International Journal of Current Pharmaceutical Research
ISSN- 0975-7066 Vol 3, Issue 1, 2011

Research Article

# POROUS MICROSPHERE OF 5-FLOURU URACIL: A TOOL FOR SITE SPECIFIC DRUG DELIVERY IN GASTRIC CANCER

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*Received: 22 July 2010, Revised and Accepted: 27 Aug 2010*

**ABSTRACT**

The objective of the present investigation to prepare 5- Fluoro Uracil loaded porous microsphere for Gastric Cancer and it's in Vitro Cytotoxicity analysis on KATO-III human gastric cancer cell lines. Porous microspheres prepared by emulsification extraction technique using different concentration of pectin and casein (250, 500 and 750mg) and different stirring speeds (500-2000rpm). The effect of formulation and process variables on the particle morphology, mean particle size, entrapment efficiency and percentage buoyancy was analyzed. In present work drug polymer interaction study, in vitro drug release study and cytotoxicity study were also performed on KATO-III gastric cancer cell lines. The microspheres were found to be regular in shape and highly porous. The yield of preparation and the entrapment efficiency were high for all porous microsphere. Microsphere prepared by using polymer: emulsifier ratio 1:1, and stirring speed 1500 rpm were selected as an optimized formulation (F2). Results of drug polymer intraction study revealed that there is no significance difference in infrared spectra of 5-FU and drug loaded microsphere whereas in case of TG-DTA spectra there is presence of a new exothermic peak with characteristic curve of the 5-FU which may be due to presence of polymer. In vitro drug release studies indicated that there is constant release of drug from microsphere upto 24 hours from F1. The 5-FU loaded optimized formulation was used to perform cytotoxicity study. Cytotoxicity study indicated that the microparticles of 5-FU had greater cytotoxic effects on KATO-III gastric Cancer cell lines in comparison to drug solution.

**Keywords:** Porous microspheres, Pectin, casein, Kato III.

## INTRODUCTION

Gastric cancer is the second most common cancer and cause of cancer related death in the world. Gastric cancer was the leading cause of cancer death in men and third leading cause of cancer of women. The delivery of drugs currently used in cancer treatment is performed mostly through intravenous drug infusion or topically. Due to unfavorable pharmacodynamics, the infusions need to be performed frequently, exacerbating the potential for side effects. Oral treatment with anticancer drugs can be preferred as this delivery route is convenient to patient, reduces administration cost and facilitates the use of more chronic treatment regimens. Thus, for low bioavaibility has usually limited the development of cancer treatment by the oral route. Attempts are being made to develop a sustain drug delivery system that can provide therapeutically effective plasma drug concentration level for larger durations, thereby reducing dosing frequency and minimizing fluctuations in plasma drug concentration at steady state by delivering drug in a controlled and reproducible manner[1,2,3].

The polysaccharide pectin is an inexpensive, nontoxic product extracted from citrus fruits has been used as food additive, a thickening agent, and a gelling agent. In addition, pectin can reduce interfacial tension between an oil phase and a water phase and is efficient for the preparation of emulsion. Pectin has a very complex structure that depends on both its source and the extraction process. Numerous studies contributed to elucidate the structure of pectin. Basically, it is polymer of ά-D-galacturonic acid with 1→4 linkages. This chain is regularly interrupted by some rhamnogalacturonan segments that combine galacturonic acid residues and ά-L-rhamnopyranose by a 1→2 linkage. The galacturonic acid of the backbone is partially methyl esterified. Low methoxy pectin with degree of esterification less than 50% can form rigid gels. It is completely degraded by colonic bacteria but is not digested in the upper GI tract. This gel is stable in low pH solution and is being investigated as a carrier material for different controlled release systems [4,10].

In this study we studied the effect of process condition on casein-pectin porous microsphere prepared. Casein by virtue of its emulsifying properties causes incorporation of air bubbles and formation of large holes in the microspheres that act as air reservoir in floating system and serve as a simple and non expensive material used in controlled oral drug delivery systems. The development of drug carrier made of biodegradable polymer like pectin, is receiving increasing attention in the field of pharmaceutical technology. These systems offer a number of advantages over the classical drug delivery systems. Like (1) by selecting the appropriate drug polymer combination it is possible to achieve encapsulation hydrophilic and hydrophobic drug simultaneously, (2) the bioactive molecule can be conveniently isolated and protected in the micro cavity, (3) the desired release rate of drug can be achieved by selecting suitable polymer[5,6].

5-FU one of the major anti metabolite used in variety of solid cancers, such as stomach, colon, lung, and breast cancer. It is usually given intravenously, as absorption of 5-FU from the gastro intestinal tract is erratic and unpredictable. The intravenous route of administration is associated with severe systemic side effects because of 5-FU's cytotoxic nature when it reaches unwanted sites. After oral administration, gastrointestinal absorption is rapid, and peak levels in the blood are reached between 15 and 60 minutes after ingestion, but much variability is seen between individuals because of first pass metabolism in the liver. After intravenous administration, the drug diffuses equally in all compartments in a volume equivalent to body fluid volume. Peak plasma levels are reached within minutes, and plasma half life is 10-20 minutes. The drug, despite low lipid solubility enters the cerebrospinal fluid and the brain. Therefore, porous microsphere of 5-FU has been prepared to reduce unwanted side effects at other sites by locoregional and controlled delivery to stomach cancer [7,8,9].

## MATERIALS AND METHODS

### Materials

5-FU was purchased from CDH, New Delhi, Casein was purchased from LOBA chemicals. Pectin was purchased from Southern Citrus products Pvt.Ltd., GUDUR, AP (India). All other chemicals used were of analytical reagent grade.

### Analytical estimation of 5- FU

The estimation of 5 Fluoro Uracil was done by UV-visible spectrophotometric method. Aqueous solution of 5-FU was prepared in distilled water and the absorbance was measured at 266 nm spectrophotometrically from 2.0 to 20 µg concentration[,10].

**Preparation of 5-FU loaded porous micro spheres**

Porous microspheres were prepared by adding 15% w/v solution of Casein and pectin in 10 ml deionized water ($60^0$C) to soya oil (60ml) preheated to $60^0$C. The dispersion was stirred to obtain emulsion and temperature was lowered to $5^0$C by rapid cooling and 100 ml previously cooled acetone was added to obtain solid microspheres that were sieved and dried under vacuum and stored in well closed container. Non porous microspheres were prepared similarly from a solution mixture of casein and pectin previously treated at reduced pressure to completely remove air bubbles. Long lasting floating systems or non floating systems in stomach can be prepared alternatively to deliver the drugs, according to the therapy requirements[6].

**Determination of Mean Particle Size and its particle size distribution**

Particle size analysis of miscrosphere was determined by using optical microscopy method. Approximately 500 microspheres were counted for particle size using a calibrated compound microscope[11,12].

**Morphological study of microspheres**

The shape and surface morphology of the microspheres was investigated using scanning electron microscopy (Leo, VP-435, Cambridge) (UK). Photomicrographs were observed at 1600 x magnification operated with an acceleration voltage of 10.0 KV and working distance 9.7 nm was maintained[13].

**Determination of percent drug entrapment**

5 FU loaded porous microspheres (200 mg) were digested in 50 ml. of distilled water. The suspension was then warmed for few min, filtered with 0.2m membrane filter and an aliquot of the filtrate was diluted appropriately with respective solvent system. Absorbance was measured at 266 nm using UV-visible spectrometer[14,15].

**Floating properties**

Microspheres 0.3 gms were spread over the surface of a USP XXIV Paddle type dissolution apparatus filled with 900 ml simulated gastric fluid PH 2 containing 0.02 % v/v Tween 20. The mixture was stirred at 100 rpm for 8 hr. After 8 hr, the layer of buoyant micro particles was pipetted out and separated by filtration. Particles in sinking particulate layer were separated by filtration. Particles of both types were dried in a dessicator until constant weight both fraction of microspheres were weighed and buoyancy was determined by the weight ratio of floating and sinking particles[15,16].

% Buoyancy = {[wf/wf + ws] x 100 }

**Infra red spectroscopy**

Drug Polymer interactions were studied by Infra Red Spectroscopy. The Spectrum was recorded for 5 FU and 5 FU loaded optimized porous microspheres (F2) using Hitachi 270-50 infrared Spectro photometer. Samples were prepared in KBr disk (2 mg sample in 200 mg KBr) with a hydrostatic pressure at a force 40 psi for 4 min. The scanning range 500-4000 $Cm^{-1}$ and the resolution was 2 $cm^{-1}$ [17].

**Thermogravamatric analysis – differential thermal analysis**

The thermal behavior of 5 FU and its optimized microsphere (F2) was examined with a Diamond TG-DT Analyzer (PERKIN ELMER , USA). Argon was used as carrier gas and the TG – DTA Analysis was carried out at a heating rate $10^0$C/min and an argon flow rate of 35CC/min. The sample size was 5mg and curves were recorded at a temperature range 30-$310^0$C[17].

**In vitro release of 5-FU from porous microspheres**

In Vitro dissolution studies were performed using US Pharmacopoeia XXIII Dissolution apparatus II (paddle type). An accurate weighed sample (40 mg) of optimized porous microsphere was dropped into 900 ml of HCl Buffer pH 2.0 maintained at a temperature at $37^0$C ± $0.5^0$C and stirred at a speed of 50 rpm. At different time intervals, a 10ml aliquot of the sample was withdrawn and the volume was replaced with an equivalent amount of plain dissolution medium kept at $37^0$C. The collected samples were filtered and analyzed at $\lambda_{max}$266nm using a UV-Visible spectrophotometer against HCl Buffer PH 2.0 taken on blank[15,16].

**In vitro cytotoxicity analysis of 5-FU loaded porous microsphere on KATO-III human gastric cancer cell line**

The KATO III human gastric cancer cell line were purchased from National Centre for Cell line Pune and cultured in Jawaharlal Nehru Cancer Research Centre and Hospital, Bhopal. To examine the effects of 5 FU and 5 FU loaded Porous microspheres, the cells were treated with 0.001, 0.01, 0.1, 1 and 10 mg/ml of 5 FU and similar concentrations of optimized microspheres (F2).

**MTT assay**

MTT [(3 – (4,5 – dimethylthiazol-2-Y) 2,5 – diphenyl tetra sodium bromide)] is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazon product. This process requires active mitochondria and even freshly dead cells do not cleave significant amount of MTT. Thus the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. This assay was performed at Jawaharlal Nehru Cancer Research Centre and Hospital, Bhopal using the standard operating procedures. Briefly the compounds were dissolved in DMSO and serially diluted with complete medium to get the concentrations a range of test concentration. DMSO concentration was kept < 0.1% in all the samples. Cell lines maintained in appropriate condition were seeded in 96 well plates is treated with different concentration of the test samples and in incubated at $37^0$C, 5% $CO_2$ for 94 hours. MTT reagent was added to the wells and incubated for 4 hours, the dark blue formazon product formed by the cells as dissolved DMSO under a safety cabinet and read at 550nm. Percentage inhibitions were calculated and plotted with the concentrations used to calculate the $IC_{50}$ values(17).

**RESULTS AND DISCUSSION**

Porous microsphere of 5-FU were successfully prepared by emulsification extraction method. The method was optimized using different stirring speed and emulsifier concentration to produce microsphere of small size and narrow size distribution, high drug loading efficiency and controlled release at the gastric pH. The mean diameter of porous microsphere varied from 30.12±3.8µm with varying pectin concentration from 500, 750 and 1000 mg. the percentage drug entrapment was found to be 77.0±0.6% in all the microsphere formulations. The highest drug loading efficiency was found with 750mg pectin.

A higher concentration of polymer produced a more viscous dispersion, which formed larger droplets and consequently larger microsphere. In the study of effect of emulsifier concentration on formation of microsphere, the mean diameter of microsphere vary from 32.2±2.3µm to 80.4±6.8µm on varying emulsifier concentration (casein) from 500, 750, and 1000mg for porous microsphere. The drug loading efficiency varied from 58.0±3.8% to 76.5±2.2% with varying emulsifier concentration. The mean diameter of porous microsphere decreased from 82.2±0.6µm to 25.0±2.1µm with increasing agitation speed of mechanical stirrer from 500rpm to 1500rpm.

This result was expected because high stirring rates provide the shearing force needed to separate the aqueous phase into smaller globules. The stirring speed 1500rpm found to be optimum for porous microsphere, as the drug loading efficiency was 71.1±1.0% at this speed. High stirring speed produced irregular shape of microsphere but slightly increased entrapment efficiency was found (table No.1). By selecting above variables three formulations F1, F2 and F3 were optimized and best formulation selected with highest percentage buoyancy (table No2). The microsphere has highly porous surface with maximum size distribution in 30-40µm range (figure No.1 and photograph no. 1).

**Table 1: Effect of fabrication variables on the particle size and percentage drug entrapment**

| SR. NO. | VARIABLES | RATIO | MEAN PERCENTAGE DIAMETER (µM) | DRUG ENTRAPMENT |
|---|---|---|---|---|
| 1. | Drug: Polymer | 200:500 | 30.12±3.8 | 63.09±4.6 |
| (mg) | 200:750 | 32.06±1.6 | 77.06±1.2 | |
| | 200:1000 | 38.00±2.9 | 74.03±2.8 | |
| 2. | Emulsifier | 500 | 32.20±2.3 | 58.0±3.8 |
| Amount(mg) | 750 | 33.60±1.6 | 76.02±5.6 | |
| | 1000 | 80.40±6.8 | 68.0±5.5 | |
| 3. | Stirring | 500 | 82.06±1.6 | 62.00±1.3 |
| speed (rpm) | 1000 | 58.50±2.0 | 69.00±2.4 | |
| | 1500 | 31.12±0.3 | 71.10±1.0 | |
| | 2000 | 25.00±2.1 | 72.00±1.3 | |

**Table 2: Percentage buoyancy of porous microsphere**

| Serial no. | Porous microsphere ( drug:polymer:emulsifiere) | Percentage buoyancy |
|---|---|---|
| 1. F1 | 200:500:750 | 62.9 |
| 2. F2 | 200:750:750 | 74.9 |
| 3. F3 | 200:1000:750 | 62.1 |

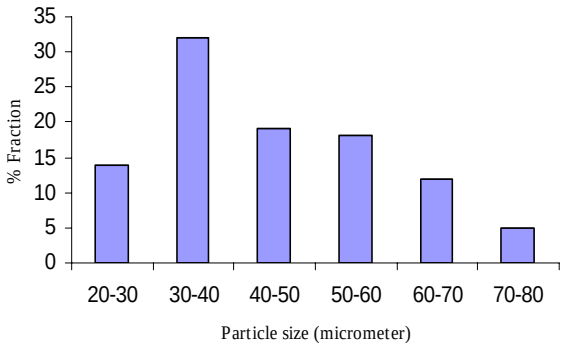



**Fig. 1: Particle size distribution of porous microsphere**

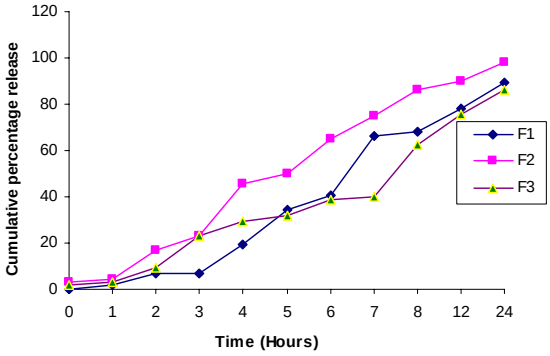



**Fig. 2: In vitro drug release study of formulation**

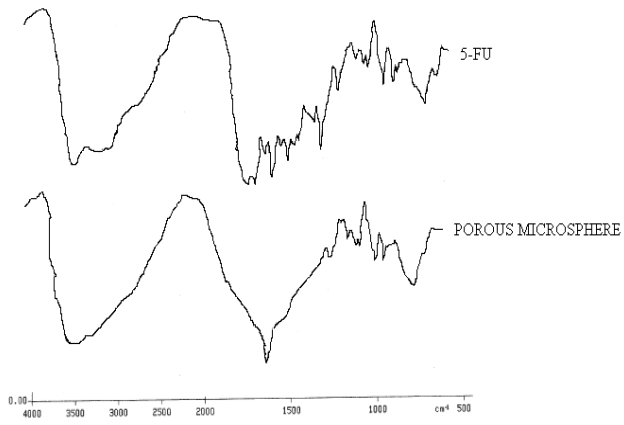



**Fig. 3: IR spectra of 5-FU and porous microsphere**

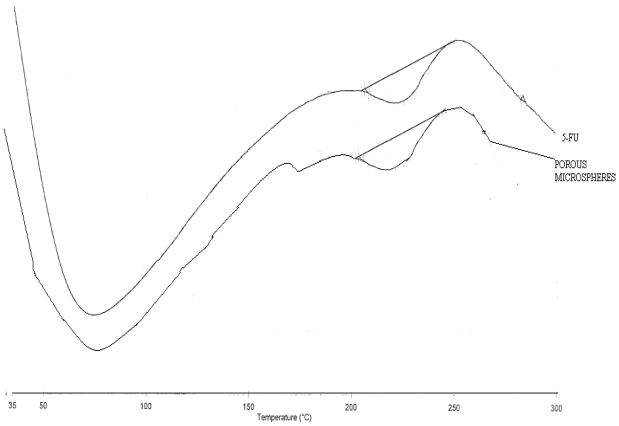



**Fig. 4: TG-DTA spectra of 5-FuU and porous microsphere**

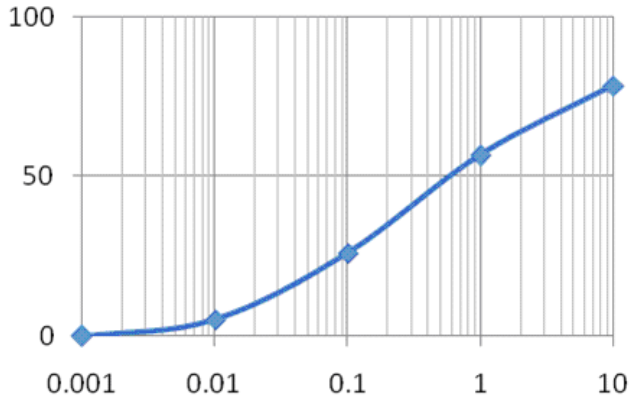



**Fig. 5: IC50 of porous microdphere**

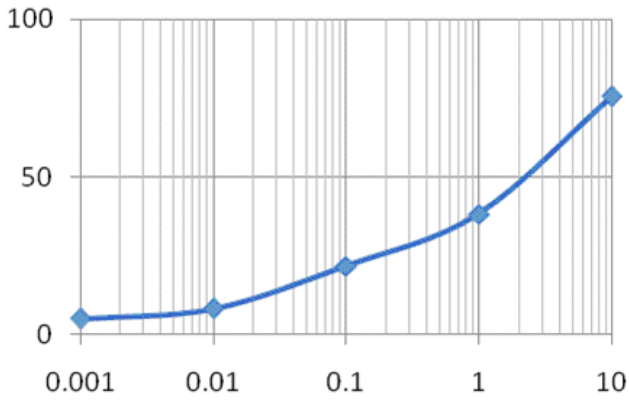
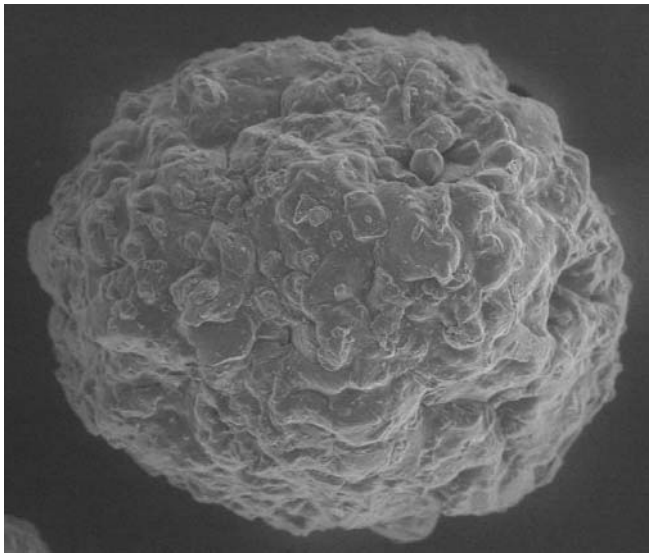



**Fig. 6: IC50 of 5-FU solutions**



**Photograph 1. Scanning electron microscopy of porous microsphere**

The porous microsphere (F1, F2 and F3) of pectin were subjected to in vitro drug release rate studies in simulated gastric fluid (pH 2.0) for 12 hrs in order to investigate the capability of formulation to withstand the physiological environment of the stomach. The amount of 5-FU released during the 24 hours studies was found to be 98.24% which attests the ability of optimized porous microsphere. The porous microsphere are having a bulk density less than 1, the air entrapped within the matrix imparts buoyancy to the microspheres. The inherent low density of dosage form helps in the buoyancy of dosage form. Data obtained by in vitro release study revealed that the release from all three formulation is constant(figure no.2).

As mentioned in the figure no.3 there was no significant difference in the infrared spectra of 5-FU and 5-FU loaded porous microspheres when both compared with each other.

Curves of TGA-DTA as shown in figure no 4, one can conclude that characteristic exothermic peak of 5-FU loaded porous microspheres curve, in which a new characteristic peak at 175.4ºC temperature appeared, which may be due to presence of polymer .

The cytotoxicity of 5-FU loaded porous microsphere and 5-FU solution was investigated using KATO III human gastric carcinoma cell line by MTT assay studying their effect on cell cytotoxicity (table). Cytotoxicity was determined by following the addition of 0.001, 0.01, 0.1, 1.0 and 10 µg equivalent of 5-FU and similar concentration of loaded microspheres. Loaded microsphere shows less cytoxicity than 5-FU solution (Figure 5 and 6).

## CONCLUSION

In the present work, porous microsphere of 5-FU were formulated by using emulsification extraction method with excellent buoyancy, % entrapment efficiency and cytotoxicity. Therefore, porous microsphere based on pectin-casein would be a great tool to provide sustained release of drug with a view to providing an effective and safe therapy for stomach cancer with a reduced dose and duration of therapy.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge the Dr. N. Ganesh, Jawaharlal Nehru Cancer Hospital and Research centre, Bhopal, India for in vitro cytotoxicity study and guidance during the work.

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