Microscope

The particle shape and morphology of the prepared 5-Fluorouracil nanoparticles were determined by Transmission electron microscopy (TEM) analysis. The nanoparticles were viewed using Philips Transmission Electron Microscope (TEM) model CM200 which is shown in Figure: 3 for morphological examination.

The Sample can be mounted on carbon/formvar coated Copper grid or can be made of disc type with a thinned (electron transparency) central area of size 3mm. Operating voltages are 20-200kv and the Resolution was 2.4 A^{o} [16].

Zeta potential measurement of the nanoparticles

The Zeta potential of the nanoparticles was determined by laser Doppler anemometry using a Malvern Zetasizer also called Doppler Electrophoretic Light Scatter Analyzer. It is used to measure velocities and thereby zeta potential of colloid particles [17]

***In-Vitro* diffusion study**

The *in-vitro* drug release profile of 5-FU--NP was determined by using dialysis membrane bag. 5-FU--NP (20mg) was placed in to dialysis bag (with a molecular cut-off of 5kDa). 5-FU--NP loaded dialysis bag was incubated in 70ml 0.1N HCL (1.2PH). The system was maintained at 37 ± 0.5^0 C with mild magnetic stirring. At appropriate time interval, 4ml of the release media was taken and equivalent volume of 0.1N HCl (1.2pH) was supplemented in order to keep the volume of the system identical. The sample was assayed at 266 nm by UV-Spectrophotometer (Lab India) and calculates the cumulative percentage of drug release. [18, 19]

I*n-Vitro* diffusion studies of Nanoparticles incorporated in Sucralfate suspension

Nanoparticles loaded sucralfate suspension dissolution was carried out for a 12 hrs in 0.1N Hcl as a medium. At the intial time the suspension dispersed at the effected part and the nanoparticles diffuse in to suspension containing suspending agents such(FS5 1%), methyl cellulose(FN9 72.75±0.5) ,Xanthan gum(0.5% FS10) (76.8±1FN10) , Sodium carboxy methyl cellulose (FS14, FS15 4% & 5%) 80.92±1, 85.33±1 and adhere to the specific site**.** The dissolution profiles were fitted to various kinetic equation and results were displayed in table: 6 .

In-vitro release kinetics of Nanoparticles incorporated in Sucralfate Suspension

The Korsmeyer's peppas equation showed that the exponential (n) value was greater than 1 indicating that supercase-II mechanism on drug release behavior .The drug release follows zero order with regression value near to (0.859 to 0.965) with diffusion type of release from the Higuchi equation regression value (0.966) and the results were displayed in table : 6.

Statistical analysis: All test and results were performed in multiple of three and expressed as mean ± SD (standard deviation). The results were subjected to statistical tests. if P value was < 0.05 they were consider to significant..

RESULTS

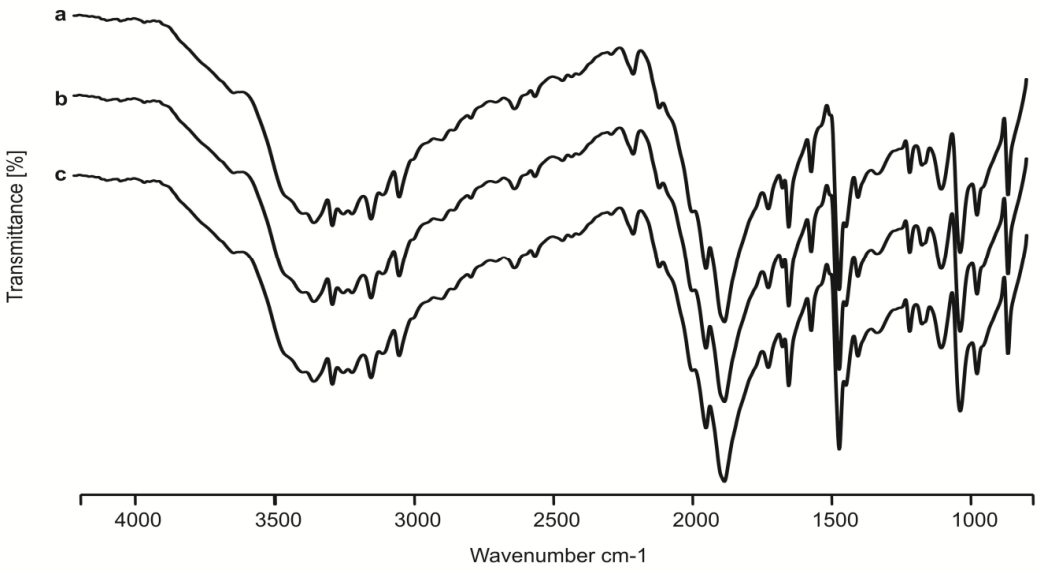

Figure : 1 a) 5-Fluorouracil pure drug, b) 5-Fluorouracil nanoparticles, c) 5-Fluorouracil nanoparticles incorporated in sucralfate suspension

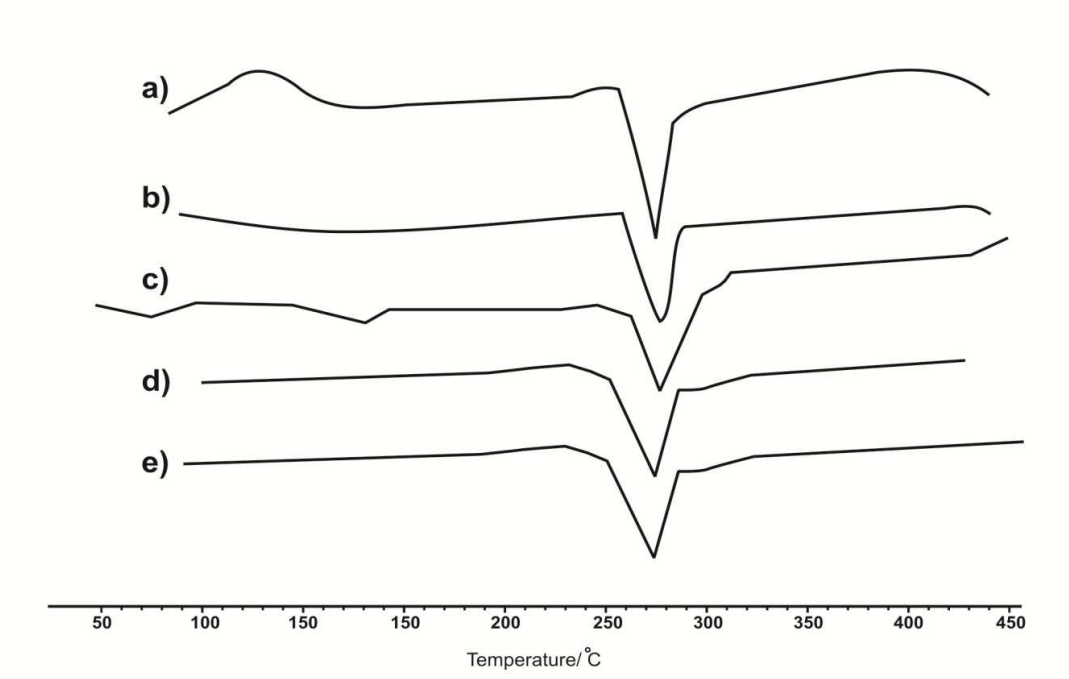

Figure : 2 a) 5-Fluorouracil pure drug, b)sodium alginate c) 5-Fluorouracil nanoparticles d) Sucralfate suspension e) 5-Fluorouracil nanoparticles incorporated in Sucralfate Suspension

FTIR & DSC STUDIES:

The FTIR spectra for pure drug 5-Fluorouracil was given in the Figure: 3 and the peaks observed are From the graph is evident that the absorption bands at 1661.51cm^{-1}, 1449.89cm^{-1}, 3136.40cm^{-1}1430.70cm^{-1} and 1246.87cm^{-1} indicate presence of C=O, C=C, N-H, C-F and C-N stretching vibrations corresponding to 5-Fluorouracil, the peak at 1349.35cm^{-1} refers to vibration of pyrimidine compound confirming 5-Fluorouracil. In Sodiumalginate sucralfate, polyvinylpyrolidone soduimcarboxy methyl cellulose and xanthangum shows the prominent peaks are observed at 3483.36 cm^{-1} , 1641.68 cm^{-1} , 1263.44 cm^{-1} , 1007.74 cm^{-1} , 810.93 cm^{-1} indicates the presence of aliphatic O-H stretching, C-O in pyrimidine ring, C-C stretching in pyramidine ring, C-C stretching in furan, C-C bending in monosubstituted ring. Identically they show the characteristics O-H stretching in nanoparticles. These peaks were retained in 5-Fluorouracil drug loaded nanoparticles and incorporated in sucralfate suspension. After binding the drug it forms an (N-O) bond between drug and polymer due to intermolecular hydrogen bonding shows the remaining characteristics.

DSC thermogram of 5-fluorouracil was shown in Figure: 4 a). This reveals that the drug shows good thermal stability up to its melting point. The onset melting peak of 5-fluorouracil is observed at about 273.47ºC. No other characteristic decomposition peak is observed in the DSC thermo gram. It suggests that the drug is stable up to 280.13 ºC and undergoes degradation after that. 5-Fluorouracil loaded nanoparticles and 5-Fluorouracil nanoparticles incorporated in surcralfate suspension were evaluated to physical form were displayed in Figure 4.c when 5-Fu nanoparticles were tested from 0 to 500 c an endothermic peak at 273.47ºC and exothermic 280.13 ºC (Fig 3) which might be due residual water left in nanoparticles

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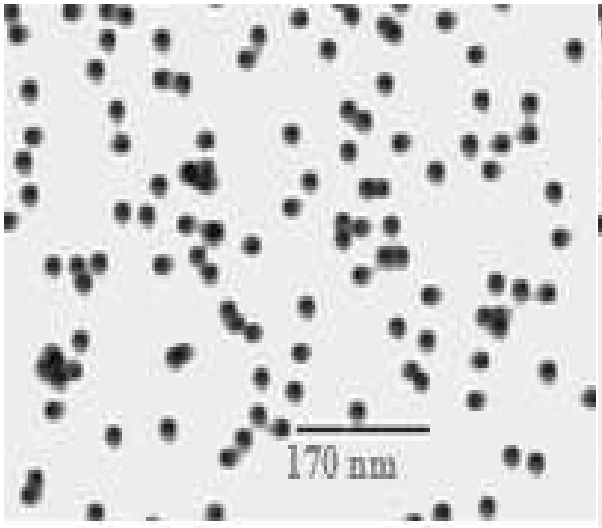

Figure 3) a.Tem image of 5-Fu nanoparticles (FN1)

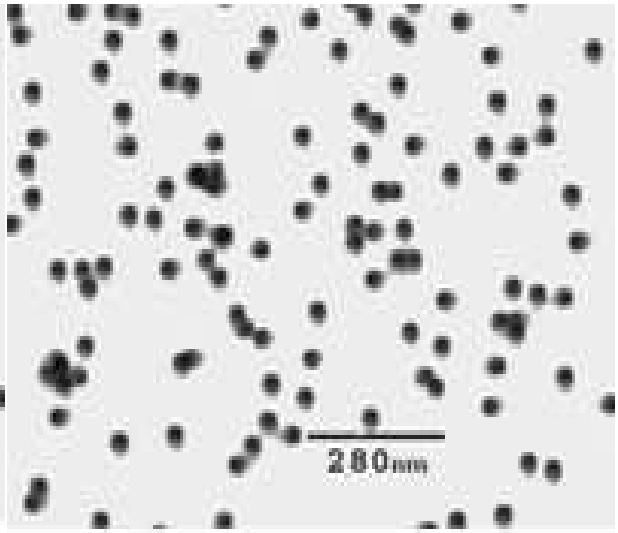

Figure 3) b.Tem image of 5-Fu nanoparticles (FN9)

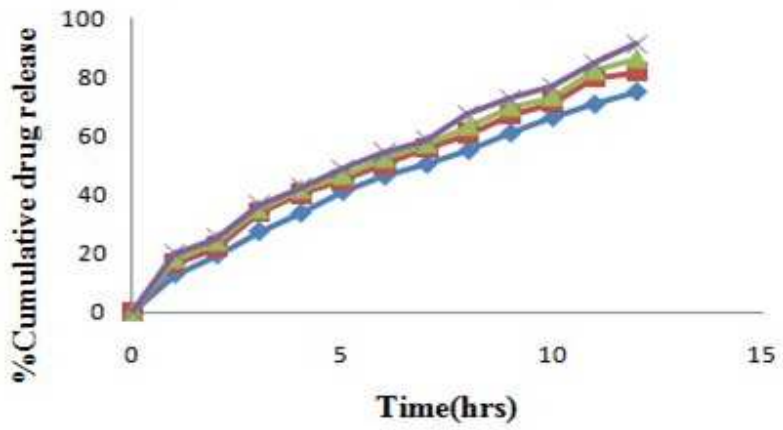

Figure: 3 *In-vitro* diffusion profiles of incorporated Nanoparticles in Sucralfate suspension (FNS9-FNS12)

In-vitro release kinetics of Nanoparticles incorporated in Sucralfate Suspension

The Korsmeyer's peppas equation showed that the exponential (n) value was greater than 1 indicating that supercase-II mechanism on drug release behavior .The drug release follows zero order with regression value near to (0.859 to 0.965) with diffusion type of release from the Higuchi equation regression value (0.966).

Table : 2 Evaluation parameters of Sucralfate suspension

Evaluation parameter	FS1	FS2	FS3	FS4	FS5	FS6	FS7	FS8	FS9	FS10	FS11	FS12	FS13	FS14	FS15
pH	4.76	5.12	5.76	6.24	6.74	4.02	4.99	5.66	6.03	6.12	5.10	5.80	6.20	6.70	7.2
Viscosity(cps)	200	210	225	238	250	110	128	136	148	180	193	204	215	222	238
Density(gm/cc)	0.689	0.966	1.322	1.399	1.66	1.2062	1.2074	1.2097	1.2132	1.2813	0.72	0.945	1.32	1.523	1.932
Sedimentation Volume H_U/H_O	0.39	0.43	0.47	0.56	0.59	0.46	0.49	0.56	0.62	0.7	0.32	0.36	0.42	0.48	0.52

Table : 3 Characterization of Nanoparticles

Parameters	FN1	FN2	FN3	FN4	FN5	FN6	FN7	FN8	FN9	FN10	FN11	FN12
Practical Yield	63.3	70	66	77.14	72.75	80	65	77.7	72.3	66.6	64.76	77
Entrapment efficiency (%)	38.5	43.21	47.31	54.34	56.13	60.33	64.6	70.2	72.0	79.0	81.6	85.4
Average Zetapotential (mv)	3.46±1.2	6.22±0.5	12.8±2.3	22.3±3.1	36.32±0.8	42.6±1.4	2.46±0.2	8.4±2.1	16.2±3.2	28.3±0.2	32.4±0.5	40±1.3
Polydispersity index(PDI±SD)	0.67±0.52	0.32±0.21	0.22±0.1	0.47±0.39	0.31±0.21	0.56±	0.14±0.11	0.22±0.23	0.37±	0.44±0.22	0.68±0.64	0.82±0.2
Average particle diameter (nm)	170±3.2	280±2.42	310±3.21	412±2.2	450±2.2	500±1.23	150±1.3	250±2.3	280±3.2	340±1.3	380±2.1	560±2.3

Table no:4 In-vitro diffusion data of 5-Fluorouracil Nanoparticles (FN1-FN12)

Time (hr)	FN1	FN2	FN3	FN4	FN5	FN6	FN7	FN8	FN9	FN10	FN11	FN12
1	0.27±0.1	3.3±0.1	0.37±0.3	1.13±0.6	0.92±0.2	1.46±0.3	0.921±0.2	1.73±0.2	2.11±0.2	2±0.1	5.53±0.6	5.31±0.5
2	0.31±0.5	5.68±0.3	0.74±0.1	2.13±0.8	2.21±0.4	2.43±0.6	2.21±0.6	2.41±0.5	3.05±0.3	4.31±0.3	14.7±0.3	10.51±0.6
3	0.387±0.5	7.89±0.5	1.2±0.2	3.16±0.2	3.04±0.5	3.45±0.5	3.04±0.5	3.85±0.8	4.5±0.5	8.9±0.5	14.7±0.3	14.97±0.2
4	7.21±0.3	12.03±0.1	7.12±0.5	7.87±0.2	7.52±0.2	9.42±0.4	7.52±0.1	11.39±0.3	14.2±0.5	18.19±0.1	19.56±1	19.32±0.5
5	10.71±0.2	15.34±0.5	10.81±1	12.4±0.1	12.35±0.6	16.4±1	12.35±0.3	19.62±0.6	17.47±0.2	23±0.5	24.41±0.8	25.07±1
6	13.64±0.4	20.03±0.3	13.75±0.4	18.56±0.5	16.55±1	24.33±1.2	16.55±0.6	24.93±0.5	22.27±0.3	29.7±1	29.6±0.6	30.02±0.3
7	18.19±0.5	23.52±0.4	18.3±1	23.9±1	24.5±0.5	30.4±0.5	23.63±0.2	32.36±0.1	28.98±0.6	39.95±1.2	36.87±0.5	37.11±0.5
8	22.88±0.6	27.36±0.2	22.99±0.5	29.27±1	29.79±0.3	35.59±0.6	29.69±0.5	38.3±0.1	39.33±0.5	49.79±0.5	42.28±0.2	45.41±1
9	26.39±1	31.9±1	27.69±0.2	36.34±0.5	35.1±0.4	41.48±1	37.6±10.3	49.5±0.6	49.08±0.5	49.79±0.5	50.99±0.8	52.78±1.2
10	33.96±0.5	35.03±0.5	32.54±0.5	42.45±0.6	40.54±0.5	48.99±1.2	48.87±0.5	54.7±0.5	56.1±1	56.71±0.6	58.84±1	61.54±0.5
11	33.96±0.5	39.32±0.5	39.24±0.6	48.31±0.5	47.74±1	55.28±0.5	57.29±1	63.93±1	64.74±1	65.33±1	70.22±0.9	72.12±0.8
12	37.54±1	42.07±1	48.78±1	53.26±1	55.59±1	60.93±1	65.49±1	69.71±1	72.75±0.5	76.8±1	80.92±1	85.33±1

Table 5 : In-vitro release kinetics of Nanoparticles incorporated in sucralfate suspension

Formulation code	Zero order		First order		Higuchi		Kersmeyer- peppas		Release Mechanism
	Slope	R^2	Slope	R^2	Slope	R^2	N	R^2	
FNS9	6.805	0.952	-0.046	0.988	22.22	0.859	1.349	0.848	Super case II Transport
FNS10	7.532	0.927	-0.056	0.968	21.82	0.964	1.349	0.848	Super case II Transport
FNS11	7.844	0.921	-0.062	0.945	22.74	0.966	1.349	0.848	Super case II Transport
FNS12	8.158	0.924	-0.068	0.904	23.64	0.965	1.349	0.848	Super case II Transport

Statistical analysis: Statistical analysis was performed using student's test with JMP soft ware .P value < 0.05 were consider to indicate statistical significance.

DISCUSSION

An increase in the concentration of polymer showed an increase in the entrapment efficiency and average particle size. FTIR and DSC studies exhibited that there is no interaction between drug and excipients. The TEM analysis showed the particle size is within the range of 170±3.2 to 560±2.3 nm, with optimum zeta-potential. From the results the formulation FN12 was found to be best formulation with 85.33±1 of drug release at the end of 12hr. The release exponents showed that the value of 'n' was greater than 1, indicating super case II mechanism on drug release behavior. The drug release follows zero order with regression value near to (0.859 to 0.965) with diffusion type of release from the Higuchi equation regression value (0.966).

In Sucralfate Suspension formulations FS5, FS10, FS15 exhibited high viscosity due to increase in concentration of Carboxy methyl cellulose, Xanthangum and Sodiumcarboxy methyl cellulose. Formulation FS15 containing 2.5% of Sodium carboxy methyl cellulose as a suspending is sufficient to remain particles dispersed in the suspending medium. Based on the above observation included in-vitro 5-Fluorouracil nanoparticles and sucralfate suspension with optimized concentration and stable suspension was selected and nanoparticles were incorporated in suspension and evaluated

CONCLUSION

The invention of this work understands that Sucralfate Suspension is a tool for carrying the nanoparticulates containing antineoplastic drug to carcinoma site and improves the patient compliance. It could be a promising carrier for oral administration of 5-Fluorouracil.

Other parameters such as histopathological studies and its pharmacokinetic parameters are under investigation.

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