International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

PREPARATION OF 5-FLOROURACIL MICROSPHERES FOR COLONIC DRUG DELIVERY

Sangeeta Mohanty*[1], Amit kumar Panigrahi[2]

[1]Department of pharmaceutics. School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Khandagiri square, Bhubaneswar, OR, India
[2]CPC India Pvt. Ltd, Ahmedabad. India

***Corresponding author e-mail:** shovankrishna1@rediffmail.com

ABSTRACT

5-Fluorouracil is widely used anticancer drug,which are effective during the S-phase of cellcycle. Since It has poor bioavailability so targeting of 5-Fluorouracil at site of action is of great beneficial. Attempt has been made to develop a stable microparticulate formulation of 5-Fluorouracil to be administered orally to target colon. Here, chitosan was chosen as polymer. Chitosan microspheres were coated with eudragit s-100.The Cross linked chitosan microspheres containing 5-Fluorouracil were prepared by emulsification method using glutaraldehyde as crosslinking agent and characterized for %Yield, ParticleSize, Surface properties and Morphology, Entrapment Efficiency and DSC. InVitro release studies of coated and uncoated chitosan microspheres was performed in pH progression medium at 37 ± 0.5 ^{0}C ,in simulated gastric fluid, simulated intestinal fluid. As compared to chitosan microspheres, coated microspheres(1: 5) showed about 7.88% drug release after 6 hours and rest of drug releases upto 24 hours. When the core:coat ratio is 1: 10 , release does not occur. Hence, we can conclude that eudragit coated chitosan microspheres prevents drug release in stomach, small intestine, targets colon only; thus avoiding systemic side-effects associated with 5-Fluorouracil.

KEYWORDS: Colon specific drug delivery system,5- Florouracil, Differential scanning calorimetry.

INTRODUCTION

Since last decades,Colon specific drug delivery has gained increased importance since it is a potential site for the systemic delivery of drugs.To achievesuccessful colon targeted drug delivery, a drug needs to be protected from degradation, release and/or absorption in the upper portion of the GI tract and then ensure controlled release in the proximal colon.The aim of present work was to study the feasibility and usefulness of eudragit coated cross linked chitosan micro spheres for encapsulation of 5 Fluorouracil to be administered orally for colonic delivery.5- Fluorouracil has been the only chemotherapeutic agent with clinical activity against Colon cancer. [1]. But as the Intravenous administration of this drug, causes severe gastrointestinal, hematological,cardiac and dermatological toxic effects.Hence, Oral site-specific rate-controlled 5-FU is considered. Due to potential benefits like increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying.[2]Multiparticulate approaches tried for colonic delivery include formulations in form of micro particles.

5- Fluorouracil (5 FU) is an anticancer drug, which is used alone or in combination in treatment of colon cancer. Fluorouracil is a pyrimidine analogue, which acts as an antimetabolite by interfering in DNA synthesis.[3] 5- FU has significant dose limiting toxicities, such as bone marrow suppression, stomatitis, nausea, and vomiting.To improve the efficacy of drug , Localized administration of chemotherapeutic agents has been recognized which is achieved by drug targeting or site specific drug delivery.

MATERIALS AND METHODS

5- Fluorouracil was received as a gift sample fromZydusCadila, Ahmedabad, Gujarat, India. Chitosan- 652 from Central institute of Fisheries technology, Cochin,India. Hydrochloric acid, Disodium Hydrogen phosphate,Potassium dihyrogen phosphate,Methanol(AR Grade), and Acetone(AR Grade) were procured from S.D. Chemicals, Mumbai,India. Pepsin 1: 10,000 and Pancreatin1:10.000 were procured from LobaChemie, Mumbai,India.

PREPARATION OF MICROSPHERES:

1. ***Preparation of microspheres by spray drying method:***

Selection of spray drying parameters

A. Inlet temperature.[4]

As,the distillation range of water is 100 ^{0} C,so to ensure complete removal of water from the product after spray drying. , 130°C, was taken as an inlet temperature.

B. Outlet temperature

At the inlet temperature of 130 °C, the outlet temperature of spray drying was kept at 50 °C.

C. Atomization Pressure was kept at 4 bars.[5]

*D. Aspiration volume***:**1500

E. Feed rate **:**5rpm

The solution prepared above was spray dried on LU-227 ADVANCE SPRAY DRYER (Labultima).

It was found that with the increase in concentration of chitosan,yield increases.With the increase in feed rate from 5 rpm to 10 rpm,there was increase in particle size.Since the yield and entrapment efficiency of microspheres obtained in spray drying method was very low, emulsification method for preparation of microspheres was employed.

2.*Preparation of Cross linked Chitosan microspheres by emulsion method*:By emulsion method, Chitosan microspheres were prepared using glutaraldehyde as cross linking agent.[6]Chitosan Solution (2.5%) was prepared in 5% aqueous acetic acid solution.5 -Fluorouracil was dispersed in this solution and mixed well. And again it is dispersed in liquid paraffin (1:1) containing span 80 (2%w/w). The dispersion was stirred using high speed stirrer at 2000 rpm for 4 hrs at room temperature. After 10 minutes of stirring, Glutaraldehyde 0.5 ml was added. Another 0.5 ml of glutaraldehyde was added after 1 hour of stirring. After some time, micro spheres were centrifuged, washed several times with n- Hexane to remove the liquid paraffin.The microspheres were then suspended in 5% w/v sodium bisulphite solution and stirred on magnetic stirrer for 10 minutes to remove residual glutaraldehyde. In vacuum desiccators, microspheres were dried for 48 hours.

2.1.*COATING OF CROSS LINKED CHITOSAN MICROSPHERES*

Coating of chitosan microspheres containing 5-Fluorouracil was performed using emulsion solvent evaporation technique.Chitosan microspheres were suspended in 10 ml of organic solvent (acetone: ethanol (2:1) in which Eudragit S-100 was dissolved to give 1: 5, 1: 10 core: coat ratio.) This organic phase was emulsified in 100 ml of liquid paraffin containing span 80.The system was stirred at 1000 rpm for 4 hrs at room temperature. Eudragit coated microspheres were collected and rinsed with n-Hexane and dried in vacuum desiccators.[7]

2.2.*OPTIMIZATION OF FORMULATION PARAMETERS*:The various process and formulation parameters studied for optimization of the formulation were:

a)Effect of drug to polymer ratio :Varying concentration of drug and polymer were taken so as to achieve optimal entrapment efficiency.

Microspheres prepared at Stirring Speed 2000 rpm.In this set of experiments,entrapment of the drug was found to be good with batch no.FD4. Thus the optimal entrapment was achieved with drug to polymer ratio 1: 5.Comparing the batches FD4 and FD5 by applying t test. For the batches FD4 and FD5 t cal=1.66 and t tab= 2.35 at $t_{0.95}$.Here t cal< t tab, so there is no significant difference between both the batches.Decrease in drug release with an increase in concentration of chitosan was observed.It is because gel like structure are formed in release studies with an increase in concentration of chitosan,hence prevents the dissolution of 5 Fluorouracil and its subsequent release.

*b) Effect of Emulsifier Concentration***:** Amongst the various available emulsifiers,span 80 (because of its HLB value) was extensively used as emulsifiers.Various concentration of Span 80 were used to prepare batches for particle size and size distribution as below:

An increase in concentration of Span 80 led to decrease in particle size.It is due to the fact that higher stirring speed provides the required energy to chitosan solution, to be dispersed as fine droplets in the external oily phase and therefore microspheres with small particle size were formed.2% w/v concentration (1ml) of Span 80 was considered to be optimum concentration as size distribution was narrow.

c*) Effect of speed on Particle size***:** **(n=3)**

Decrease in the particle size of microspheres with an increase in concentration of Span 80 occurs**,**It isbecause of decrease in interfacial tension between aqueous droplets and organic suspension medium.Due to narrow size distribution of emulsion particles,2% v/v solution of span 80 was found to be suitable.

d) Effect of Cross linking agent:

The glutaraldehyde concentration employed for cross linking had no significant effect on particle size of microspheres.The decrease in drug release with an increase in concentration of glutaraldehyde is because of swelling ability of microspheres which in turn lead to slower release rate.When 1.5 ml solution of glutaraldehyde was used, the microspheres formed were of black colour hence, 1 ml concentration of glutaraldehyde(25%w/w) was considered to be the optimal concentration.

The Final optimize process condition for emulsification method using glutaraldehyde as cross linking agent are as follows:

CHARACTERIZATION OF MICROSPHERES:

The Cross linked chitosan microspheres containing 5 -Fluorouracil were prepared by emulsification method using glutaraldehyde as cross linking agent and characterized for:

- % Yield
- Particle Size
- Surface properties and Morphology
- Entrapment Efficiency
- Differential Scanning Calorimetry
- In Vitro drug release profile

a)Yield of microspheres: Microspheres collected at the end of preparation were weighed and the yield was calculated.The yield of microspheres was calculated by formula

% Yield= Practical Yield/ Theoretical Yield x 100

Where,

Theoretical Yield= Amount of Polymer taken+ amount of excipients added+ amount of drug taken.

Practical Yield= Amount of microspheres actually produced by experimental method

b)Particle size analysis:By a laser light scattering technique using Mastersizer (Malvern Instruments, London, UK) operating at a beam length of 2.40 mm and range of lens at 300 mm. The mean particle size of prepared microspheres was determined

***c)Surface morphology of microspheres*:** The formulation prepared by emulsification method was studied for shape,surface properties and surface morphology by optical microscopy (Olympus microscope BX-40, Japan) and Scanning electron microscopy (JEOL JSM-5610 LV, Japan)The optical microscopy was performed by taking small amount of microspheres dispersions in water on the glass slide and photographs were taken under 40 x resolution.The SEM studies of chitosan and Eudragit S100 coated microparticles were carried out by gently sprinkling the powder previously kept in desiccator on the double adhesive tape which was fixed on the dies followed by application of vacuum and high voltage for taking the images under high and low resolution.

d) Entrapment Efficiency:

Analysis of entrapped drug : Drug loaded Chitosan microspheres (25 mg)were dispersed in methanol (25ml) and kept for digestion with continuous stirring upto 24 hours, then sample was centrifuged at 1000 rpm for 10 minutes to remove any insoluble solids , the supernatant layer was removed and filtered. The drug content was determined using UV- Visible spectrophotometric method.Entrapment efficiency was measured as follows;

$$\text{Entrapment Efficiency} = \frac{\text{Entrapped Drug}}{\text{Total Drug}} \times 100$$

***e)Invitro drug release studies*:**

In Vitro drug release study from core chitosan microspheres: Uncoated Chitosan microspheres were evaluated for in vitro drug release in pH Progression medium at 37 ^{0}C±0.5 ^{0}C. 100 mg of microspheres were weighed accurately and were placed in 100 ml dissolution medium. The content was rotated at 100 rpm. The simulation of GI Transit condition was achieved by altering pH of dissolution medium at different time interval.The pH of dissolution medium was kept 1.2 for 2 hour using Simulated gastric fluid .Then Simulated intestinal fluid (KH_2PO_4 and $Na_2HPO_4.2H_20$)were added to dissolution medium,adjusting the pH to 5.0 with 1N NaOH and release rate study was carried for further 2 hours.Then it was transferred to PBS 7.4 and maintained upto 24 hours.The samples were withdrawn from the dissolution medium at various time intervals. The rate of 5 FU release was analyzed using UVspectrophotometric method at λ max.

***In Vitro drug release study from coated Chitosan Microspheres*:**

In Vitro drug release study of coated chitosan microspheres was performed similar to that of core microspheres.It was performed in pH progression

medium at 37^0 C ± 0.5 ^{0} C. All dissolution studies were performed in triplicate.

f) Differential Scanning Calorimetry:The microspheres containing the drug and polymer as well as individual ingredients were characterized by DSC (Shimadzu ,Japan) in the range of 25-350 ^{0} C at a heating rate of 10 ^{0} C per minute with a average sample weight of 4 mg. The glass transition temperature of polymer as well as the presence of any interaction between the drug and excipients was characterized.

RESULTS AND DISCUSSION

a) % Yield of microspheres

The results of % yield of microspheres prepared are tabulated as below

It was observed from the experimental work that yield was found ranging from 50 % to 65 % .

b) Particle Size:

The mean particle size of optimized Chitosan microparticle was determined by a laser light scattering technique using Mastersizer and was found to be 105.621µm .

The mean particle size of optimized eudragit coated chitosan microspheres was determined by a laser light scattering technique using Mastersizer and was found to be 388.152 µm.

c)Surface Properties and Morphology:The shape and surface characteristics of microspheres were observed by Scanning electron microscopy and Olympus microscope.The samples were imaged using a 15-KV electron beam.It was found that both chitosan microspheres and eudragit coated chitosan microspheres were spherical in shape.

d) Entrapment Efficiency

The entrapment efficiency of optimized chitosan microspheres was found to be 65.7±1.11 %.

e) In Vitro drug release studies of Chitosan microspheres **:**In Vitro release study of cross linked chitosan microspheres were carried in 100 ml dissolution medium which was stirred at 100rpm at 37 ±0.5 ^{0} C. The scheme of using simulated fluids at different pH was as follows:[8]

1st and 2nd hour – Simulated gastric fluid of pH 1.2

3rd, 4th, 5th and 6th hours- Simulated Intestinal fluid

7th hours onwards- PBS 7.4

Release Studies of Coated cross-linked Chitosan microspheres **:**Optimized formulation was coated with Eudragit S100 using oil in oil solvent evaporation method.E1(1: 3) E2 (1:5) E3 (1:10).The release from microspheres was affected by the core : coat ratio.

In Vitro release **:**In Vitro release studies of coated and uncoated chitosan microspheres was performed in pH progression medium at 37 ± 0.5 ^{0} C in simulated gastric fluid, simulated intestinal fluid. As compared to chitosan microspheres, coated microspheres(1: 5) showed about 7.88% drug release after 6 hours and rest of drug releases upto 24 hours.When the core : coat ratio is 1: 10 , release does not occur, So we can conclude that eudragit coated chitosan microspheres prevent the drug release in stomach and small intestine and when the formulation reaches colon , drug starts releasing, provides local action. Thus avoiding systemic side effects associated with 5 Fluorouracil.As compared to plain 5 Fluorouracil,chitosan microspheres give better drug release. Optimized coated crosslinked chitosan microspheres formulation was further evaluated for drug release kinetics.In this study, result obtained for core microspheres was as follows:

Higuchi(R^2)	**KorsemeyerPeppas(R^2)**	**Hixon Crowell(R^2)**
0.9817	0.9754	0.9702

This shows release kinetics follows Higuchi model.

f) Thermal Studies[9]**:**Thermograms of samples were obtained by differential scanning calorimeter (shimadzu, DSC, Japan). Samples were placed in aluminium pans and heremetically sealed with aluminium lids. Over a temperature range of 50 to 350 ^{0} C,the thermograms of samples were obtained at a scanning range of 10 ^{0} C/ min. All tests were performed twice.In this investigation, DSC thermogram of Pure drug, polymer (chitosan) , chitosan microspheres and eudragit S-100 coated chitosan microspheres were performed.

Sharp endotherm of 5 FU at 288.88 ^{0} C was found , exothermic peak of chitosan at 303.96 ^{0} C was found. In the DSC analysis of chitosan microspheres,the endothermic peak of drug is not as sharp as that of pure drug and in the Differential scanning calorimetry of eudragit coated chitosan microspheres the peak of drug was not there, showing the entrapment of drug.

Stability Study: To study the effect of storage conditions on the formulation properties, Stability studies of the microsphere formulation were carried out.During stability studies, the formulations were placed in hard gelatin capsules and sealed in aluminium packing,(coated inside with polyethylene). As per ICH guidelines,these studies

were performed at 2-8 ^{0} Cand at ambient temperature.The stability studies were carried out for 3 months. The various parameters evaluated to check the stability of the formulation were:

a. Percentage drug retained

b. In Vitro release profile of the formulation

a. Drug retained

The formulations under stability study was analyzed for Drug Content after 1,2 and 3 months. AboveTable shows that there was no significant reduction in percentage drug retained and also there was no significant difference in drug release profile for sample store at 2-8 ^{0} c and at ambient temperature.

CONCLUSION:

Eudragit coated crosslinked chitosan microspheres successfully protect the drug from being released under conditions mimicking mouth to colon transit, these microspheres are expected to decrease parenterally administered side effects of 5 Fluorouracil. However, actual performance evaluation studies first in animals followed by clinical trials in cancer patients are required to substantiate this claim and establish clearly the utilities of this formulation for oral therapy of 5-Fluorouracil.

Table 1. Product Optimization

Drug:Polymer (wt/wt)	Entrapment Efficiency (%)	Mean ParticleSize µm
1:3	40.16 ± 1.25	29.56 ± 1.07
1:4	42.90 ± 1.26	36.02 ± 1.54
1:5	44.63 ± 1.57	36.46 ± 1.82

Table 2. Optimization of Aspiration Volume n=3

Drug: Polymer (wt/ wt)	Outlet temp ^{0} C	Inlet temp ^{0} C	Atomization Pressure (Bar)	Aspiration Volume	Yield(%)
1:3	50	130	4	1000	7.25
1:4	50	130	4	1000	10.80
1:5	50	130	4	1000	18.50
1:3	50	130	4	1500	21.25
1:4	50	130	4	1500	23.09
1:5	50	130	4	1500	25.00

Table 3. Optimization of feed rate n=3

Drug: Polymer (wt/ wt)	Outlet temp ^{0} C	Inlet temp ^{0} C	Atomization Pressure (Bar)	Aspiration Volume	Flow rate (rpm)	Particle Size (µm)	Yield (%)
1:3	50	130	4	1500	5	29.48 ± 1.03	21.35
1:4	50	130	4	1500	5	35.04 ± 1.47	24.08

1:5	50	130	4	1500	5	36.67 ± 1.80	25.52
1:3	50	130	4	1500	10	32.07± 1.08	21.09
1:4	50	130	4	1500	10	40.88 ± 0.98	20.92
1:5	50	130	4	1500	10	42.48 ± 0.68	22.83

Table 4. Optimization of drug to polymer ratio for optimal entrapment efficiency

n = 3

Batch no.	**Drug: Polymer (w/w)**	**Mean Partcle Size (µm)**	**Entrapment Efficiency(%)**	**% release after 10 hours**
FD1	1:2	65.32 ± 0.98	36.16 ± 1.18	90.57±1.97
FD2	1:3	73.25 ±0.81	44.27 ±0.96	86.67±1.57
FD3	1:4	93.32 ±1.42	57.40 ±1.34	84.63 ±1.72
FD4	1:5	105.621 ±0.98	65.70 ±1.11	78.88 ±1.53
FD5	1:6	104.34 ±0.88	66.08 ±1.08	74.08 ±1.22

Table 5. Optimization of Emulsifier Concentration: (n=3)

Drug:Polymer (w/w)	**Emulsifier Conc. (ml) Span 80**	**Mean Particle Size (µm)**	**Entrapment Efficiency(%)**
1:2	0.75	71.11 ± 1.92	36.16 ± 1.18
1:2	1	65.32 ± 0.98	36.07 ±1.17
1:2	1.25	61.21 ±1.27	34.20 ±1.08
1:3	0.75	80.11± 1.80	46.40 ±0.82
1:3	1	73.25 ±0.81	44.27 ±0.96
1:3	1.25	72.12 ±1.13	43.28 ±0.96
1:4	0.75	98.89 ± 1.21	59.72 ±1.13
1:4	1	93.32 ±1.42	57.40 ±1.34
1:4	1.25	90.41 ± 1.83	55.44 ±1.34
1:5	0.75	110.58 ±1.42	66.14 ±0.98
1:5	**1**	**105.621 ±0.98**	**65.70 ±1.11**
1:5	1.25	101.11 ±1.88	62.77 ±0.12

Table 6. Optimization of speed of high speed stirrer

Speed of stirrer (rpm)	Mean Particle Size (µm)
500	388.24 ± 1.23
1000	348.13 ±1.36
1500	209.69 ±1.55
2000	105.621 ±0.98
2500	83.52±1.20

Table 7. Optimization of glutaraldehyde 25% w/w n=3

Drug: Polymer ratio	**Volume ofGlutaraldehyde (25%w/w)**	**% release after 10 hours**	**Particle Size(µm)**
1:5	0.5 ml	84.65	105.56 ±0.98
1:5	1ml	78.75	103.42 ±0.48
1:5	1.5 ml	75.07	102.06 ±0.50

Table8. Particle Size and Entrapment efficiency of coated cross linked chitosan microspheres

Core: Coat ratio (Eudragit S100)	**Mean Particle size(µm)**	**Entrapment Efficiency(%)**
1:3	230.77±1.03	60.08 ±1.95
1:5	388.152 ±2.07	64.78 ±1.45
1:10	555.96±2.17	62.07 ±1.69

Table 9. Final optimize process conditions

Stirring speed	2000 rpm
Emulsifier Concentration	2% (v/v)
Drug to Polymer ratio	1:5
Conc. Of Cross linking agent	1ml

Table:10. % yield of microspheres

Formulation Code	**% Yield**
FD1	50.22
FD2	51.90
FD3	65.63
FD4	57.36
FD5	60.01

Table 11.In Vitro drug release studies of Chitosan microspheres

Time	Medium	FD1	FD2	FD3	FD4	FD5	FD6
1		28.73 ±1.11	25.89±1.10	24.79±0.89	20.52±0.98	21.09±1.09	19.57 ±1.20
2	SGF	36.24±1.07	31.21±1.22	30.66±0.96	27.55±1.07	25.06±1.21	24.35 ±1.31
3		45.18±1.16	40.89±1.08	37.57±0.58	38.69±0.97	35.50±1.41	32.02 ±1.20
4	SIF	54.24±1.22	46.91±1.07	46.59±1.06	47.57±1.02	42.79±1.33	39.12 ±1.41
5		65.49±1.31	59.08±1.76	52.86±1.01	52.17±1.12	48.98±1.23	44.08 ±0.98
6		74.36±1.07	67.03±1.12	61.91±1.02	60.66±1.08	57.49±1.11	52.32 ±0.81
7		80.32±1.11	75.67±1.11	72.58±1.11	70.78±0.98	65.43±1.08	62.35±1.06
8	PBS 7.4	87.52±1.53	84.02±0.98	78.67±0.89	76.59±0.91	70.08±1.42	67.89 ±0.96
10		94.38±1.20	90.58±0.78	87.66±0.85	84.67±1.22	78.21±1.21	77.86 ±1.02

Table 12. Release Studies of Coated cross-linked Chitosan microspheres

Time	E1(1:3)	E2(1:5)	E3(1:10)
0.5	---		
1	---		
2	1.01 ±0.08		
3	1.91 ±1.02	0.73 ±0.67	
4	5.31 ±1.04	3.56 ±0.57	0.57 ±0.87
6	15.22 ±1.11	7.88 ± 0.84	3.11±0.26
8	38.14 ±1.90	28.98 ±1.44	20.88 ±0.96
10	58.10 ±1.04	42.15±1.11	35.09 ±1.09
24	98.12 ±1.03	95.37 ±1.09	83.78 ±1.12

Table13. Percentage drug content before and after 3 months storage

Time(Month)	**Drug Content (% Assay)**	
	2-8 ^{0} C	**Ambient Temp.**
0	99.93%	99.93%
1	98.99%	98.96%
2	98.88%	98.77%
3	98.65%	98.43%

Table 14. Percentage 5 Fluorouracil released after storing at different temperatures for a period of 3 months.

Time	% Drug released	
	2-8 ^{0} C	Ambient Temp.
0	95.43%	95.43%
1	95.24%	95.22%
2	95.19%	95.15%
3	95.14%	95.10%

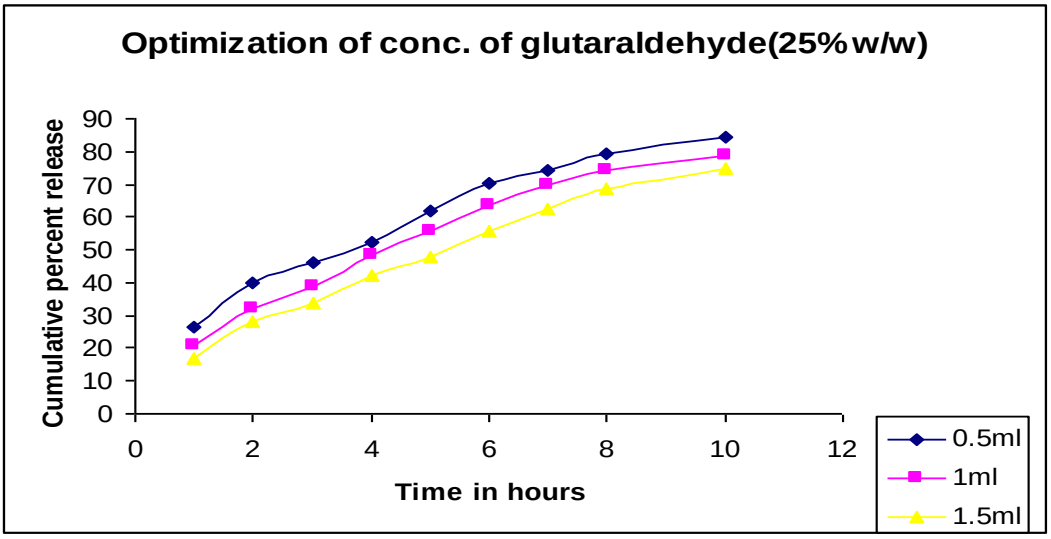

Figure no 1.Optimization of Glutaraldehyde(25%w/w).

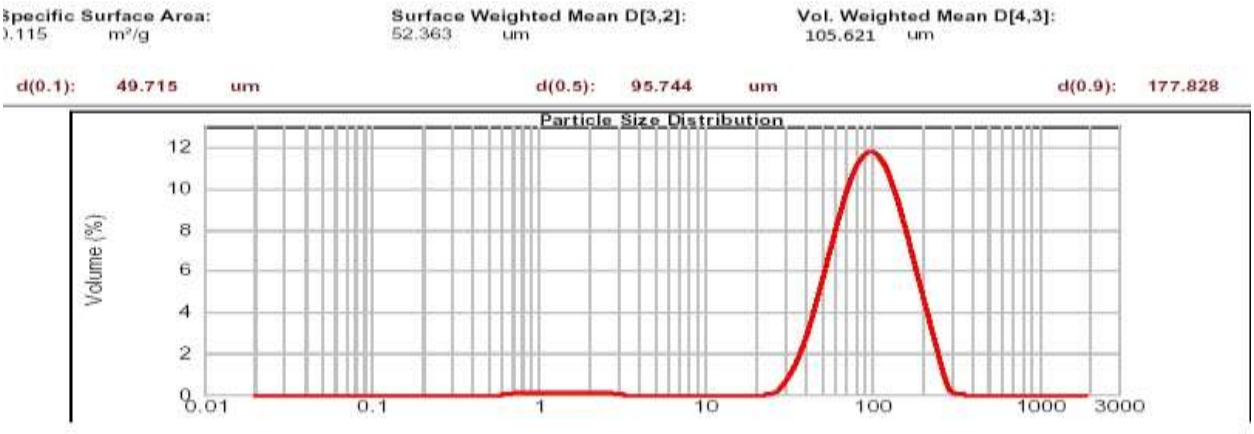

Figure no 2. Mean Particle size of optimized chitosan microspheres.

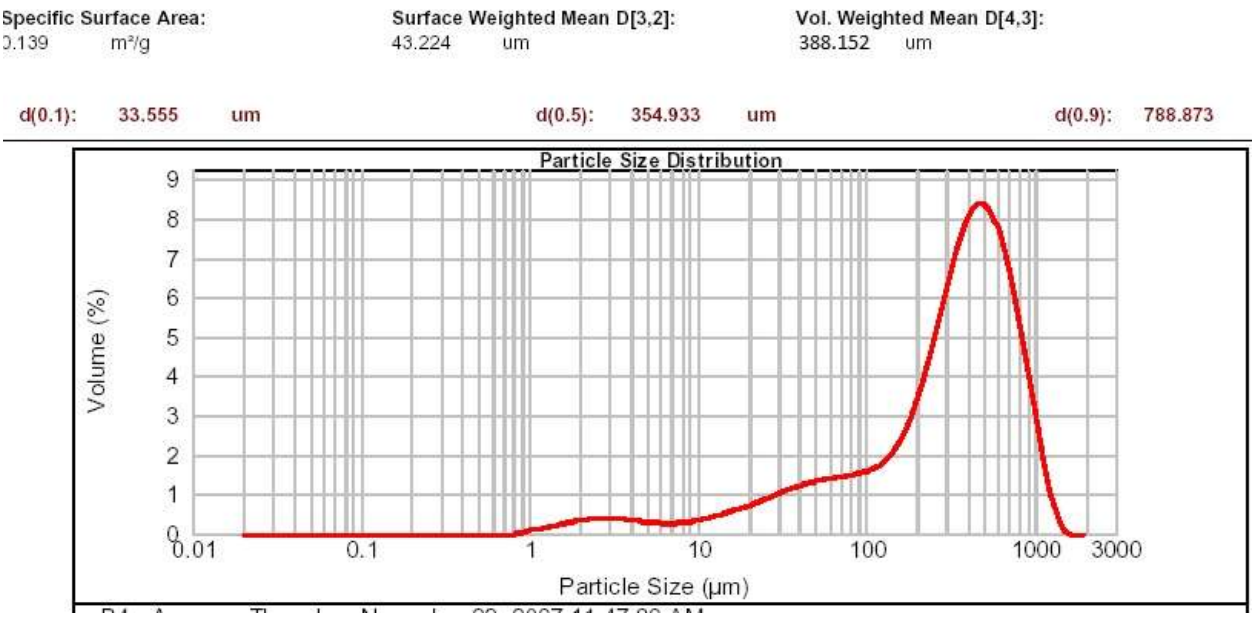

Figure no 3 . Mean particle size of optimized eudragit coated chitosan microspheres.

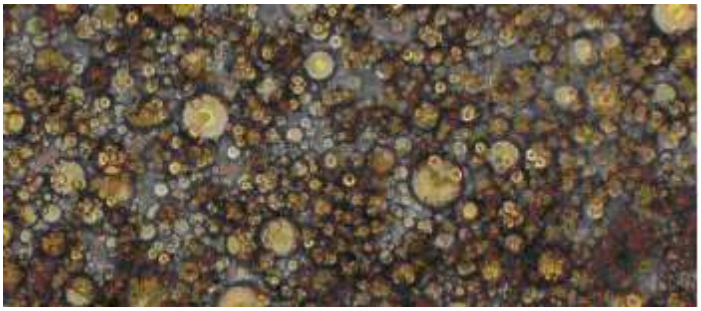

Figure no 4**. Optical microscopy study of chitosan microspheres by Olympus microscope**

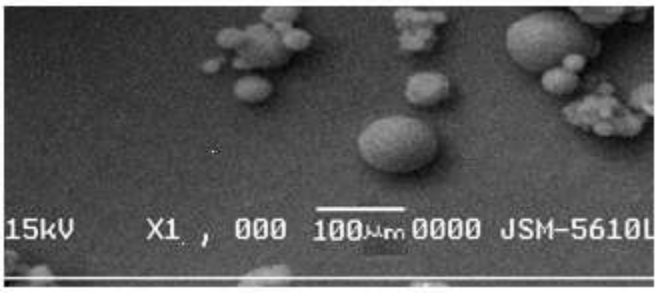

Figure no 5**. Morphological studies of chitosan microspheres by scanning electron microscopy**

Figure no 6**. Morphological Studies of Eudragit coated chitosan microspheres by scanning electron microscopy.**

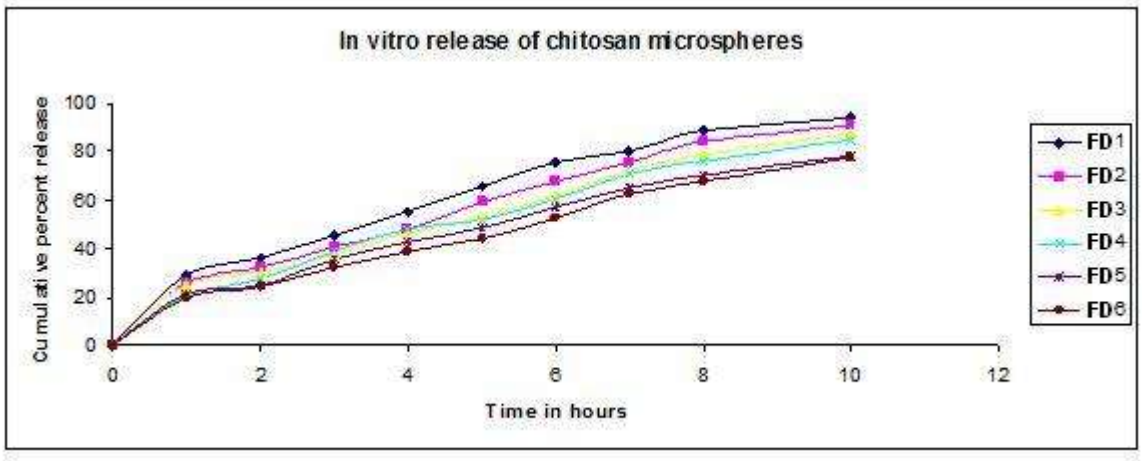

Figure no 7**. In Vitro release studies of Chitosan microspheres**

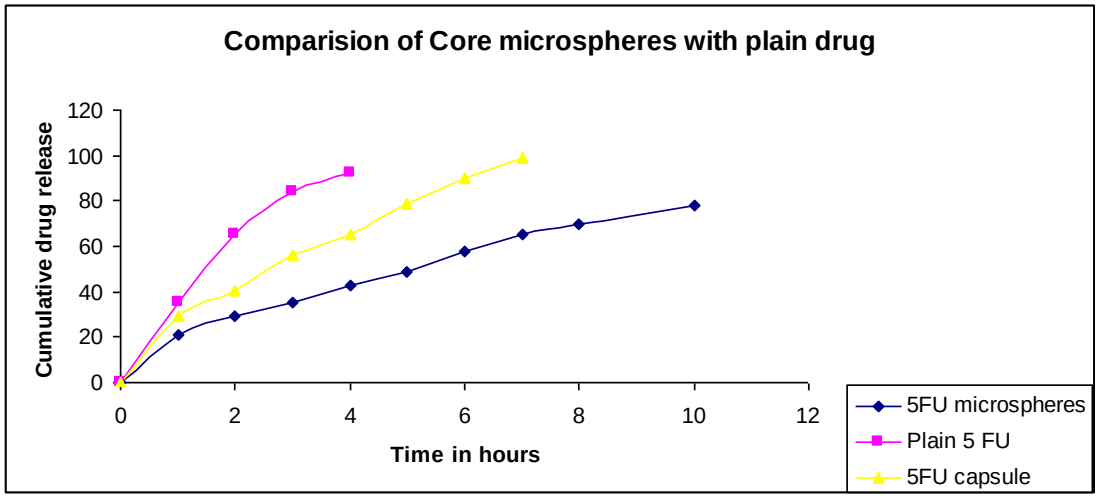

Figure no 8**. Comparison of the formulation**

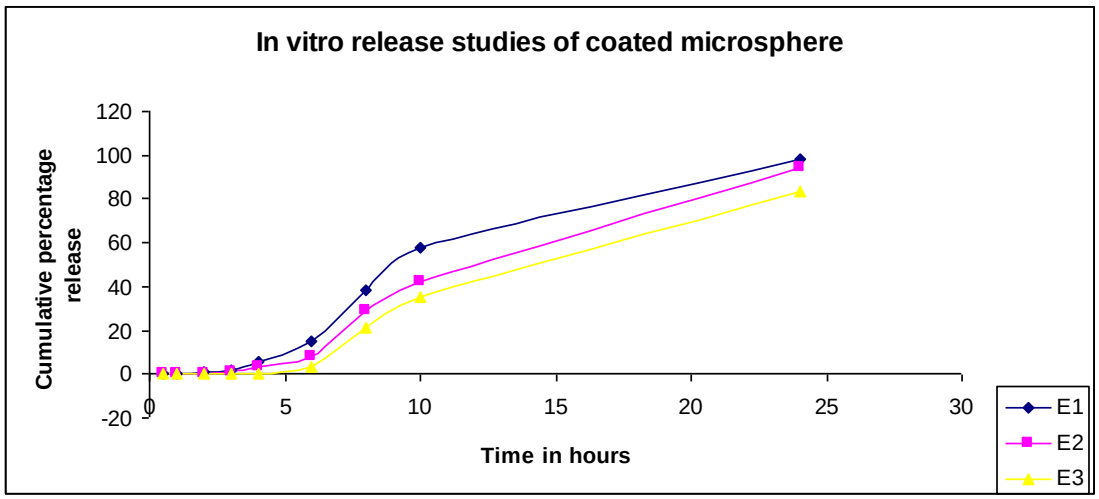

Figure no 9**. In Vitro release of coated Chitosan microspheres**

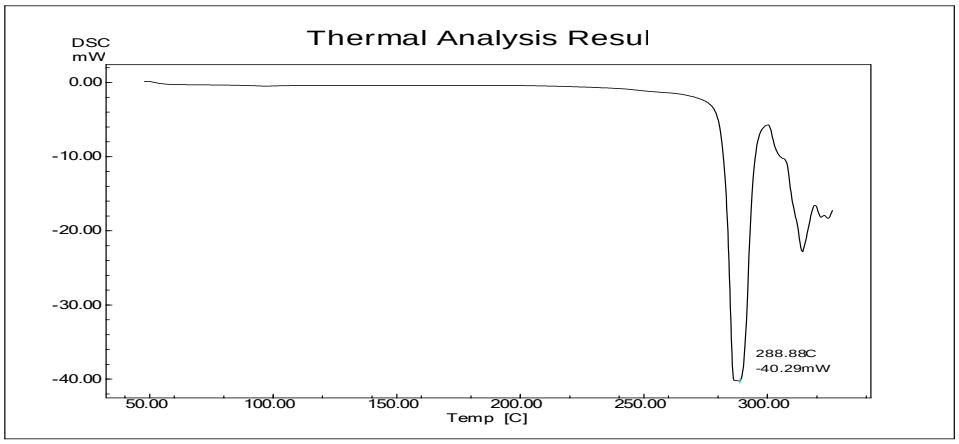

Figure no 10**. DSCThermogramof 5-Fluorouracil**

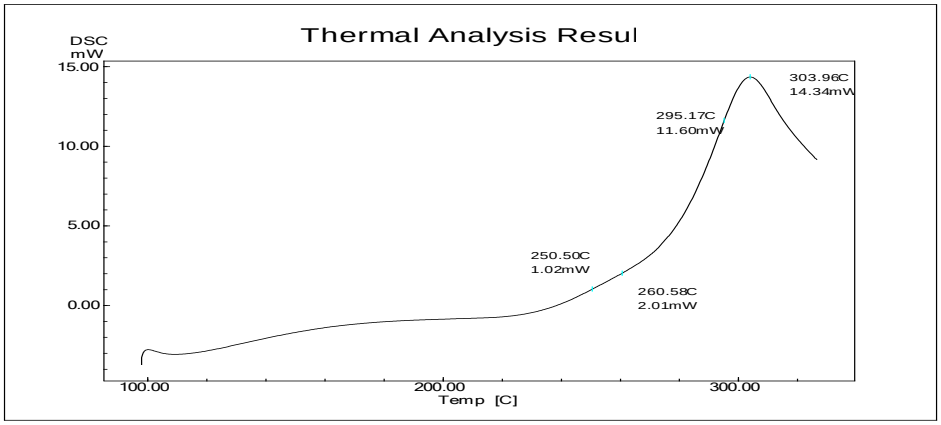

Figure no 11**. DSC Thermogram of Chitosan-652**

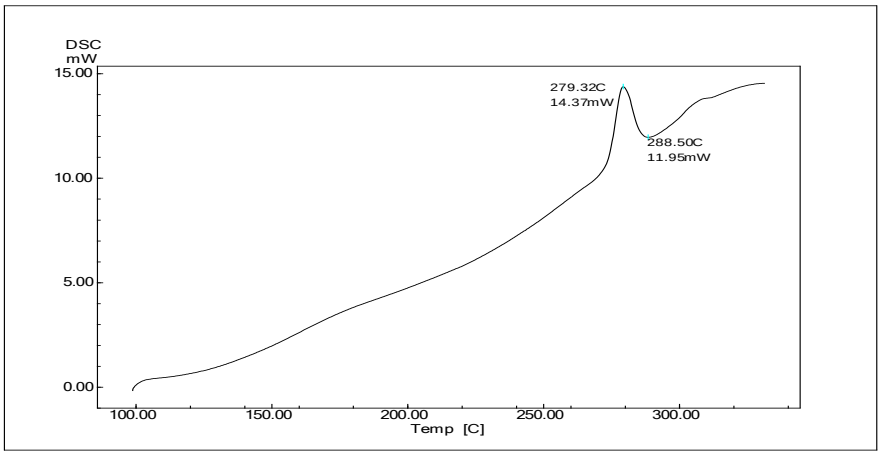

Figure no 12**. DSC Thermogram of Cross linked Chitosan microspheres**

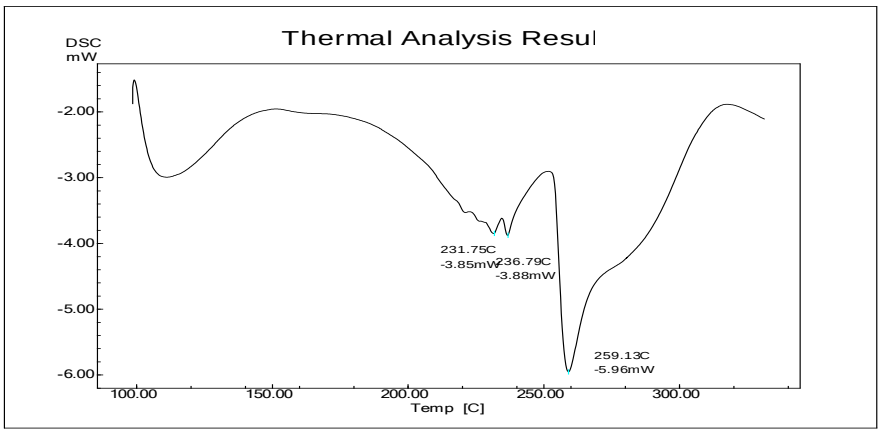

Figure no 13**. DSC Thermogram of eudragit coated cross linked chitosan Microspheres**

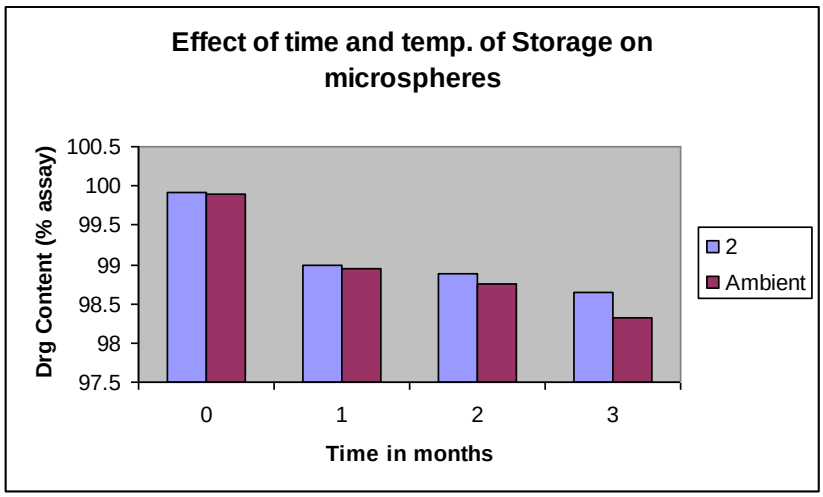

Figure no 14**. Percentage drug content before and after 3 months storage**

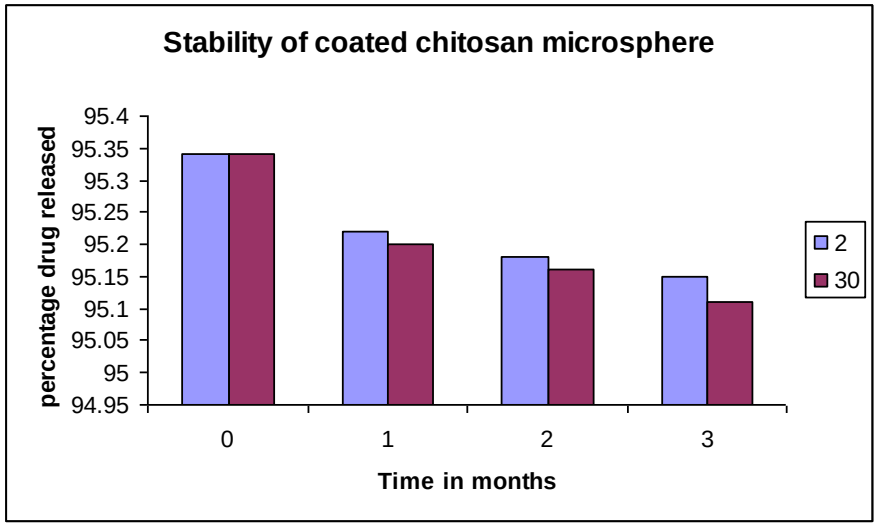

Figure no 15**. Percentage 5-Fluorouracil released after storing at different temperatures at a period of 3 months**

REFERENCES

1. P. Calabresi, B.A. Chabner, Chemotherapy of neoplastic diseases, in: J.G. Hardman, L.E. Limbird, B.M. Perry, W.R. Raymond (Eds.), Goodman *and Gilman's the Pharmacological Basis of Therapeutics*; 9th edition, McGraw-Hill, New Delhi**,** 1996; 1225– 1232.
2. Ibekwe, V.C, Kendall, R.A, Basit, A.W, Drug Delivery to colon, The Drug Delivery Companies, Report, *spring/ Summer*, *Pharmaventures Ltd***;** 2004.
3. Reynolds J.E.F (Ed) "Martindale- The Extra pharmacopoeia" *thirtieth Edition the pharmaceutical press London*; pg 480-481.
4. Ping He, Stanley S. Davis, Lisbeth Illum" Chitosan microspheres prepared by spray drying"*Int.J. Pharmaceutics*;1999;187: 53-65.
5. M.L Lorenzo –Lamosa, C.remunan- Lopez, J.L Vila –Jato. M.J. Alonso."Design of microencapsulated chitosan microspheres for colonic drug delivery"*Jof Controlled release* 1998; 52: 109-118.
6. Thanoo, B.C, Sunny, M.C and Jayakrishnan(A" cross linked chitosan microspheres preparation and evaluation as a matrix for controlled release of pharmaceutical " *J. of Pharm. Pharmacol*,1992; 44: 283-286.
7. Chourasia, M.K and Jain, S.K," Pharmaceutical approaches to colon targeted drug delivery systems"*J. pharmaceutical Sci* 2003; 6 (1) : 33-66.
8. M.K. Chaurasia, S.K. Jain; 2004; Design and development of multiparticulate system for targeted drug delivery to Colon *Drug delivery* 11:3; 201-207.
9. Dubernet C; 1995; Thermo analysis of microspheres. *ThermochiActa;* 248:259-269.