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DESIGN AND DEVELOPMENT OF 5 - FLUOROURACIL LOADED BIODEGRADABLE MICROSPHERES

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ABSTRACT
The present study is aimed at the overall improvement in the efficacy, reduction in toxicity and enhancement of therapeutic index of 5-fluorouracil. Biodegradable microparticulate delivery system of 5-fluorouracil has been developed by solvent evaporation technique by using polymethacrylate polymers like eudragit L100, eudragit S100, eudragit P4135F and methylcellulose. Four different formulations were prepared by using these polymers in drug to polymer ratio of 1:2. The formulations were evaluated with respect to particle size analysis, entrapment efficiency, *in vitro* drug release studies*, in vivo* drug targeting studies and stability studies. The formulated magnetic microspheres were found to be spherical with average particle size of 3-12 µm in diameter and incorporation efficiency up to 78.80%. *In vitro* drug release after 12 hr was 86.41 %, 92.84 %, 79.88 % and 82.38 % for formulation F1, F2, F3 and F4 respectively. Formulation F2 with highest drug content was selected for *in-vivo* drug targeting studies. The average targeting efficiency of drug loaded microspheres was found to be 26.16 % of the injected dose in liver, 11.40 % in lungs, and 15.08 % in spleen, whereas the concentration of pure drug was 15.52 % in liver, 9.0 % in lungs, and 9.50 % in spleen. These results reveal that the drug loaded microspheres showed preferential drug targeting to liver followed by spleen and lungs. Stability studies revealed that 4º C is the most suitable temperature for storage of 5 - fluorouracil loaded microspheres. Overall, this study showed that the 5 - fluorouracil can be formulated in a microparticulate drug delivery system by using various polymers and it showed significant prolonged drug release.

KEYWORDS: 5-Fluorouracil, biodegradable, microparticulate drug delivery system.

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INTRODUCTION

The necessity and advantages of colon-specific drug delivery systems have been well recognized and documented. In addition to providing more effective therapy of colon related diseases such as irritable bowel syndrome, inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis, colon specific delivery has the potential to address important unmet therapeutic needs including oral delivery of macromolecular drugs. It has been reported that at least 1 million Americans are believed to have IBD with 15000 - 30000 new cases diagnosed annually[1]. Studies indicated that absorption enhancers performed more effectively in the colon than in the upper gastrointestinal (GI) tract [2]. Drug delivery devices that specifically deliver active agents to the colon have been recognized as having important therapeutic advantages. A large number of colonic conditions could effectively be treated more efficaciously if the active ingredient is released locally. Examples of such colonic disorders include Crohn's disease, ulcerative colitis, colorectal cancer and constipation[3]. Most of the conventional drug delivery systems for treating the colon disorders such as bowel disease (e.g. irritable bowel syndrome, ulcerative colitis, crohn’s disease etc), infectious diseases (amoebiasis) and colon cancers are failing as the drugs do not reach the site of action in appropriate concentrations. Thus, an effective and safe therapy of these colonic disorders, using site specific drug delivery system is a challenging task to the pharmaceutical technologists[4]. Colon cancer is the second most cause of death after lung cancer by cancer diseases. Many different drugs or drug combinations have been tested for a successful therapy. At present, the standard regimen is an intravenous bolus injection of 5-fluorouracil (5-FU) modulated by leucovorin (LV)[5-6.] Only few approaches for an oral administration of anticancer drugs in the treatment of colon cancer have been described in literature. Recently, enzyme-dependent tablet-based systems have been proposed, which might allow an efficient treatment combined with a reduction of adverse effects[7]. Alternatively, pH-dependent drug release systems have been developed for the 5-FU release in the colon [8]. For at least forty years, 5-fluorouracil (5-FU) has been the mainstay of treatment for patients with metastatic colorectal cancer. Biochemical modulation of 5-FU has been investigated in an attempt to identify the most efficacious form of administration. Folinic acid (FA) is shown to increase the rate of objective response when given in combination with 5-FU and prolongs progression free survival with a slightly improved overall survival [9]. 5-Fluorouracil (5-FU) is an antimetabolite of the pyrimidine analog class which is widely used alone or in combination chemotherapy regimens. After intravenous injection of 5-FU, it is rapidly distributed and eliminated with an apparent terminal half-life of 8-20 m [10]. 5-FU is poorly absorbed after oral administration with extremely variable bioavailability [10]. These disadvantages make it an appropriate candidate for microencapsulation. Indeed, microencapsulation using biodegradable and non biodegradable polymers has already been employed to achieve sustained release of anticancer drugs such as 5-FU[11-12]. Furthermore, there is no controlled-release dosage form of 5-FU for oral delivery, although this would be particularly useful in cancer therapy. Hence in this study, an attempt is being made to prepare microparticulate drug delivery system of 5 – fluorouracil using solvent evaporation technique employed for drug targeting and as drug carriers.

MATERIALS AND METHODS

5-Fluorouracil was a kind gift sample from Intas Pharmaceuticals Ltd. Ahmedabad. Eudragit L100, Eudragit S100 and Eudragit P4135F were received from Degussa India Pvt. Ltd, Mumbai. Ethyl cellulose, Liquid paraffin (light), n- hexane and petroleum ether were generously gifted by Hi media Laboratories Pvt Ltd. Mumbai.

Formulation of Microspheres

Emulsification - solvent evaporation method

Accurately weighed polymers were dissolved in 10 ml of acetone and 5 ml of ethanol in drug: polymer ratio of 1: 2 to form a homogenous polymer solution. Core material i.e. 5-Fluorouracil was added to the polymer solution and mixed thoroughly. The organic phase was slowly poured at 15^0C in to liquid paraffin (100 ml) containing 1 % w/w of span 20 with stirring at 800 rpm to form a smooth emulsion. Thereafter, it was

allowed to attain room temperature and stirring was continued until residual acetone evaporated and smooth walled, rigid and discrete microspheres were collected by decantation and the product was washed with petroleum ether (40-60^{0}C) or n- hexane or any other organic solvents, washing was repeated for four times and dried at room temperature for 3 h. The microspheres were then stored in a desiccator over fused calcium chloride. Three batches were prepared with different proportion of core to coat materials (drug: polymer) [13-15].

Evaluation of Magnetic Microspheres

Particle size analysis

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large part to simplicity of sample preparation. Scanning electron was carried out by using JEOL JSMT-330A scanning microscope (Japan). Dry microspheres were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of microspheres was taken by random scanning of the stub[14,16].

Estimation of drug encapsulation efficiency

To determine the entrapment efficiency, 50 mg of drug loaded microspheres were washed with 10 ml of phosphate buffer to remove the surface- associated drug. The absorbance of the filtrate was taken at 266 nm to estimate the surface drug content. The filtered microspheres were then digested in a small amount of phosphate buffer to release the entrapped drug from the microspheres. The drug was then extracted into the buffer by making up the volume to 100ml with the phosphate buffer and keeping it overnight in a metabolic shaker with slight shaking. The solution was then filtered and the drug content analyzed spectrophotometrically at 266 nm[17]. The results are shown in Table no. 1.

$$\text{Percentage entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Entrapped drug content}} \times 100$$

In vitro drug release studies

The drug dissolution tests of microspheres were carried out by the paddle method specified in the US Pharmacopoeia XXI. Microspheres were weighed (weight equivalent to 50 mg of drug) were filled in tea bags .The tea bag tied using thread with paddle and 900 ml of different buffers (pH 1.2 & 7.4) as dissolution medium. Rotated at 100 rpm and thermostatically controlled at 37°. Perfect sink conditions prevailed during the dissolution tests. The sample was withdrawn at a suitable interval from the dissolution vessel and assayed at 266 nm by using UV-Visible spectrophotometer (UV-1201 Shimadzu, Japan) at 266 nm[13, 18, 14, 19].

In vivo drug targeting studies

This study was carried out after obtaining the due permission for conduction of experiments from relevant ethics committee (K.L.E.S's College of Pharmacy, Belgaum) which is registered for "Teaching and Research on Animals" by committee for the purpose of control and supervision of experiments on animal, Chennai (Registration number 221/CPCSEA).

This study was carried out to compare the targeting efficiency of cisplatin loaded magnetic microspheres with that of free drug in terms of percentage increase in targeting to various organs of reticuloendothelial system like liver, lungs, spleen and kidneys. Experiments were performed on rats of 225-235 g weight. All the experiments were carried out in accordance with the protocols approved by the Institutional animal ethics committee (K.L.E.S College, Belgaum, India).

Ten healthy male Albino Wistar rats of weighing 200-250 gm were selected, a constant day and night cycle was maintained and they were fasted for 12 hr. The animals were divided into 2 groups. Each containing five mice group I received microspheres equivalent to 2.5 mg of 5-fluorouracil in the caudal vein after redispersing them in sterile phosphate buffer saline solution. Microspheres from batch F2 were selected for the study. Group-II received 2.5 mg of pure 5-fluorouracil intravenously.

After 6 h the mice were sacrificed and their liver, lungs, and spleen were excised, rinsed, blotted, dry and weighted. The individual organs of each mouse were homogenized in 8.0 ml PBS (pH 7.4) and centrifuged to obtain supernatant [20-21].

Extraction procedure
The plasma or supernatant of tissue homogenate was mixed with 0.1 ml of 0.25 M NaH_2PO_4 buffer and extracted with 6.0 ml of ethyl acetate. After centrifugation at 5000 rpm for 5 min, 3 ml organic layer was evaporated at 55° C. The residue was redissolved in triple distilled water and analyzed with UV detector at 280 nm.

Stability studies
Information on the stability of drug substance is an integral part of systematic approach to stability evaluation. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under influence of the variety of environmental factors such as temperature, humidity and light, and to establish a shelf life for the drug product and recommended storage conditions. All 4 batches of microspheres were tested for stability. All the preparations were divided into 3 sets and were stored at, 4ºC in refrigerator, 30º C ± 2ºC / 65 % ± 5 % RH in humidity control oven (GINKYA IM 3500 series), ambient temperature and humidity. Drug content of all the formulations was determined after 15 day, 30 day and 60 day. *In vitro* release study of a selected formulation was also carried out after storage for 1 month[22].

RESULTS AND DISCUSSION
Different magnifications were used while taking these photomicrographs. Average particle size of magnetic microspheres of 5 - fluorouracil was found to be 3.27 ± 0.0766, 5.39 ± 0.1932, 7.61 ± 0.846, 12 ± 0.1066 μ for F1 to F4 respectively. Particles of formulations F1, F3 and F4 were found to be smooth, oval and discrete whereas particles of formulation F2 were slightly rough surfaced but discrete. Scanning electron photomicrographs of formulations F2 is shown in **Fig. 1.**

Drug entrapment efficiency was calculated from the drug content. The mean practical yield for all the formulations was more. A positive correlation between solid content and percentage yield was observed. This may be explained by the fact that through a constant amount of material is always lost in processing, this loss is proportionately less significant when the solid content is more (e.g. if the loss in processing is 15 mg then it is significant for a 100 mg sample, but much less significant for 500 mg sample). It was observed that entrapment efficiency was increased with increase in concentration of polymer added in formulations. The maximum drug content was found in F2 i.e. 89.59 % and minimum drug content was found in F1. The percentage drug loading and drug entrapment efficiency of all the formulations were given in the **Table1.**

Pure 5 – fluorouracil, marketed preparation and all the four formulations of 5 - fluorouracil loaded microspheres were subjected to *in vitro* release studies. These studies were carried out in PBS pH 7.4. The cumulative percent drug release of pure drug and marketed preparation was found to be 91.3 % and 85.58 % respectively at 3 hr. Cumulative percent drug release after 12 hr was 86.41 %, 92.84 %, 79.88 % and 82.38 % for F1, F2, F3 and F4 respectively by UV spectroscopy.

The release data obtained for pure drug, marketed preparation and formulations F1, F2, F3 and F4 are showed in Fig. 2 It shows plots of cumulative percent drug released as a function of time for pure drug and for different formulations of 5 – fluorouracil loaded microspheres. When compared with pure drug and

marketed formulation, the release of 5-fluorouracil loaded microspheres was prolonged over a period of 12 hr or more. The *in-vitro* release of all the four batches of microspheres showed an interesting bi-phasic release with an initial burst effect. In the first 30 mins, drug release was 18.4 %, 20 %, 14.4 % and 16.4 % for F1, F2, F3 and F4 respectively. This was followed by a steady drug release pattern, which approximated zero order release. The mechanism for the burst release can be attributed to the drug loaded on the microspheres or imperfect entrapment of drug. It was observed that the drug release from the formulations decreased with increase in the amount of polymer added in each formulation.

Formulation F2 with highest drug content was selected for *in-vivo* drug targeting studies. The comparison between the amount of drug targeted from microspheres and free drug in various organs is presented in Fig. 3. The average targeting efficiency of drug loaded microspheres was found to be 26.16 % of the injected dose in liver, 11.40 % in lungs, and 15.08 % in spleen, whereas the concentration of pure drug was 15.52 % in liver, 9.0 % in lungs, and 9.50 % in spleen. These results reveal that the drug loaded microspheres showed preferential drug targeting to liver followed by spleen and lungs. It was also revealed that as compared to pure drug, higher concentration of drug was targeted to the organs after administering the dose in form of microspheres. Higher drug targeting in liver and spleen as compared to lungs may be attributed to high macrophages load in these organs and large size of liver as compared to spleen and lungs. Accumulation of drug in lungs could be also due to physical entrapment of the microspheres.

Stability studies of the prepared microspheres were carried out, by storing all the formulations F1 to F4 at 4ºC in refrigerator, ambient temperature and humidity and 30ºC ± 2ºC / 65 % RH ± 5 % RH in humidity control oven for 60 day. Two parameters namely residual percent drug content and *in vitro* release studies were carried out. The results of drug content after 15 day, 30 day and 60 day are shown in Table no.2. These studies reveal that there is a reduction in drug content after storage for 60 days at 4ºC, ambient temperature and humidity and 30ºC ± 2ºC / 65 % RH ± 5 % RH. It was also revealed that out of the four formulated batches, the one stored at 4º showed maximum residual drug followed by that stored at ambient temperature and humidity and 30º C ± 2ºC / 65 % RH ± 5 % RH. *In vitro* release studies, which were carried out after storing a selected formulation F2 at 4º, ambient temperature and humidity and 30ºC ± 2ºC / 65 % RH ± 5 % RH for 60 day. *In vitro* release studies reveal that the formulation stored at 4º showed 86.15 % release, the one which stored at ambient temperature and humidity showed 85.13 % and formulation stored at 30ºC ± 2ºC / 65 % RH ± 5 % RH showed 83.93 % release after 12 hr. On comparing this data with the previous release data of F2, it was observed that there was maximum drug content and closest *in vitro* release. Thus, it can be concluded that 4°C is the most suitable temperature for storage 5-FU loaded microspheres.

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Table No.1- Percentage Practical Yield and Drug Entrapment Efficiency

Formulations	Practical yield (%)	Drug entrapment efficiency (%)
F1	79.4 ± 0.221	63.2 ± 0.168
F2	89.59 ± 0.223	68.9 ± 0.214
F3	88.33 ± 0.268	79.4 ± 0.257
F4	83.76 ± 0.321	78.8 ± 0.355

Each value represents mean ±SD for (n=3)

Table No. 2 - Stability studies for percent drug content
(After storage at 4ºC, ambient temperature and humidity and at 30ºC / 65 % RH)

Formulations	Percent drug content at 4º C			Percent drug content at ambient temperature and humidity			Percent drug content at 30º C / 65% RH		
	After 15 days	After 30 days	After 60 days	After 15 days	After 30 days	After 60 days	After 15 days	After 30 days	After 60 days
F1	39.74 ± 0.322	39.62 ± 0.300	38.88 ± 0.315	39.36 ± 0.325	39.10 ± 0.291	38.54 ± 0.285	39.30 ± 0.301	38.95 ± 0.299	38.30 ± 0.283
F2	46.64 ± 0.385	46.38 ± 0.389	45.64 ± 0.379	46.29 ± 0.380	45.89 ± 0.382	45.12 ± 0.370	46.25 ± 0.387	45.76 ± 0.368	44.62 ± 0.360
F3	53.02 ± 0.522	52.88 ± 0.495	52.26 ± 0.530	52.96 ± 0.520	52.32 ± 0.548	51.96 ± 0.498	52.88 ± 0.495	52.15 ± 0.501	51.58 ± 0.488
F4	65.18 ± 0.551	56.04 ± 0.549	55.38 ± 0.541	56.14 ± 0.545	55.94 ± 0.540	54.78 ± 0.499	56.08 ± 0.501	55.70 ± 0.544	54.42 ± 0.498

Each value represents mean ±SD for (n=3)

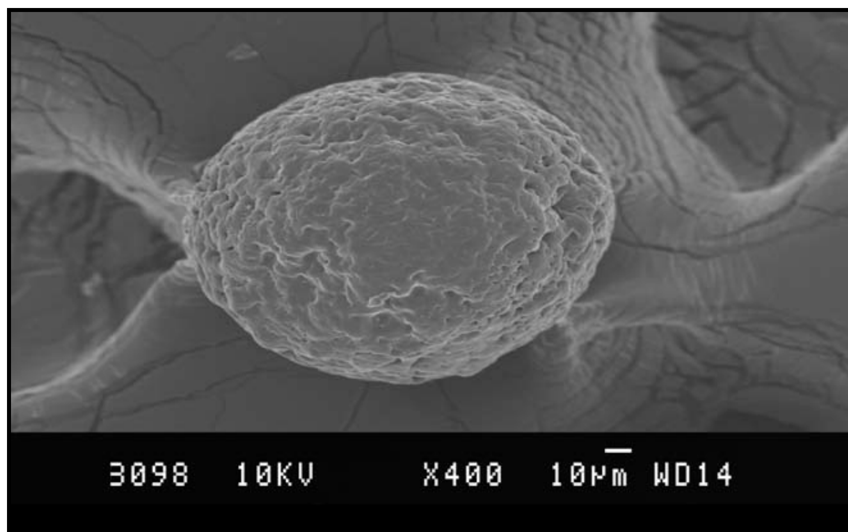

Fig 1. Scanning electron photomicrographs of formulations F2

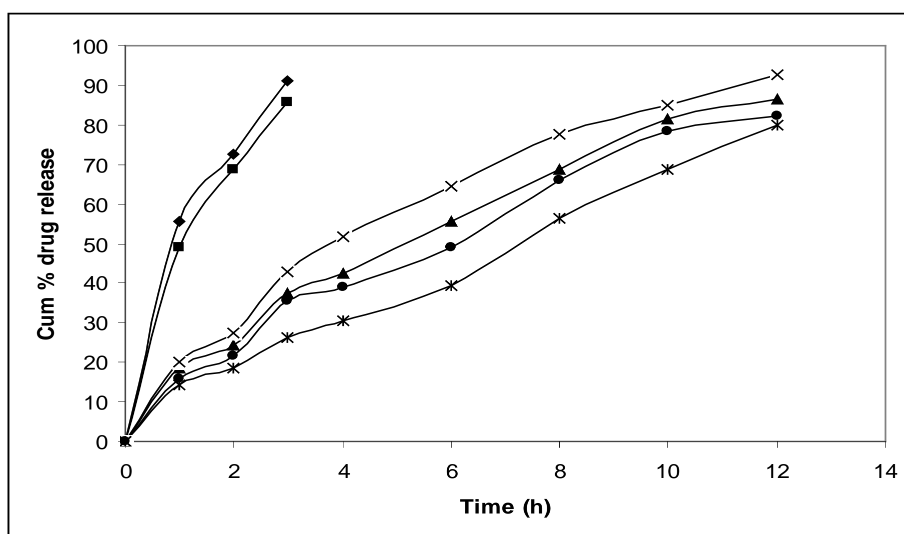

Fig 2. *In vitro release* profile for different formulations of 5-fluorouracil from microspheres.
Pure drug (-◆-), Marketed preparation (-■-), Formulation F1 (-▲-),
Formulation F2 (-×-), Formulation F3 (-●-), Formulation F4 (-*-)

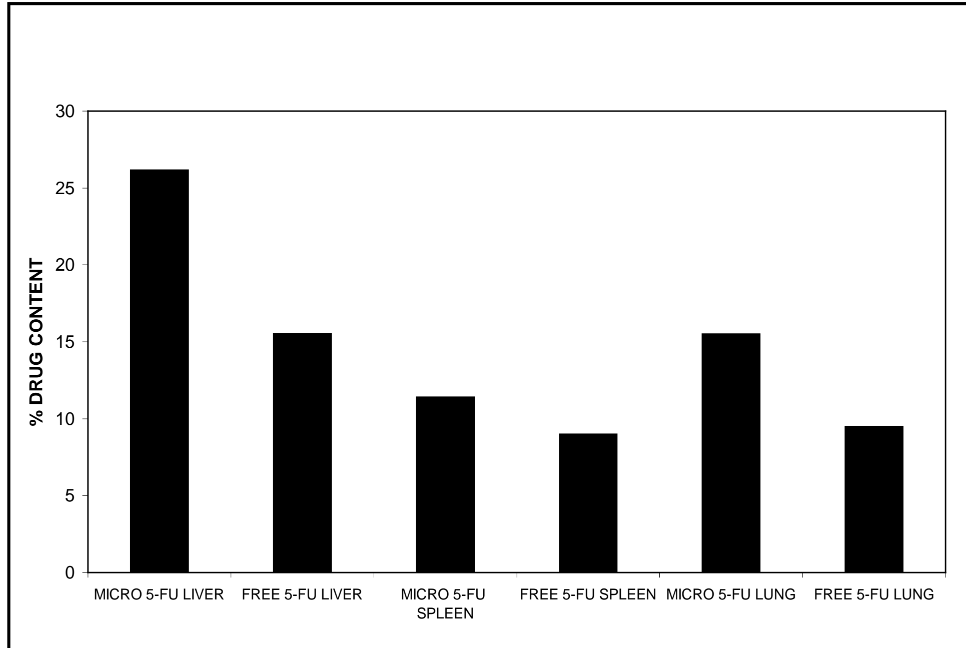

Fig 3. *In vivo* profile of formulation F2 in various organs.
Comparison of % drug content and free drug in various organs

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