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# FABRICATION AND EVALUATION OF CIPROFLOXACIN LOADED NANOSPONGES FOR SUSTAINED RELEASE

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## ABSTRACT

Nanotech materials can be easier to target for specific cells such as those in cancerous tumors. They can be shaped into containers - miniscule pockets to contain drugs, especially those for cancer that are toxic to healthy tissues and need to be encapsulated until they reach the target. The present research study is related with sustained release of an antiulcer drug i.e., proton pump inhibitor. The drug is acid labile and hence it is entrapped with ethyl cellulose for its sustained release. As the drug made into nanoparticle the density was found to be increased. Nanosponge is water soluble. This does not mean the molecules chemically break up in water, but it means that nanosponge particles can mix with water and use it as transport fluid, for example to be injected. So in theory nanosponge has several advantages over other delivery methods. Like all nanomedical materials nanosponge will need lengthy phased trials, which means that commercial availability is still years away. In the present study the formulated nanosponge loaded with ciprofloxacin antibiotic used resulted in sustained release. Among all the formulated batches starting from F1 through F5 the final batch (F5) is considered as the best entrapped (90.80%) nanosponge with greater percentage drug release (99.4%). The characterization by SEM finally concluded the appearance as a "Nanosponge".

## KEYWORDS

Nanoparticle, Nanosponge, Scanning Electron Microscopy and Characterization.

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## INTRODUCTION

Targeting the drug delivery has long been a problem for medical researchers how to get them to the right place in the body and how to control the release of the drug to prevent overdose[1]. The development of new and complex molecules called Nanosponges has the potential to solve these problems. Nanosponges are a new class of materials and made of microscopic

particles with few nanometers wide cavities in which a large variety of substances can be encapsulated[2]. These particles are capable of carrying both lipophilic and hydrophilic substances and of improving the solubility of poorly water soluble molecule[3]. Nanosponges are tiny mesh like structures. The nanosponge is about the size of a virus with a backbone of naturally degradable polyester. The long strength polyester strands are mixed in solution with small molecules called cross linkers that have an affinity for certain portions of the polyesters. They cross link segments of the polyester to form a spherical shape that has many pockets / cavities where drug can be stored. The nanoscale materials are small enough to be effective in attaching to or passing through cell membranes. The nanosponge can be engineered to be of specific size and to release drugs over time- not just in the 'burst' mode common with other delivery methods[4]. The engineering capacity of nanosponge is due to the relatively simple chemistry of its polyesters and linking material (peptides). Compared to many other nanoscale drug delivery systems, nanosponge should be able to scale (e.g. ramp up to commercial production levels) without requiring unusual equipment or procedures. The polyster is predictably biodegradable, which means that when it breaks up in the body, the drug can be released on a known schedule[5]. Proteolytic enzymes can be used to treat cancer or type I muco polysaccharidosis, whole DNA and oligonucleotides are used in gene therapy. The administration of these molecules presents various problems and limitations. Most protein drugs are poorly absorbed through the biological membranes due to the some factors such as large molecular size, hydrophilic nature, degree of ionization, high surface charge, chemical and enzymatic instability and low permeability through mucous membranes[6]. Following i.v. administration, protein molecules may be rapidly cleared from blood, bind to plasma proteins, and sensitive toward proteolytic enzymes. With oral administration bioavailability is the problem. Various approaches exist for therapeutic use, such as increasing the dose or using absorption promoters, which can cause toxicity problems. Cyclo-dextrin based nanosponges are particularly suitable carrier to adsorb proteins, enzymes, antibodies and macromolecules[7]. It's a good example of the spreading use of nanotechnology - artificial blood platelets composed of nanoparticles. Platelets are a small blood-cell type and one of the principle components of blood coagulation, the process used by the body to stop bleeding. One of the more common techniques evolving from the use of nanoparticles to study cell biology is the ability to 'tag' cells with colored (dyed) nanoparticles[8].

**Classification of Nanosponges**

Nanosponges are encapsulating type of nanoparticles which encapsules the drug molecules within its core. By method of associating with drugs, the nanoparticles can be classified into the following:-

**1. Encapsulating nanoparticles**

This type is represented by nanosponges and nanosponges and nanocapsules. Nanosponges such as alginate nanosponge, which are sponge like nanoparticles containing many holes that carry the drug molecules in their aqueous core.

E.g. Nanosponges such as alginate nanosponge, which are sponge like nanoparticles containing many holes that carry the drug molecules. Nanocapsules such as poly (iso-butyl-cyanoacrylate) (IBCA) are also encapsulating nanoparticles.

**2. Complexing nanoparticles**

This type of nanoparticles attracts the molecules by electrostatic charges.

**3. Conjugating nanoparticles**

This type of nanoparticles links to drugs through covalent bonds. As compared to the other nanoparticles, they are insoluble both in water and organic solvents, porous, non-toxic and stable at high temperature up to 300$^{o}$C. They are able to capture, transport and selectively release a huge variety of substances because of their 3D structure containing cavities of Nanomeric size and tunable polarity.

## METHODS

**Calibration curve of Ciprofloxacin**

Stock solution of Ciprofloxacin was prepared by dissolving 100 mg of accurately weighed amount of Ciprofloxacin 10 ml of distilled water and then the volume was adjusted to 100 ml with the same solution[9, 10].

**Procedure**

The above stock solution of drug was subsequently diluted with distilled water to get 2 µg, 4 µg, 6 µg, 8 µg and 10 µg, of drug per ml. Then the absorbance of these dilute solutions was measured at a λ max of 284 nm by using double beam U.V. spectrophotometer against a blank of distilled water. Average of triplicate readings was taken and tabulated (Table No.1 and Figure No.1).

**Solubility studies**

The solubility of ciprofloxacin was determined in distilled water, different buffers, viz., pH 2.5, pH 7.5, pH 8.0 and pH 9.0. Triplicate readings were taken and average was calculated**.**

**Preparation of nanosponge by solvent evaporation method**

Five batches of nanosponges using different proportions of ethyl cellulose and polyvinyl alcohol were prepared by solvent evaporation method [11, 12]. Disperse phase consisting of Ciprofloxacin (1gm) and requisite quantity of ethyl cellulose (Table No.2) dissolved in 10 ml solvent (dichloromethane or ethanol) was slowly added to a definite amount of PVA in 100ml of aqueous continuous phase, prepared by using microwave oven. The reaction mixture was stirred at 1000 rpm for three hours on a magnetic stirrer. The nanosponges formed were collected by filtration through whatman filter paper and dried in oven at 50ºC for 2 hours. The dried nanosponges were stored in vaccum desicator to ensure the removal of residual solvent (Figure No.2 and 3).

**EVALUATION OF NANOSPONGES**

The Prepared nanosponges were evaluated for the following:

1. Characterization by scanning electron Microscopy[13]
2. *In vitro* dissolution studies[14]
3. Drug Entrapment efficiency[15]

**Drug Entrapment Efficiency**

For the drug entrapment efficiency tests, the nanosponges of F1- F5 were performed. Before starting the chemical (spectrophotometric) analyses for the drug entrapment efficiency, the repeatability of measurements between different batches was ensured by repeated analyses. The 100mg of the nanosponge suspension was analyzed by dissolving the sample in 10ml of distilled water. After the drug was dissolved, 10 mL of clear layer of dissolved drug is taken. Thereafter, the amount of drug in the water phase was detected by a UV-spectrophotometric method at 284 nm (U.V Spectrophotometer, systronics). The test was repeated with another nanoparticulate sample.

The amount of the drug in the suspension was analyzed by centrifugation at 500rpm for 5 mins and by measuring the concentration of the drug in the clear supernatant layer by the UV-spectrophotometric method. The test was again repeated with another sample. The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase of the suspension from the total amount of the drug in the nanoparticle suspension. The entrapment efficiency (%) of drug was calculated by the following equation.

$$\% \text{ of Drug entrapment} = \frac{\text{Mass of drug in nanosponge}}{\text{Mass of drug used in formulation}} \times 100$$

***In vitro* Dissolution**

| | | |
|---|---|---|
| Apparatus (Paddle) | - | USP-II dissolution apparatus |
| Medium | - | pH 6.8 buffer |
| Temperature | - | 37ºC |
| Time | - | 12 hours. |

***In vitro* release studies**

*In vitro* release studies were performed in triplicate using USP Paddle method at 100 rpm and 37±0.2ºC in 900ml of phosphate buffer (pH 6.8). 100 mg of the formulated nanoparticles is used for each experiment. Samples were taken at appropriate time intervals for 5, 15 min interval in the first hour, half hour in next 3 hours, hourly sampling for up to 6 hours and finally for twelfth hour. The samples were measured spectrophotometrically at 284nm. Fresh dissolution medium was replenished each time when sample is withdrawn to compensate the volume.

**Phosphate buffer (pH 6.8)**

Place 50ml of 0.2M Potassium dihydrogen phosphate in 200ml volumetric flask. Add 22.4ml of 0.2M NaOH. Then add distilled water to make up the volume as per I.P.

## RESULTS AND DISSCUSSION

**Solubility studies**

Solubility of ciprofloxacin in distilled water, acidic and alkaline pH buffers was studied. It was found to be 100 mg/10ml in distilled water, 0.395 mg/ml in Ethanol, 100mg/10 ml in Dichloromethane but the solution was not clear.

**Solubility by co-solvency**

The solubility of drug in ethanol is increased by water as co-solvent, 100mg/ml.

**Characterization**

The nanosponges can be characterized for morphology by Scanning Electron Microscopy (Figure No.4).

***In vitro* release studies**

*In vitro* release for the prepared nanosponges is done by using USP dissolution apparatus II. The samples are withdrawn at specific time intervals and analysed UV spectrometrically at 254nm. The values are given in Table No.5. The release of drug was found to be more for F1 and F5 formulation, but F5 formulation drug release was found to be more sustained when compared with other formulation. The *in vitro* release of drug was found to be more in samples with less drying time. Though the other batches of formulation were found to release in sustained pattern, the F5 batch is considered as best sustained release of drug for 12hrs (Table No.3 and Figure No.5).

**Drug Entrapment Efficiency**

The drug entrapment efficiency tests, the nanosponges of F1- F5 were performed (Table No.4 and Figure No.6).

**Table No.1: Calibration curve of Ciprofloxacin**

| S.No | Concentration (µg) | Absorbance (284 nm) |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 2 | 0.114 |
| 3 | 4 | 0.234 |
| 4 | 6 | 0.362 |
| 5 | 8 | 0.487 |
| 6 | 10 | 0.607 |

**Table No.2: Formulation aspects**

| S.No | Materials | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|---|
| 1 | Drug | 1gm | 1gm | 1gm | 1gm | 1gm |
| 2 | Solvent | Dichloromethane | Acetone | Ethanol | Ethanol | Dichloromethane |
| 3 | PVA | 2gm | 2gms | 3gm | 3gm | 3gm |
| 4 | Drug: Polymer | 1:1 | 1:1 | 1:2 | 1:2 | 1:2 |
| 5 | Ethyl cellulose | 1gm | 1gm | 2gm | 2gm | 2gm |
| 6 | Water (co-solvent) | 20ml | __ | 20ml | ___ | ___ |

**Table No.3: Comparative dissolution study of different formulations F1-F5**

| S.No | Time(mints) | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 5 | 1.4 | 0 | 1.4 | 1.6 | 0 |
| 3 | 10 | 2.6 | 1.4 | 2.6 | 3.2 | 1.6 |
| 4 | 15 | 5.7 | 4.7 | 3.8 | 7.2 | 3.7 |
| 5 | 30 | 9.2 | 12.3 | 10.6 | 9.3 | 7.5 |
| 6 | 45 | 16.2 | 14.4 | 13.8 | 10.1 | 9.2 |
| 7 | 60 | 26.1 | 24.4 | 27.9 | 11.7 | 24.7 |
| 8 | 90 | 34.2 | 34.2 | 36.7 | 24.4 | 47.2 |
| 9 | 120 | 55.8 | 36.1 | 45.8 | 32.1 | 68.5 |
| 10 | 180 | 62.5 | 45.7 | 65.7 | 35.8 | 78.3 |
| 11 | 240 | 65.7 | 62.5 | 77.6 | 70.5 | 86.8 |
| 12 | 300 | 79.2 | 70.2 | 85.7 | 94 | 96.2 |
| 13 | 360 | 95.4 | 88.4 | 96.33 | 95.2 | 97.7 |
| 14 | 720 | 97.51 | 94.47 | 97.21 | 95.2 | 99.4 |

**Table No.4: Drug Entrapment Efficiency**

| S.No | Formulation | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|---|
| 1 | % Drug Entrapment Efficiency | 90.20 | 88.01 | 74.40 | 76.60 | 90.80 |
| 2 | Drying Time (hours) | 1 | 2 | 1 | 2 | 2 |

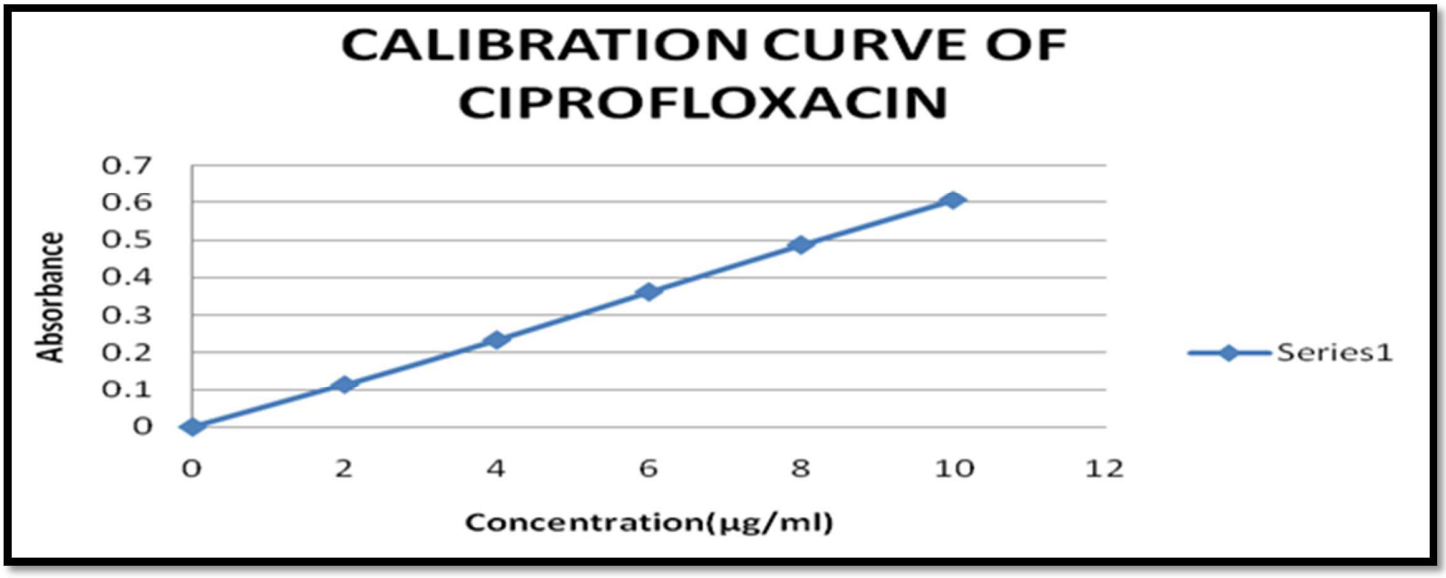


**Figure No.1: Calibration curve of Ciprofloxacin**

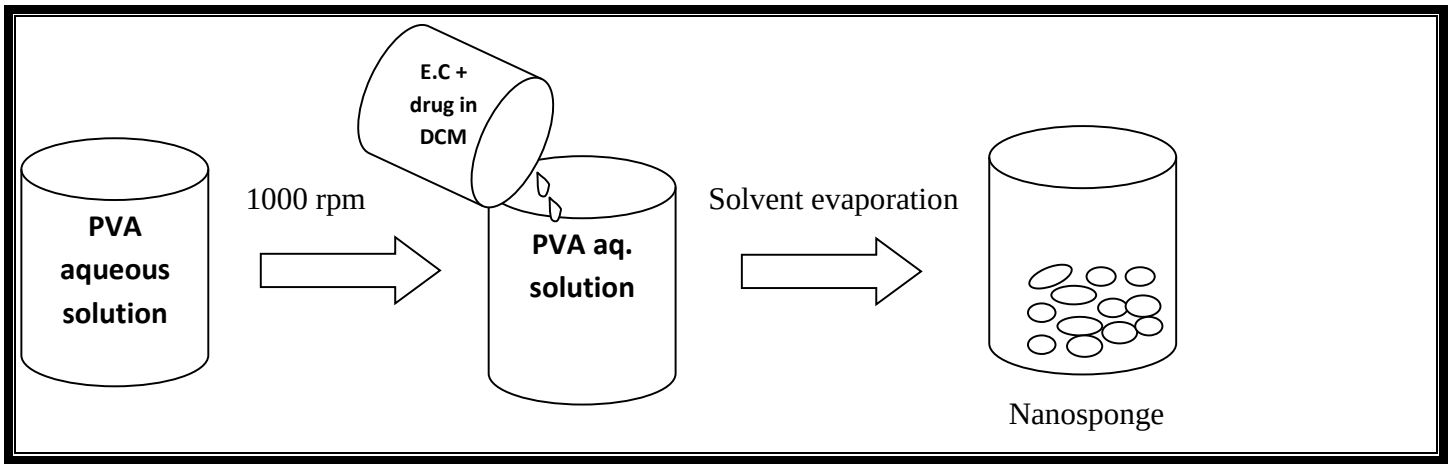


**Figure No.2: Preparation of Ciprofloxacin Nanosponge by Solvent evaporation method**

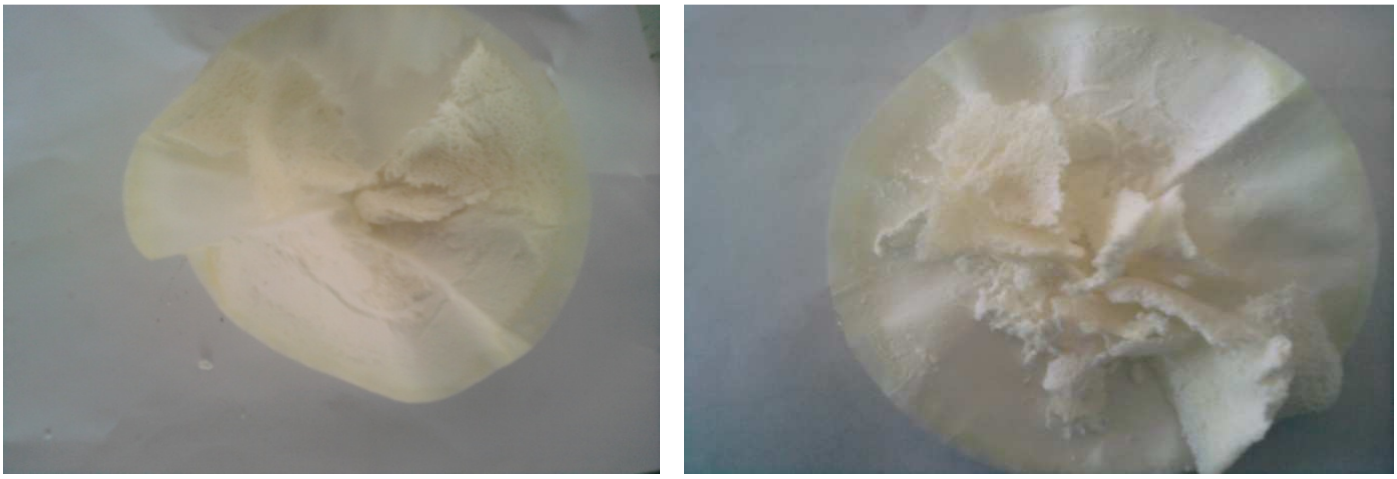

**Figure No.3: Formulated Ciprofloxacin Nanosponge (Batch-F5)**

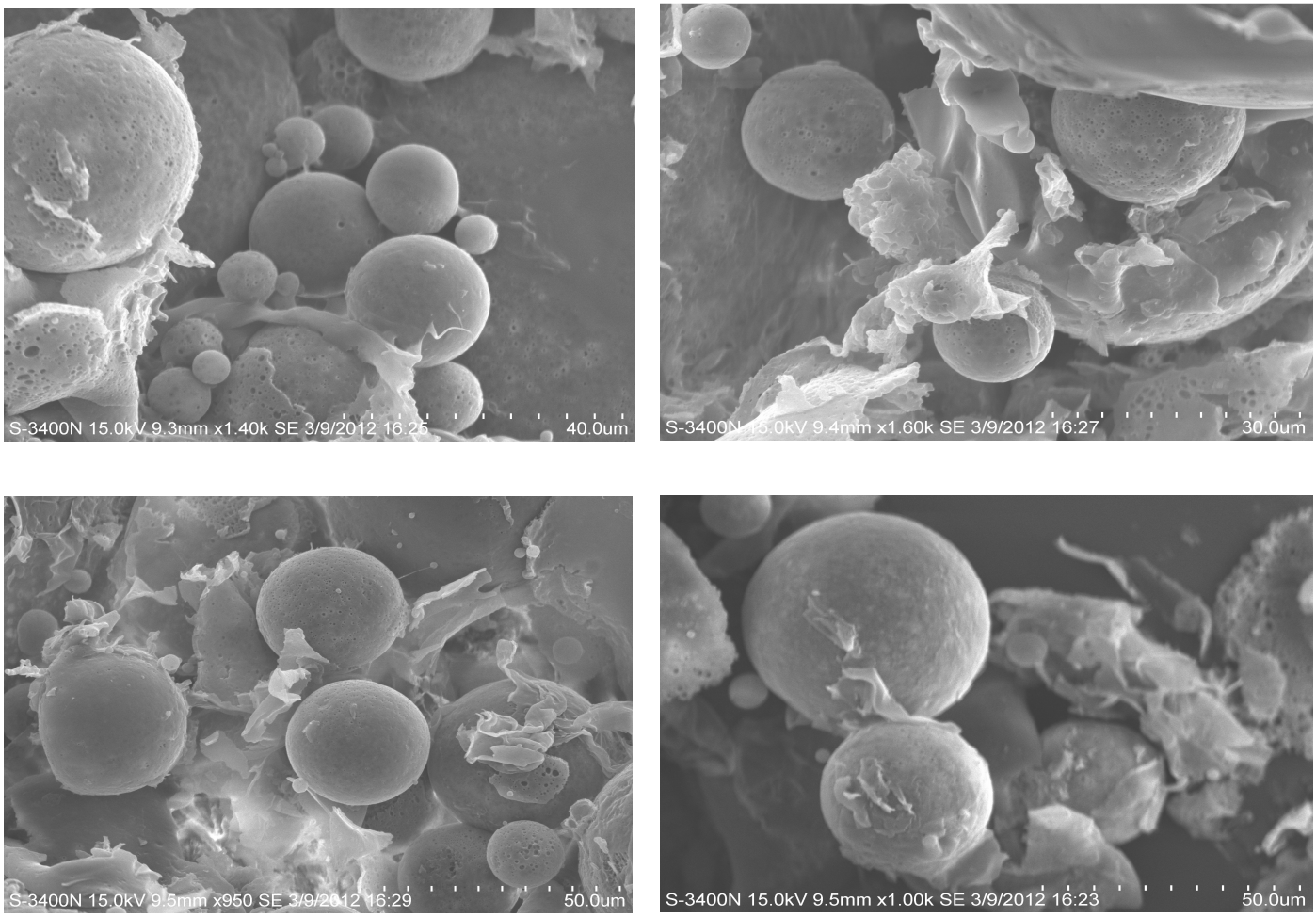


**Figure No.4: SEM Analysis Photographs of Nanosponges**

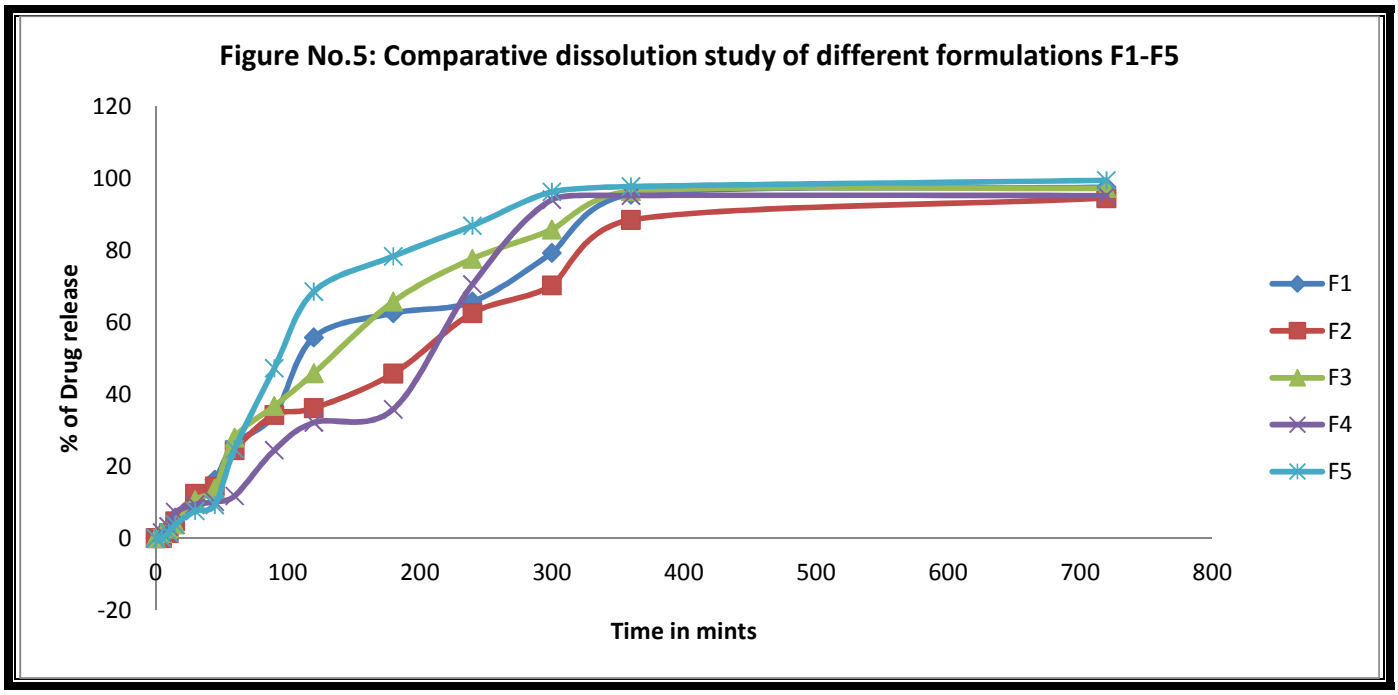


**Figure No.5: Comparative dissolution study of different formulations F1-F5**

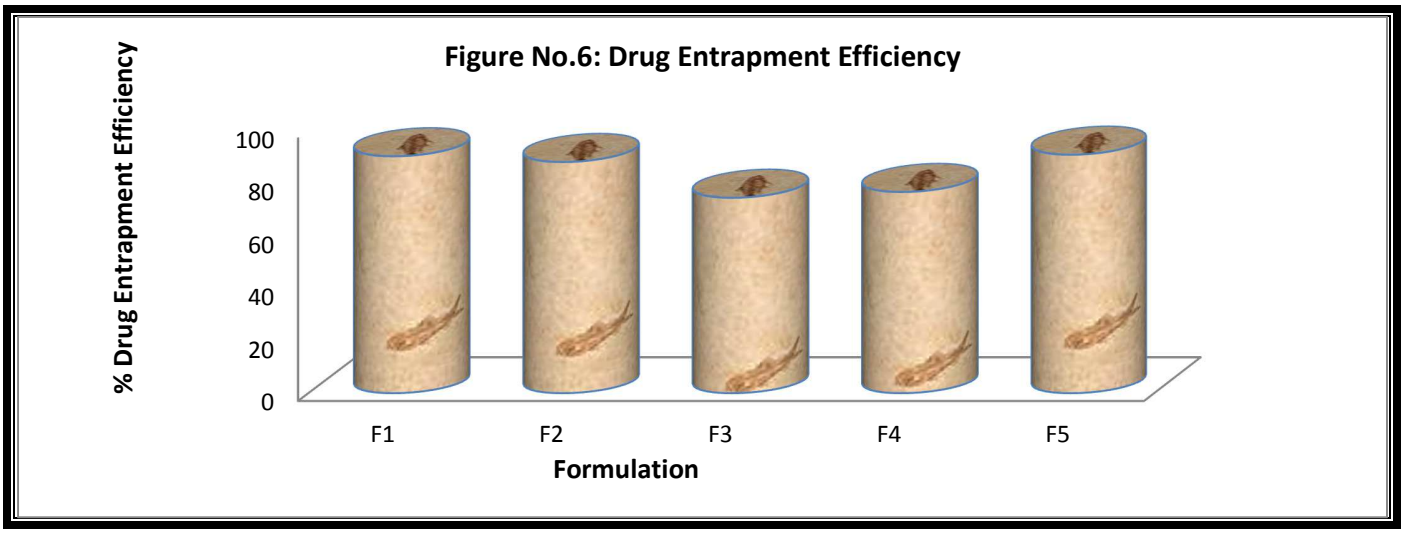


**Figure No.6: Drug Entrapment Efficiency**

## CONCLUSION

In the present study the formulated nanosponge loaded with ciprofloxacin antibiotic resulted in sustained release. Among all the formulated batches starting from F1 through F5, the final batch (F5) is considered as the best entrapped (90.80%) nanosponge with greater percentage drug release (99.4%). The characterization by SEM finally concluded the appearance as a “Nanosponge”.

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**CONFLICT OF INTEREST**

We declare that we have no conflict of interest.

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