ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2011; 2(3):926-931

ORIGINAL RESEARCH ARTICLE

Formulation of 5-fluorouracil Loaded Chitosan Nanoparticles By Emulsion Droplet Coalescence Method For Cancer Therapy

Anto Shering M[1*], Kannan C[2], Sabari Kumar K[1], Sathish Kumar V[1], Suganeshwari M[3]

[1] *Medisas Pharmaceuticals, Puducherry, India*
[2] *Vathsalya College of Pharmacy, Bhonhir, Andhra Pradesh, India*
[3] *Sri Ramachandra College of Pharmacy, Vedaranyam, Tamilnadu, India*

Received 02 May 2011; Revised 05 Jun 2011; Accepted 19 Jun 2011

ABSTRACT

Nano technology is the one of the emerging field in the World also the Medical sciences. Since, it over come most of the disadvantages of conventional therapy. There are many products related to this technology is already marketed, and giving good results in patient compliance with related to side effects and sustained release. The goal of the present investigation was to formulate and evaluate nanoparticles of 5-Fluorouracil using Emulsion droplet coalescence method. In the present study we have taken chitosan, a natural marine polymer for the preparation of Nanoparticles. Eudragit S 100 was used to produce secondary coating upon the chitosan layer and drug used is 5-fluorouracil. Emulsion droplet coalescence method was used for the preparation. This formulation meant for IV use. So the pH maintained at 7.4. DSC graph shows that there is no interaction between Polymer and drug in the nanoparticles. *Invitro* diffusion study was carried out using Franz diffusion cell and the formulation CF 2 shows good encapsulation capacity, sustained drug release up to 12 Hours. The goal of the present investigation was to formulate and evaluate nanoparticles of 5-Fluorouracil using Emulsion droplet coalescence method.

Key words: Emulsion droplet coalescence method, 5-Fluorouracil, Chitosan, Eudragit S 100.

INTRODUCTION

Nano particle drug delivery system:

The substances, which size ranges from 1 to 1000 nanometers, are called nanoparticles. Micro- and nano- particles with a diameter of less than 10 µm, particles can penetrate the mucus layer more deeply. Furthermore, microparticles of several hundred nanometers to several micrometers are subject to uptake by leukocytes such as macrophages. These materials are mainly used in the oncology for early detection of malignancy and precise localization of cancer therapeutics without or with minimal adverse effect to the somatic tissues. In addition, therapeutic or bioactive molecule to be protected from degradation and it should reach the target region or cell. These carriers are used to protect drugs, vaccines, nutrients and cosmetics. In these years, nanomaterials and nanotechnology are in leading position in terms of extensive research, application and products in pipeline. Nano technology is the emerging technique for the delivery for all kind of products. The bioavailability and solubility of drugs can be increased by these techniques. It can use in the several formulations as injections, topical creams, tablets, capsules or as solutions. The several process including simple laboratory methods can prepare them. Although it has more advantages than other pharmaceutical dosage forms. Formulation cost and stability are the problems in these products. The microencapsulation systems are investigated and extensively using for the bioactive agents protecting against degradation and inactivation. Nano formulations are used to provide targeted and controlled release. Particle size is the main responsibility for the delivery of the drug within the body. Nano sized materials have more advantages than micrometer size materials because it is provide more surface area and have the potential to increase solubility also increasing controlled drug release and enable

***Corresponding Author:** Anto Shering M, **Email:** anto_pharma@rediffmail.com

precision targeting of the entrapped compounds to a greater extend. When the drug is capsulated it improve the stability, avoiding the RES, targeting and the amount required is less. Mainly it has more advantages in delivery of biological materials, reactive and sensitive material such as polynucleotides, anti biotics, cytostatins, proteins, nuclic acids and polypeptides. There by it can be changed into stable ingredient than before encapsulation. This can be prepared by using biodegradable polymers. Their diameter is depending upon the materials and methods using in the process. Mainly the particles prepared by the biodegradable polymers are has the higher advantages than the non-biodegradable polymers. Due to their small size, prolonged circulation time and sustained drug profile nano sizing of drugs used mainly in the treatment of many diseases like anticancer.

Biodegradable polymers

In micro- and nanoparticles poly (D, L- lactide), poly (lactic acid) PLA, poly (D, L-glycolide), PLG, poly (lactide-co-glycolide), PLGA, and poly- (cyanoacrylate) PCA are used. The present review details the latest developments on the above mentioned polymers as well as nanoparticles based on chitosan, gelatin, sodium alginate and other hydrophilic / biodegradable polymers. The use of biodegradable polymers precludes the need for retrieval at the conclusion of the dosing regimen, thereby avoiding the potential complications associated with the use of non-degradable systems. Degradation may take place by a variety of mechanisms, although it generally relies on either erosion or chemical changes to the polymer. Degradation by erosion normally takes place in devices that are prepared from soluble polymers. In such instances, the device erodes as water is absorbed into the system causing the polymer chains to hydrate, swell, disentangle, and, ultimately, dissolve away from the dosage form. Alternatively, degradation could result from chemical changes to the polymer including, for example, cleavage of covalent bonds or ionization / protonation either along the polymer backbone or on pendant side-chains. A number of degradation schemes have been described that characterize how chemical degradation of the polymer or of polymer–drug conjugates can be utilized to achieve drug releae[1,2,3].

MATERIALS & METHODS

5-Fluorouracil was a gift sample from Sun Pharmaceuticals, Pune, India. Chitosan was procured from CIFT, Cochin, India. Eudragit S 100 was obtained from SD Fine Chemical Maharashtra, India. Liquid paraffin, tween 20, sodium chloride was obtained from Spectrum Chemicals and Reagents, Cochin, India.

Preparation of drug loaded nanoparticles by emulsion-droplet coalescence method [4,5]

Chitosan was dissolved in 1% acetic acid and 50 mg of 5-Fluorouracil in phosphate buffered saline. This solution was added to 10 ml of liquid paraffin containing 0.5% v/v tween 20. This mixture was stirred using a homogenizer for 3 minutes to form water in oil (w/o) emulsion. Similarly, another w/o emulsion consisting of 1% Eudragit S 100 in 3M sodium hydroxide solution was prepared. Then these two emulsions were mixed and stirred using homogenizer. As a result of coalescence of the droplets, chitosan in the system was solidified to produce nanoparticles. Eudragit S 100 producing second coating over chitosan nanoparticles. The resultant 5-Fluorouracil nanoparticles were centrifugated at 3000 rpm was 60 mts (REMI, India) and washed using ethanol and water, consecutively to remove the remaining surfactant and liquid paraffin. Later they were dried in air for 3 hour followed by hot air oven at 50° for 4 hour and stored in a dessicator. The prepared nanoparticles were evaluated.

Table 1 Formulation Design of 5-Flurouracil Nanoparticles

Formulation code	Amount of drug	Concentration of Chitosan (%)	Concentration of Eudragit S 100(%)
CF 1	50 mg	0.5	1.0
CF 2	50 mg	1.0	1.0
CF 3	50 mg	1.5	1.0
CF 4	50 mg	2.0	1.0
CF 5	50 mg	2.5	1.0

Detection of shape and morphology (SEM Analysis)

The particle shape and morphology of the prepared 5-Fluorouracil nanoparticles were determined by SEM analysis. The nanoparticles were viewed using a Jeol-5610 L V (Tokyo, Japan) for morphological examination. Powder samples of dried 5-Fluorouracil nanoparticles were mounted onto aluminium stubs using double side adhesive tape and then sputter coated with a thin layer of gold at 10 Torr for vaccum before examination. The specimens were scanned with an

© 2010, IJPBA. All Rights Reserved.

electron beam of 1.2 kv acceleration potential and images were corrected in secondary electron mode [6].

Determination of loading/entrapment efficiency [7,8,9]

The amount of 5-Fluorouracil entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticles and the amount of non entrapped drug remaining in the aqueous supernatant. The latter was determined following the separation of drug loaded nanoparticles from the aqueous medium by centrifugation at 5000 rpm for 30 min. The supernatant was collected and the particles were washed with water and then subjected to another cycle of centrifugation. The amount of free 5-Fluorouracil in the supernatant was determined by UV-Visible spectrophotometer (UV1 v 7.07 Thermo Scientific, Germany).

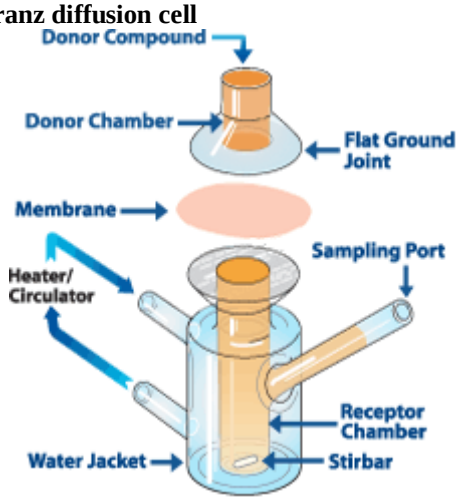
$$\text{Loading capacity (\%)} = \frac{\text{Amount of 5 FU added} - \text{Amount of free 5 FU}}{\text{Weight of nanoparticles}} \times 100$$

$$\text{Entrapment efficiency (\%)} = \frac{\text{Amount of 5 FU added} - \text{Amount of free 5 FU}}{\text{Amount 5 FU added}} \times 100$$

Invitro drug release study using diffusion cell

A Franz diffusion cell was used to monitor 5-Fluorouracil release from the nanoparticles.13 The receptor phase was phosphate buffered saline (PBS, pH 7.4) thermostatically maintained at 37°C, with each release experiment run in triplicate. Dialysis membrane (Hi Media, Mumbai, India) with molecular weight cut off 12,000 to 14000 Daltons was used to separate receptor and donor phases. The latter consisted of a 2ml suspension of nanoparticles containing 10 mg of 5-Fluorouracil, mixed for 5 seconds to aid re-suspension, in a 1% w/v tween 80 solution in PBS. Samples (1ml) from the receptor phase were taken at time intervals and an equivalent volume of PBS replaced into the receiver compartment. Diffusion of 5-Fluorouracil into the receptor phase was evaluated spectrophotometrically[10].

Fig 1 Franz diffusion cell



RESULTS AND DISCUSSION

Nanoparticles were prepared by emulsion droplet coalescence method. It is a laboratory method proved for the preparation of nanoparticles. The concentration of the polymer chitosan and Eudragit S 100 were selected based on the results on preliminary screening. The surfactant used for the preparation was 0.5 % tween 20. The time taken to complete preparation was around 2 hours. In total five formulations of 5-Fluorouracil loaded nanoparticles of chitosan were prepared and evaluated for various parameters such as particle size, morphology, drug entrapment efficiency, *invitro* drug release study.

The data of drug entrapment efficiency and drug loading capacity for drug loaded nanoparticles were as shown in the (**Table 2**). The formulation CF 2 showed around 78.2 % of drug loading,

Table 2 Summary of results of Nanoparticles

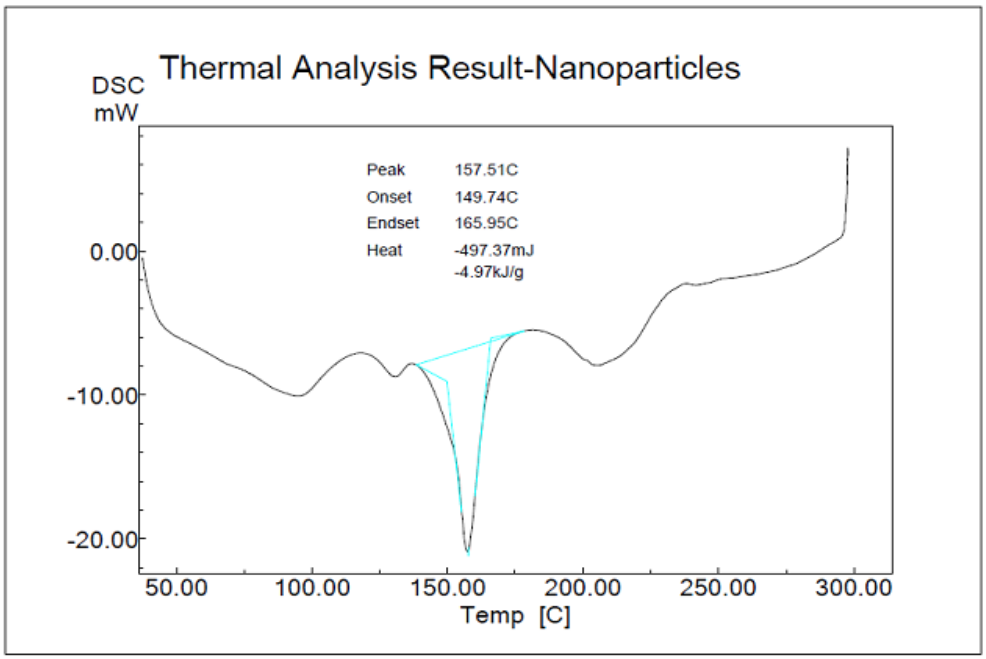
Parameter	CF 1	CF 2	CF 3	CF 4	CF 5
pH	7.1	7.5	7.7	7.6	7.8
Practical Yield (mg)	130	175	130	165	175
Efficiency of particle Recovery (mg)	86.67	87.50	86.66	88.33	84.28
Unencapsulated drug (mg out of 50 mg)	8.5	10.9	11.8	13	14.35
Encapsulation efficiency (%)	73.00	78.20	76.40	74.00	71.30
Loading capasity (%)	31.92	22.34	29.38	13.96	12.08

© 2010, IJPBA. All Rights Reserved.

Figure 2 Photographs of Prepared Nanoparticles

As DSC is useful tool to monitor the effect of additives on the thermal behavior of materials, these techniques were used to derive qualitative information about the physicochemical status of drug in particles. The peak for 5-Fluorouracil pure sample was obtained in 152.04°C. The peak in physical mixture and nanoparticles were 154.68°C and 157.51°C respectively. It indicates no interaction between polymers and Drug.

Figure 3 DSC of 5-Fluorouracil Nanoparticles



The drug release profile from the nanoparticles was as shown in the Table 3 & graph (Figure 4). The formulation CF2 showed good drug release from the polymer. The percentage cumulative drug release after 12 hours 74.06%. However

© 2010, IJPBA. All Rights Reserved.

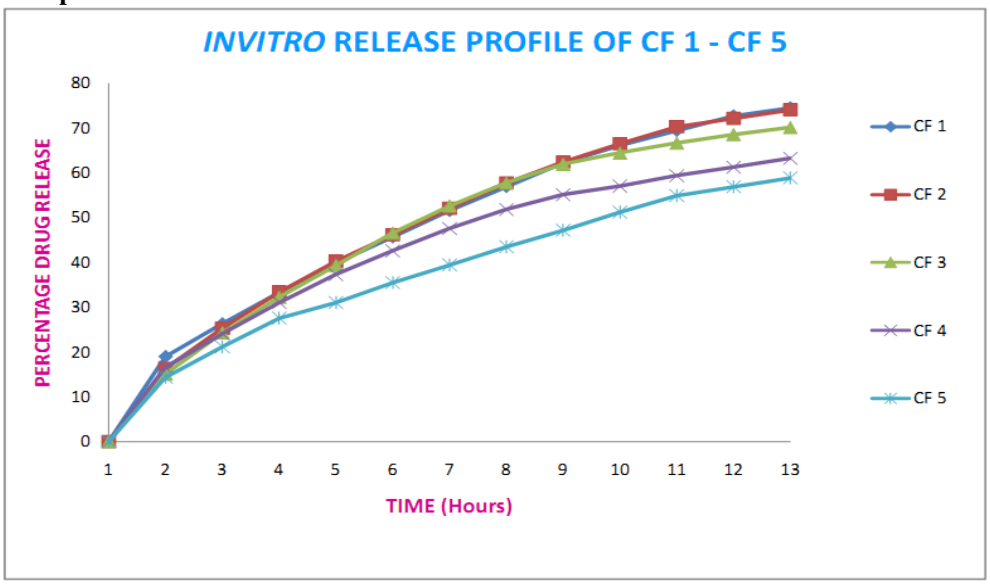
IJPBA, May - Jun, 2011, Vol. 2, Issue, 3

about 15 % initial burst release was found at 1 hour in all formulations.

Table 3 *Invitro* Diffusion Study of 5-Fluoruracil Nanoparticles

Time (Hrs)	Cumulative drug release (%)				
	CF 1	CF 2	CF 3	CF 4	CF 5
1	18.97	16.28	15.05	16.55	14.37
2	26.28	25.32	24.27	24.08	21.09
3	33.31	33.41	32.22	31.02	27.49
4	39.87	40.28	39.32	37.30	31.01
5	45.65	52.11	46.57	42.59	35.45
6	51.55	57.75	52.65	47.55	39.45
7	56.90	62.45	57.77	51.85	43.46
8	62.26	66.52	61.92	55.13	47.14
9	66.13	70.31	64.45	57.06	51.18
10	69.42	72.18	66.67	59.35	54.89
11	72.7	72.18	68.57	61.29	56.85
12	73.49	74.06	70.14	63.24	58.83

Fig 4: Invitro release profile of CF1-CF5



CONCLUSION

On preliminary screening different formulations were developed with various ratios of polymers and different surfactants. It revealed that formulations with the polymer concentration (1.0-2.5%) with tween 20 had better drug release and entrapment efficiency. So the formulations were designed with that polymer concentration and surfactant. Prepared five formulations were evaluated and among CF 2 (1% Chitosan & 1% Eudragit S 100) showed maximum drug release in 12 hours diffusion study and good entrapment efficiency. The work on formulation development of 5-Fluorouracil nanoparticles was very much advantageous than the existing dosage forms as the drug is targeting to the cancerous cells, hence better action.

The future studies are *Invitro* & *invivo* anticancer studies and estimation of targeting capacity of the nanoparticles to use it for cancer treatment for human.

REFERENCES

1. J. Kreuter, Nanoparticles, in: J. Kreuter (Ed.), Colloidal Drug Delivery Systems, Marcel Dekker, New York, 1994, pp. 219–342.
2. L.B, Peppas, Recent advances on the use of biodegradable microparticles and nanoparticles in the controlled drug delivery, Int. J. Pharm. 116 (1995) 1–9.
3. P. Couvreur, L. Grislain, V. Lenaerts, F. Brasseur, P. Guiot, P. Couvreur (Eds.), Polymeric Nanoparticles a Microspheres, CRC Press, Boca Raton, Florida, 1986.
4. Lowman A M; Biodegradable nanoparticles for drug delivery and

© 2010, IJPBA. All Rights Reserved.

targeting, Current Opinion in Solid State and Material Science 6 (2002) pp319-327.

5. Scholes P D, Coombes A G A, Illum L, Davis S S, Vert M, Davies M C; The preparation of sub-500 nm poly(lactide co-glycolide) microspheres for site soecific drug delivery, J. Control. Rel. 25 (1993) pp145-153.
6. Damge C, Michel, C, Aprahamian M, Couvreur P, Devissaguet J P; Nanocapsules as carriers for oral peptide delivery. J. Conntrol. Rel. 1990, 13, pp 233-239.
7. Leroux J C, Allemann E, Doelker E, Gurnay R; New approach for the preparation of nanoparticles by an emulsification-diffusion method, Eur. J. Pharm. Biopharm. 41 (1995) pp14-18.
8. Niwa T, Takeuchi H, Hino T, Kunou N, Kawashima Y; Preparations of biodegradable nanospheres of water soluble drugs with D,L- lactide/ glycolide copolymer by a novel spontaneous emulsification solvent diffusion method and the drug release behaviour, J. Control. Rel. 25 (1993) pp 89-98.
9. Ganta S, Paxton J W, Baguley B B and Garg S; Formulation and pharmacokinetic evaluation of an asulacrine nanocrystalline suspension for intravenous delivery, International Journal of Pharmaceutics 367 (2009) pp 179-186.
10. Fraser I S, Titinen A, Affandi B, Brache V, Croxatto H B, Diaz S, Ginsburg J, Gu S, Holma P, Johansson E, Meirik O, Mishell D R, Nash H A, Von Shoultz I, Sivin; Norplant- consensus statement and background review.Contraception 1998, 57, 1-9.

© 2010, IJPBA. All Rights Reserved.