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Formulation and characterization of colon specific 5-flourouracil tablets using pectin- phenylalanine azo polymeric conjugate

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ABSTRACT

The present research work aims the synthesis of an azo polymeric conjugate using pectin with phenylalanine and preparation of compression coated tablets of 5-fluorouracil (5-FU) using the synthesized conjugate. The azo polymeric conjugate was synthesized using pectin, phenyl alanine and other co-processing reactants. The resulting azo conjugate was evaluated for colour, percent yield, melting point, R_f value, solubility, spectral analysis and single dose acute toxicity studies. Compression coated tablets of 5-fluorouracil were prepared using this conjugate and subjected to various evaluation parameters like weight variation, hardness, thickness and friability. The stability studies of tablets were conducted at different conditions of temperature and relative humidity. Finally i*n-vitro* dissolution studies of tablets were performed in simulated gastric fluid, simulated intestinal fluid and a dissolution medium containing rat fecal contents. The tablets exhibited no release in simulated gastric as well as simulated intestinal fluid but an appreciable release in the dissolution medium containing rat fecal contents; indicating the colon specific nature of synthesized azo polymeric conjugate.

Keywords:

Colon targeted drug delivery, Pectin, Azo conjugates, Polymers.

1. Introduction

Colonic drug delivery has gained a great importance for the targeted delivery of the drugs to colon for the treatment of colonic diseases like Crohn's diseases, ulcerative colitis, colorectal cancer and amoebiasis[1, 2]. The advantages of targeting drugs specifically to the colon include reduced incidence of systemic adverse effects and the ability to cut down the required dose of drug. In order to achieve successful colonic delivery of orally administered drug, the drug needs to be protected from the environment of upper gastrointestinal tract (GIT). The various strategies for targeting orally administered drugs to the colon include covalent linkage of drug with a carrier, coating with pH-sensitive polymers, formulation of time release systems, exploitation of carriers that are degraded specifically by colonic bacteria, bioadhesive systems and osmotic controlled drug delivery systems[3, 4]. The bacteria present in the colon secrete a wide range of reductive and hydrolytic enzymes such as β-glucuronidase, azoreductase, β-xylosidase, β-galactosidase, α-arabinosidase, nitroreductase, deaminase and urea hydroxylase[5, 6]. These enzymes are responsible for biodegradation of polysaccharides. Based on this approach, we have synthesized an azo polymeric conjugate using pectin and phenylalanine, which would be acted upon by azoreductase enzyme in the colonic environment.

Pectin is a structural heteropolysaccharide contained in the primary cell walls of terrestrial plants. It was first isolated and described by Henri Braconnot in 1825. It is produced commercially as a white to light brown powder; mainly extracted from citrus fruits. It is also used in fillings, medicines, sweets, as a stabilizer in fruit juices and milk drinks and as a source of dietary fibre. Pectin is mainly used as a gelling agent, thickening agent and stabilizer in food. Pectin increases viscosity and volume of stool so it is used against constipation and diarrhea. Pectin is also used in wound healing preparations and specialty medical adhesives, such as colostomy[7, 8]. The aim of this research work is to synthesize azo polymeric conjugate using pectin with phenylalanine and utilize it for preparing compression coated 5-fluorouracil tablets for colonic delivery.

2. Materials and methods

2.1 Materials

All chemicals used in the research work were of AR grade. 5-fluorouracil was obtained as gift sample from Dabur Pharmaceuticals Ltd. India and sodium starch glycolate was obtained as a gift sample from Wockhardt Research Centre; Aurangabad; India. Pectin, phenylalanine, microcrystalline cellulose and talc were purchased from Loba Chemie; Mumbai; India. Sodium nitrite, hydrochloric acid, potassium dihydrogen phosphate, potassium chloride and sodium hydroxide were purchased from Central Drug House (P) Ltd. New Delhi; India. Distilled water was procured from institutional laboratory. IR spectra of the synthesized azo conjugate was performed in Laureate Institute; Himachal Pradesh; India. ^{1}H NMR of synthesized azo conjugate was performed in SAIF, Panjab University; Chandigarh; India. SEM & elemental analysis of the conjugate was provided by Wadia Institute; Dehradun; India.

2.2 Synthesis of pectin - phenylalanine azo polymeric conjugate

1 ml freshly distilled thionyl chloride was slowly added to 20 ml methanol with cooling in ice bath. 3.3 g of phenylalanine was added to it with stirring. The mixture was refluxed for 7 hours at 70^0C to produce methyl ester hydrochloride of phenylalanine. The resulting product was recrystallized by ether[9, 10] **(Figure 1)**.

2.1 g phenylalanine methyl ester hydrochloride was dissolved in a suitable volume of water containing 1.7 ml of dilute hydrochloric acid and then solution was cooled in ice. The beaker containing above solution was placed in a cryostatic bath and temperature was constantly maintained at $0\text{-}5^0$C. An aqueous solution of 1.4 g sodium nitrite in 10 ml water was added to it through a syringe. The temperature was maintained $0\text{-}5^0$C and precaution was taken that tip of the syringe lies completely dipped in the solution. The addition was continued until the solution gave an immediate positive test with moist potassium iodide-starch paper. To stabilize the diazonium salt and to minimize secondary reactions; proper conditions of acidity were maintained by adding excess of hydrochloric acid. The reaction mixture was kept at $0\text{-}5^0$C during the course of reaction in order to avoid the hydrolysis of diazonium salt to corresponding phenol[9, 10] **(Figure 2)**.

2 gm pectin was suspended in sodium hydroxide solution (0.08 g/ml) and stirred well on a magnetic stirrer. The solution was cooled below 5^0C. It was then slowly added to diazotised salt with continuous stirring maintaining the temperature $0\text{-}5^0$C in cryostatic bath. After completing the reaction, crude product was recovered and recrystallized by dissolving in methanol followed by cooling[9, 10] **(Figure 3)**.

2.3 Characterization of synthesized azo polymeric conjugate

The pectin- phenylalanine azo polymeric conjugate was evaluated for colour, percentage yield, melting point and solubility[11, 12, 13]. Its R_f value was determined by thin layer chromatography (TLC) using precoated silica gel plates- 60 F_{264} (Merck) in chloroform: methanol (5:2) as the solvent system. It was further subjected for various spectral analyses like IR spectroscopy, ^{1}H NMR spectroscopy, SEM and elemental analysis. The spectra obtained were subjected for interpretation and the results were inferred[14, 15].

2.4 Acute toxicity studies

The synthesized azo polymeric conjugate was evaluated for acute toxicity studies. The study protocol was approved by the Institutional Animal Ethical Committee (Registration No. 1156/AC/07/CPCSEA). The procedure followed was as per OECD 423 guidelines. Two groups of 6 wistar rats (either sex, 200-250 gm); one for test and other for control; were used for the study. The study was performed by administering the azo polymer conjugate at 2 gm/kg body weight for the test group animals. The acute toxicity study was done for a period of 14 days by observing body weight, changes in the skin, corneal reflex, behavioural patterns, and convulsions. The results were compared with the control group animals[16, 17].

2.5 Drug polymer interaction studies

Initially λ_{max} of pure drug was determined after scanning by UV Spectroscopy (Shimadzu 1800 UV-Visible spectrophotometer). The drug & azo polymeric conjugate were mixed three different ratios 1:1, 1:3, 3:1. The λ_{max} of mixtures was also determined by UV Spectroscopy. The λ_{max} of mixtures was compared with that of pure drug[18, 19].

2.6 Preparation of 5-fluorouracil tablets using synthesized conjugate

2.6.1 Preparation of 5- fluorouracil core tablets

The core tablets of 5- fluorouracil were prepared by direct compression **(Table 1)**. All the ingredients given in table 1 were mixed in a geometric progression and passed through sieve # 120. The mixture was compressed into core tablet by a ten station rotary tablet machine (Shakti Engineering, Gujarat, India) using 9 mm punches[20].

2.6.2 Compression coating of 5- fluorouracil core tablets with synthesized conjugate

The composition of compression coating material is shown in **Table 2.** Initially, the ingredients were accurately weighed and then mixed in geometric progression. 50% of the total weight of coating mixture was placed in the die cavity of tablet punching machine; the core tablet was placed on it at the centre, remaining 50% of the coating mixture was added to the die cavity and tablets were compressed using 12 mm punches[20].

2.7 Evaluation of prepared 5- fluorouracil tablets[20, 21]

The tablets were subjected to various evaluation parameters like weight variation, hardness, thickness and friability. For testing the weight variation, twenty tablets from each batch were selected randomly and weighed individually. The mean weights were calculated for each batch. The hardness of the tablets was determined using Monsanto Hardness tester. It is expressed in Kg/cm^2. Ten tablets were randomly picked from each formulation and the mean of three readings was calculated. The tablet thickness was measured using vernier callipers.

For testing friability, twenty tablets were weighed initially and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes. The tablets were weighed again and percentage friability was calculated by the formula:

$$F = \frac{W_{initial} - W_{final}}{W_{initial}} \times 100$$

$W_{initial}$ = Initial weight of tablets

W_{final} = Final weight of tablets

2.8 *In- vitro* release studies of prepared 5- fluorouracil tablets

2.8.1 *In-vitro* release studies in simulated gastric and simulated intestinal fluid

Release study was carried out on USP dissolution (Rotating paddle) apparatus. Initially release study was performed in 900 ml of simulated gastric fluid (HCl buffer pH 1.2; prepared as per I.P using Hydrochloric acid, potassium chloride and distilled water) for 2 hours in a constant temperature bath at 37 ± 1^0C. The solutions were occasionally stirred and 5 ml aliquot portions were withdrawn at various time intervals. The aliquots were directly estimated on Shimadzu 1800 UV-Visible spectrophotometer at 267 nm[9, 22].

Further release study was performed in 900 ml of simulated intestinal fluid (phosphate buffer pH 7.4; prepared as per I.P using potassium dihydrogen phosphate, sodium hydroxide and distilled water) for 10 hours in a constant temperature bath at 37 ± 1^0C. The solutions were occasionally stirred and 5 ml aliquot portions were withdrawn at various time intervals. The aliquots were directly estimated on Shimadzu 1800 UV-Visible spectrophotometer at 292 nm[22].

2.8.2 *In-vitro* release studies in rat fecal contents

Initially, phosphate buffer (pH 7.4) was prepared as per I.P using potassium dihydrogen phosphate (KH_2SO_4), sodium hydroxide (NaOH) and distilled water[23]. 5 gm of fresh fecal content of rats was weighed; added to 1000 ml of phosphate buffer (pH 7.4) and mixed well. It was incubated at 37^0C for 1 hour and filtered. It was used as the dissolution medium for carrying out *in-vitro* drug release study of prepared tablets. The release study was conducted on USP dissolution apparatus II (Rotating paddle type) at a temperature of 37 ± 0.5°C. The 5 ml sample was withdrawn at regular intervals and replaced by fresh dissolution medium. The absorbance of filtered solution was measured by UV spectroscopy at λ_{max} 292 nm and % Drug Release was calculated[9, 23].

2.9 Stability Studies

The tablets were subjected for stability studies at various conditions of temperature and Relative Humidity (5°C ± 3°C / 60% RH, 25°C± 2°C / 60 ±5% RH and 40°C± 2°C / 75% RH) for three months. The changes were observed in the characteristics of tablets and the results were reported[19, 24].

3. Results & discussion

3.1 Characterization of azo polymeric conjugate

The experimental results reveal that pectin- phenylalanine azo polymeric conjugate was deep orange in colour. It showed 72% yield and 241^0C melting point (with darkening). Its R_f value was 0.74. It exhibited solubility in water, insolubility in chloroform, benzene and acetone.

3.2 Spectral analysis

The IR spectra of pectin- phenylalanine azo conjugate showed peaks at 3464 cm^{-1}; 3383 cm^{-1} (OH stretching aromatic ring), 2927 cm^{-1} (CH stretching aromatic ring), 1600 cm[-1;] 1562 cm^{-1} (C=C aromatic ring stretching), 1492 cm^{-1}; 1427 cm^{-1} (-N=N-stretching), 1338 cm^{-1}; 1296 cm^{-1} (C-N stretching), 1153 cm^{-1}; 1114 cm^{-1} (C-O stretching alcohol), 825 cm^{-1}; 783 cm^{-1} (CH bending aromatic ring) **(Figure 4)**. The ^{1}H NMR spectra showed peaks at δ 1.2 ppm (-CH saturated proton), δ 2.1 ppm (-C≡CH, acetylenic proton), δ 3.1-3.8 (-CH₃OR, ether proton), δ 4.5-4.8 (-C=CH, vinylic proton), δ 5.1-5.2 (R-OH, hydroxyl proton) **(Figure 5)**. SEM analysis of azo conjugate showed that it has slender rod shaped particles with smooth surface **(Figure 6).**

3.3 Acute toxicity studies

The acute toxicity studies of azo polymeric conjugate revealed that there was no toxic effect of the conjugate on experimental animals. The animals did not reveal any change in body weight, locomotion, body temperature or behavioural pattern. There was no symptom of redness drowsiness, lacrimation and inflammation in animals. Hence the synthesized azo polymeric conjugate is devoid of toxicity.

3.4 Drug polymer interaction studies

The drug interaction study revealed that there was no interaction between the drug and the synthesized polymeric conjugate because there was no change in the λ_{max} value, which was observed at 266 nm prior to and after the test.

3.5 Evaluation of 5- fluorouracil tablets

The prepared tablets passed the weight variation as well as friability test. The hardness and thickness of the tablets was also in acceptable range. Thus all the tablet formulations were found to comply with the IP standard **(Table 3)**.

3.6 *In- Vitro* Release Studies

The prepared tablets using 5-fluorouracil with the synthesized pectin-phenyl alanine conjugate did not show any release in simulated gastric and intestinal fluid. It implies that azo polymeric conjugate does not degrade in stomach as well as small intestine. Thus, the objective of bypassing the upper GIT was achieved. The azo polymeric conjugate showed a significant release of 80.25% (FP1) and 90.5 % (FP2) in the rat fecal contents. The rat fecal material contains azoreductase enzyme and the same enzyme is also synthesized by colonic bacteria in colon. Hence it reveals that the synthesized conjugate shows degradation in colon by means of azoreductase enzyme **(Figure 7).** The kinetics of release data was analyzed by BIT software (BIT-SOFT 1.12). The drug release kinetics followed Peppas Korsmeyer equation and mechanism of drug release was fickian diffusion with R^2 value 0.9699.
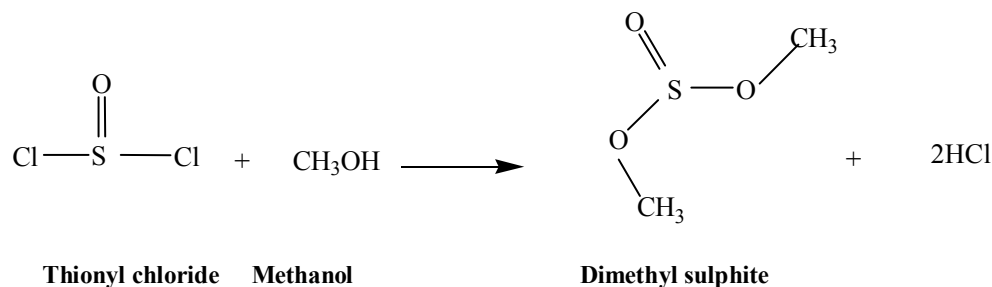
3.7 Stability Studies

All the tablets were investigated for changes in the appearance, drug content and *in-vitro* release during three months stability testing conditions**.** During regular observation till three month, the tablets showed no significant changes in the colour, drug content and *in-vitro* drug release. Thus all the tablets passed the stability test.

4. Conclusion

The pectin- phenylalanine azo conjugate was successfully synthesized with a good percentage yield. Its IR spectra revealed the peak for -N=N- stretching, thus confirming the formation of azo conjugate. Since the tablets prepared using azo conjugate did not show any release in simulated gastric fluid as well as simulated intestinal fluid; the conjugate protected the 5-fluoro uracil tablets from environment of upper GIT. The tablets showed a significant release in rat fecal matter; so the synthesized conjugate has colon specific nature. Hence conclusion was drawn that the synthesized pectin- phenylalanine azo conjugate can serve as a potential excipient for delivering drugs to colon. Since the azo polymeric conjugate did not show any toxicity in animals, it is safe for its active initiations in human volunteers for clinical trials.

Thionyl chloride   Methanol   Dimethyl sulphite

Figure 1: Proposed mechanism for esterification of phenylalanine

Phenylalanine methyl ester   **Diazonium salt**

1.1.

1.2. Figure 2: Proposed mechanism for diazotization of phenylalanine methyl ester

Diazonium salt   **Pectin**   **Pectin -phenyl alanine conjugate**

Figure 3: Proposed mechanism for coupling of diazonium salt with pectin
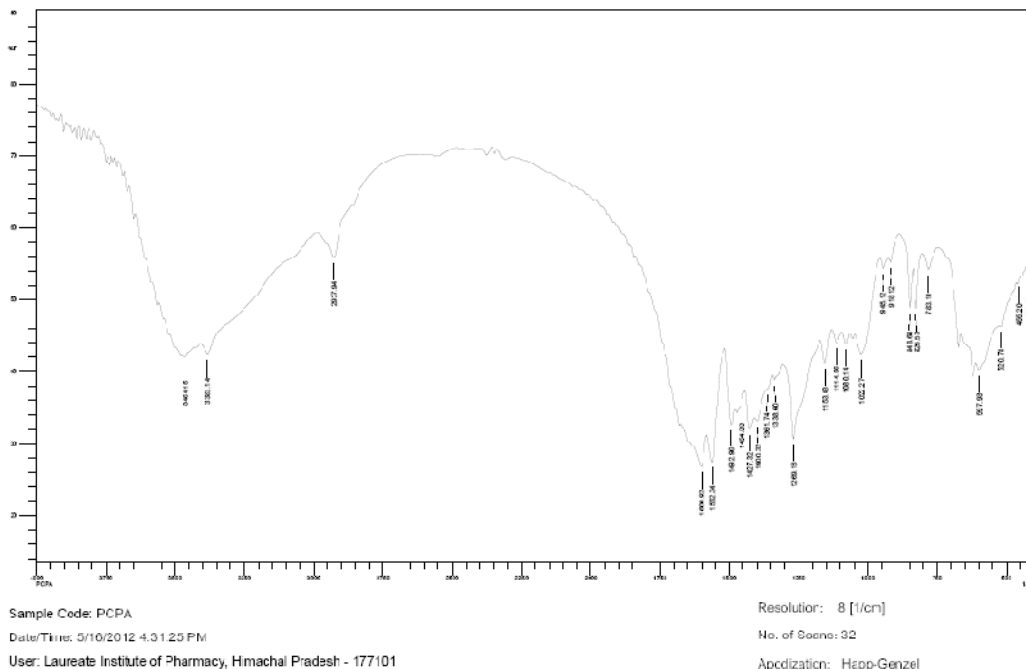
Figure 4: IR spectra of pectin- phenylalanine conjugate
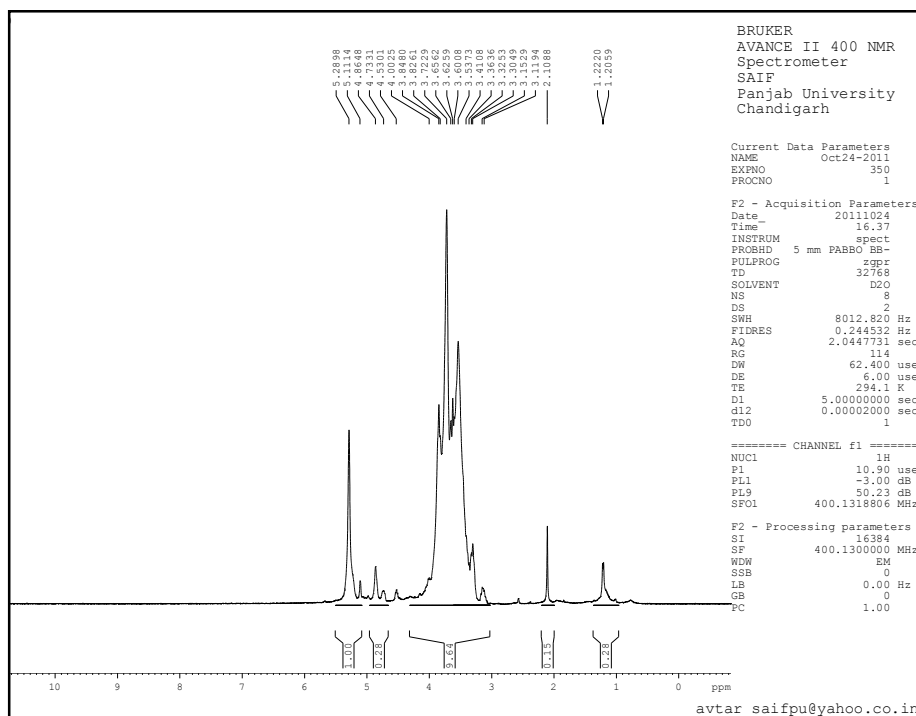

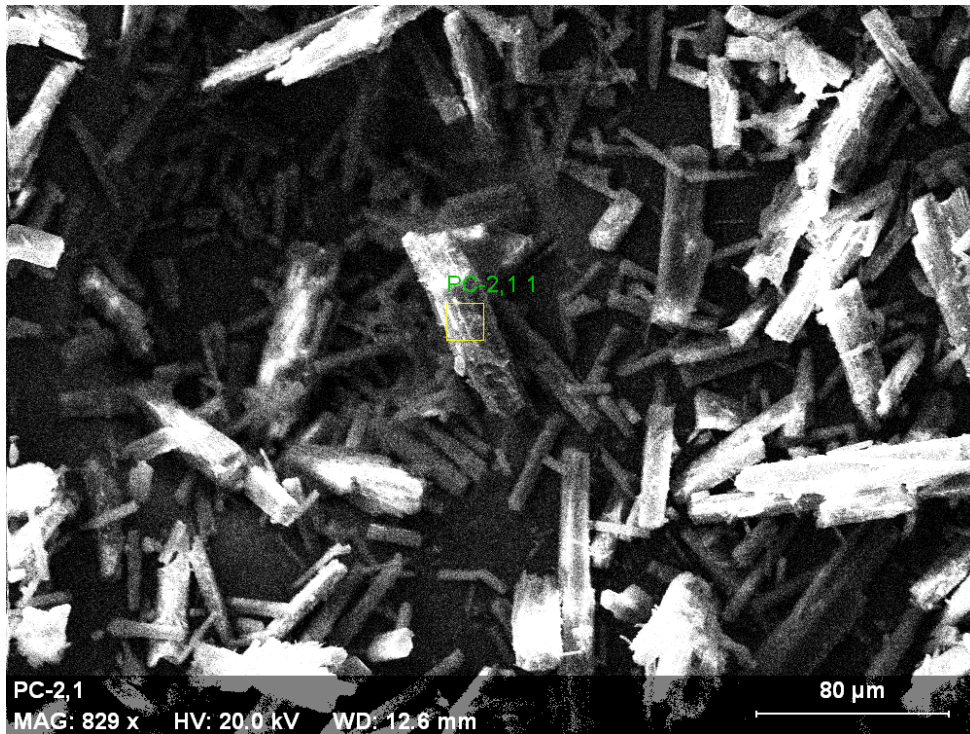**Figure 5: ¹H NMR spectra of pectin- phenylalanine conjugate**

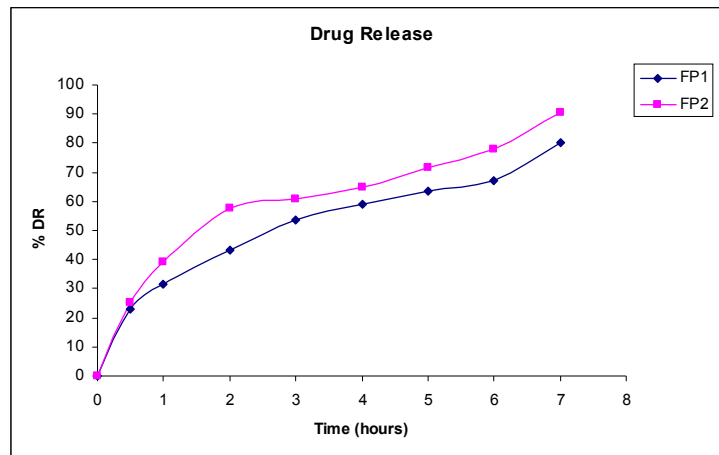**Figure 6: SEM analysis of pectin- phenylalanine conjugate**

Figure 7: *In-vitro* release of tablets in dissolution medium containing rat fecal contents

S.No.	Ingredients	Amount (mg)
1	5-Fluoro-uracil	50
2	Sodium starch glycolate	30
3	MCC	15
4	Mg stearate	2.5
5	Talc	2.5

Table 1: Formula for preparing 5-fluorouracil core tablet

S.No.	Ingredients	FP1	FP2
1	Pectin-Phenylalanine conjugate	100	200
2	Starch	180	80
3	MCC	100	100
4	Mg stearate	10	10
5	Talc	10	10

Table 2: Formula for preparing compression coated 5-fluorouracil tablets

Parameters	FP1	FP2
Weight variation* (mg)	401.22± 1.058	400.84±0.96
Hardness* (Kg/cm^2)	6.70 ± 0.10	5.33 ± 0.06
Thickness* (mm)	2.54±0.02	2.54± 0.01
Friability (%)	0.62	0.49

* Average of three determinations

Table 3: Evaluation parameters of prepared tablets

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