

## Research Article

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# Formulation and Evaluation of Naproxen Sodium Loaded Bovin Serum Albumin Nanoparticles by Desolvation Technique

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### ABSTRACT

The objective of the present study is to prepare Naproxen sodium loaded BSA nanoparticles by desolvation technique using different desolvating agent. Naproxen sodium loaded BSA nanoparticles were prepared using desolvation technique. In desolvation technique two methodologies i.e continuous addition and intermittent addition method were adapted for the preparation of naproxen nanoparticle. The formulations were prepared at three different RPM and using different desolvating agents. The obtained formulation were evaluated for drug content, entrapment efficiency, loading capacity and characterized for mean particle diameter and stability. Among all these formulation Acetone (P2) formulation prepared by intermittent addition method at 800 rpm was showing promising results with mean particle diameter of 556 nms. It was able to sustain the drug release up to 9 hrs with 98% following first order rate constant with Fickian diffusion. From the result it was observed that intermittent addition method and Acetone were considered as best method and desolvating agent for the preparation of Naproxen sodium nanoparticle.

**Key-words:** Naproxen Sodium, Bovin Serum Albumin Nanoparticles, Desolvation Technique, etc.

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### **Introduction<sup>1-3</sup>:-**

Nanoparticles are drug loaded particles with diameter ranging from 1 to 1000nm. Nanoparticles are defined as solid, sub micro-sized drug carrier that may or may not be biodegradable. Nanoparticle is a collective term used for both nanospheres and nanocapsule<sup>4</sup>. Nanospheres have a matrix type structure and the drug may be adsorbed at the sphere surface or encapsulated within the particle. Nano capsules are the vesicular system in which the drug is confined to a cavity consisting of an inner liquid core surrounded by a polymeric membrane<sup>5</sup>. In this case the drug is usually dissolved in the inner core but may also be adsorbed to the capsule surface. Naproxen sodium is non-steroidal anti-inflammatory drug and used as first line therapy for the treatment of arthritis, dysmonorrhoea and ankylosing spondylitis. It has a major side effect of gastric irritation. The drug used in the investigation is Naproxen sodium. Naproxen sodium is available as tablets, capsules and suspensions. It is a BCS class II (low solubility and high permeability) drug<sup>6-9</sup>. Naproxen sodium has a wide range of gastrointestinal disorders, like gastrointestinal bleeding, gastric upset and cardiovascular side effects. Frequent administration of drug is required to maintain the desired steady state level. So in order to get a sustained release effect and to reduce the dose, dosing frequency to minimize the side effects there is a need to formulate Naproxen nanoparticle drug delivery system<sup>10</sup>.

### **Materials:-**

Naproxen sodium (DIVIS Laboratories limited), Bovine Serum Albumin (Hi Media Laboratories Pvt. Ltd., Mumbai), Glutaraldehyde solution 25% (SD fine-chem Limited, Mumbai), Ethanol (SD fine-chem Limited, Mumbai), Acetone (SD fine-chem Limited, Mumbai), Potassium Dihydrogen Phosphate (SD fine-chem Limited, Mumbai), Sodium Hydroxide (SD fine-chem Limited, Mumbai).

### **Methodology<sup>7</sup>:-**

Preparation of Naproxen sodium loaded BSA nanoparticles was carried out by Desolvation technique. Aqueous drug polymer dispersion was prepared and pH was adjusted to 7 (away from iso-electric point). The desolvating agent was added under continuous mechanical stirring at 800rpm. In continuous addition method the desolvating agent was added at a rate of 1ml per min. In intermittent addition method the desolvating agent was added at a rate of 1ml per 5 mins. The formation of insoluble precipitate was observed as the end point of the reaction. A cross-linking agent (glutaraldehyde 25%) was added and stirring was continued for next 8 hrs. The solvent was removed by rotary evaporation at a vacuum pressure of 760 mm Hg. Free flowing amorphous nanoparticles were obtained<sup>2,11</sup>.

### **Optimization of Stirring Speed (Rpm):**

Three formulations F1, F3 and F6 were prepared by varying stirring speed i.e. 400, 600 and 800 rpm respectively.

### **Optimization of Different Desolvating Agent:**

Three formulations were prepared by using three different desolvating agent i.e Acetone, Ethanol, Isopropanol.

### **Evaluation and Characterization of Naproxen Sodium Nanoparticles:**

#### **Study of Interaction between the Drug and the Excipients Using FTIR Spectroscopy:**

Naproxen sodium, BSA prepared nanoparticles were mixed separately with IR grade KBr and compressed into pellets by applying 8000 metric tons of pressure in a hydraulic press and the pellets were scanned over a wave number range of 4000 to 400 cm<sup>-1</sup> in a FTIR instrument.

#### **Study of Surface Morphology of Nanoparticles by Scanning Electron Microscope (SEM):**

The prepared amorphous nanoparticles were dispersed in deionized water and sonicated for 30 minutes. A circular metal plate is taken on to which carbon double tape (1mm×1mm) is stickered; a drop of the resultant nano dispersion is placed on to the tape and allowed to dry for a while. Then it is scanned under SEM for morphology.

#### **Determination of Size Distribution, Polydispersity Index (PDI) and Zeta Potential:**

The prepared nanoparticles were dispersed in deionized water and sonicated for 30 minutes. The resultant dispersion was diluted and observed for particle size and zeta values.

**Drug Content and Entrapment Efficiency Study:**

The nanoparticle formulations were examined for drug content. Prepared nanoparticle were added to equivalent quantity of Ethanol and kept for magnetic stirring at 600 rpm for 3 hrs separately. The resultant samples were observed under UV spectrophotometer for concentrations. The nanoparticle formulations were examined for entrapment efficiency. Entrapment efficiency is conducted by taking prepared particles in equivalent quantity of 7.4 pH phosphate buffer. The nanoparticles suspension is ultra-centrifuged at 17000 rpm and temperature of -4oC for 40 minutes.

The entrapment efficiency can be expressed as follow;

$$\text{Entrapment Efficiency} = \frac{\text{Total amount of the drug entrapped}}{\text{Total amount of the drug initially taken}} \times 100$$

$$\text{Loading Capacity} = \frac{\text{Total amount of the drug entrapped}}{\text{Total weight of the nanoparticles taken}} \times 100$$

***In vitro* drug release study from nanoparticle formulations in Phosphate Buffer Saline (pH 7.4)**

For the nanoparticles both the drug release and polymer degradation are two important considerations. *In vitro* drug release studies were conducted by means of Arbitrary shaker in 7.4 pH buffer at a temperature of 37 (+/-) 0.5oc and rotation speed of 100 rpm. Samples were withdrawn at regular time interval and replaced with equal quantity of buffer solution. Then the withdrawn samples were centrifuged at 3000 rpm for 15 minutes after which the clear supernatant was collected. The drug concentration in the supernatant was observed under UV spectrophotometer at a wavelength of 271nm.

**Results and Discussion:****Optimization of Process Parameter:-****Effect of Stirring Speed (Rpm):**

Formulations F1, S1 and P1 were prepared by varying stirring speed.at 400, 600,800 respectively.

TABLE 1:-Optimization of stirring speed (rpm):-

FORMULATION	RPM	DRUG:POLYMER
F1	400	1:1
S1	600	1:1
P1	800	1:1

The prepared by varying stirring speed using BSA as polymer three formulations F1, S1, P1 were evaluated for product yield,drug content, particle size , zetapotential ,entrapment efficiency,and *In vitro* drug release. The product yield of F1, S1, P1was found to be 93%, 98%, 98.1%. Drug content result for F1, S1, P1was found to be 83%, 88%, 90.4%. The particle diameter of F1, S1, P1 was found to be 745, 753, 708nm. The zeta potential of F1, S1, P1was found to be 17, 14,25.2 mv .Entrapment efficiency of F1, S1, P1 was found to be 27.9%, 31.8%, 46.9% . Loading capacity of F1, S1, P1 was found to be 12%, 14%, 21.2%. *In vitro* drug release of F1, S1, P1was found to be 95.7% in 5 hrs,9 7% in 6 hrs, 95.5% in 7 hrs .Among all the formulations of nanoparticles P1 formulation was found to be best with drug content of 90.4%, entrapment efficiency of 46.9%, minimum particle diameter of 708nm and showing better stability,drug release 95.5% for 7 hours followed first order release with fickian diffusion mechanism. FTIR studies revealed no drug polymer interactions.

**Scanning electron microscopic:-**

The bovine serum albumin nanoparticles prepared by desolvation technique (continuous addition method) were characterized for surface morphology using Scanning electron microscopy.

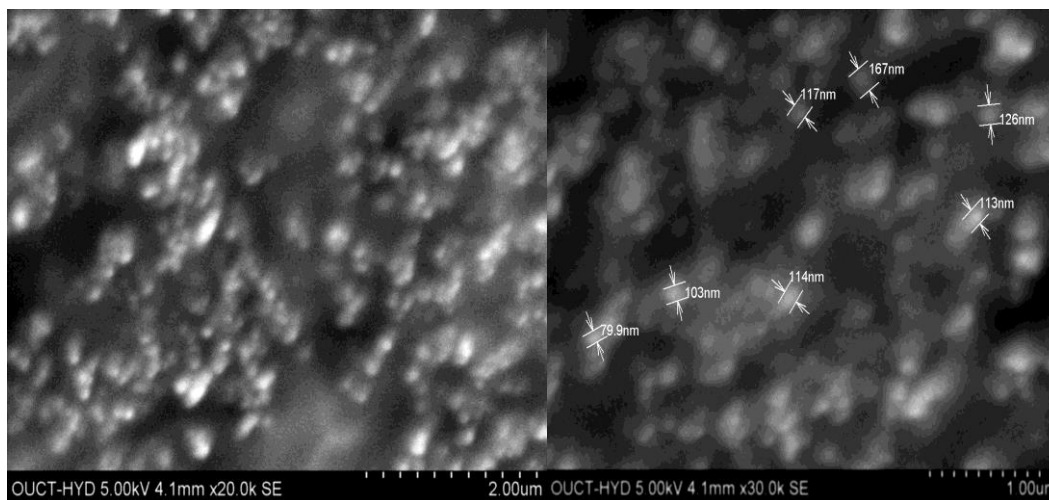
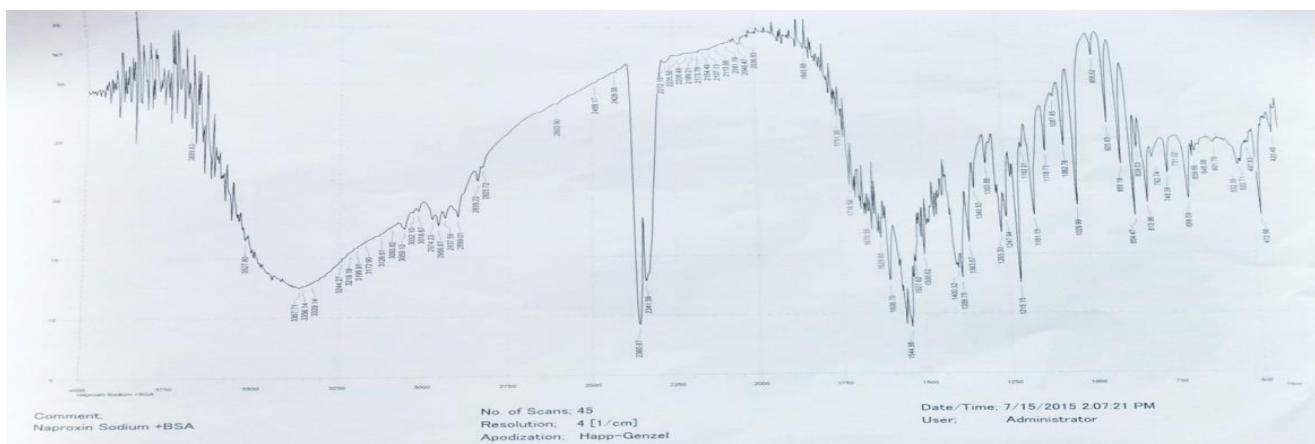


FIG 1: SEM images of P1 formulation of Naproxen sodium loaded BSA (continuous addition method) nanoparticles

**FTIR Spectrum:-**The prepared formulations were characterized for drug-polymer interactions using FT-IR.



### **Preparation of Naproxen Sodium Loaded BSA Nanoparticle by Desolvation Method Using Different Desolvating Agent:-**

Two methods that is continuous addition method and intermittent addition method were adapted for the preparation of naproxen nanoparticle.

#### **1) Preparation of Naproxen Sodium Loaded BSA Nanoparticle by Desolvation Method Using Acetone As Desolvating Agent:-**

The prepared nanoparticles using Acetone as desolvating agent two formulations P1, P2 were evaluated for product yield, drug content, entrapment efficiency, and *In vitro* drug release. The drug content results for P1, P2 was found to be 90.4 %, 96.15 %. The mean diameter of P1, P2 was found to be 708, 556nm. Zeta potential of P1, P2 was found to be -25.2, -26.7mv. Entrapment efficiency of P1, P2 was found to be 46.9%, 60%. Product yield of P1, P2 was found to be 98%, 98.6%. Loading capacity of P1, P2 was found to be 21.2%, 30%. *In vitro* drug release of P1, P2 was found to be 95.5% in 7 hrs, 98% in 9 hrs. Among all the formulations of nanoparticles P2 formulation was found to be best with drug content of 96.15%, entrapment efficiency of 60%, small particle size of 556nm, drug release 98% for 9hours.

#### **2) Preparation of Naproxen Sodium Loaded BSA Nanoparticle By Desolvation Method Using Ethanol As Desolvating Agent:-**

The prepared nanoparticles using Ethanol as desolvating agent two formulations FA, FB were evaluated for product yield, drug content, entrapment efficiency, and *In vitro* drug release. The drug content results for FA, FB was found to be 81.9%, 82.9%. The mean diameter of FA, FB was found to be 942.4, 605.3nm. Zeta potential of FA, FB was found to be -12.8, 14.1 mv. Entrapment efficiency of FA, FB was found to be 31.3%, 33.5%. Product yield of FA, FB was found to be 91%, 92%. Loading capacity of FA, FB was found to be 13.5%, 15%. *In vitro* drug release of FA, FB was found to be 97% in 6 hrs, 96% in 7 hrs. Among all the formulations of nanoparticles FB formulation was found to be best with drug content of 82.9%, entrapment efficiency of 33.5%, small particle size of 605.3 nm, drug release 96% for 7 hours.

#### **3) Preparation of Naproxen Sodium Loaded BSA Nanoparticle by Desolvation Method Using Isopropanol As Desolvating Agent:-**

The prepared nanoparticles using Isopropanol as desolvating agent two formulations FC, FD were evaluated for product yield, drug content, entrapment efficiency and *In vitro* drug release. The drug content results for FC, FD was found to be 78%, 85.7%. The mean diameter of FC, FD was found to be 805.4, 788.8 nm. Zeta potential of FC, FD was found to be -16.5, 18.8 mv. Entrapment efficiency of FC, FD was found to be 30.2%, 31.5%. Product yield of FC, FD was found to be 90.1%, 94.3%. Loading capacity of FC, FD was found to be 13%, 14.2%. *In vitro* drug release of FC, FD was found to be 93.2% in 5 hrs, 98.7% in 6 hrs. Among all the formulations of nanoparticles FD formulation was found to be best with drug content of 85.7%, entrapment efficiency of 31.5%, small particle size of 788.8 nm, drug release 98.7% for 6 hours.

#### **Comparative Study of Best Formulation of All Three Desolvating Agent:-**

Naproxen sodium loaded BSA nanoparticles were prepared by desolvation technique using three different desolvating agents (Acetone, Ethanol and Isopropanol). With each desolvating agent two formulations were prepared by adapting continuous addition method and intermittent addition method. On comparing continuous addition method and intermittent addition method, intermittent addition method was showing better stability and less particle size diameter. A comparative study was performed among the formulation prepared by intermittent addition of Acetone, Ethanol, Isopropanol as desolvation agent to determine the best desolvating agent to prepare naproxen sodium nanoparticles.

#### **Scanning electron microscopic:-**

The bovine serum albumin nanoparticles prepared by desolvation technique (intermittent addition method) were characterized for surface morphology using Scanning electron microscopy (S-3700N, Hitachi, Mumbai).

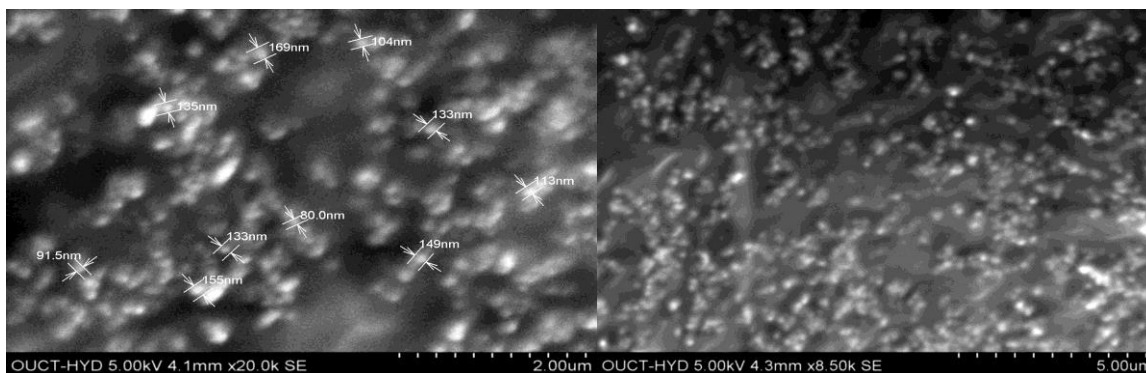


FIG 4:SEM pictures of P2 formulations of Naproxen sodium loaded BSA (intermittent addition method) nanoparticles.

From the resultant images the particles of the entire formulations show spherical surface ranging in nano meters.

**FTIR Spectrum:-**

The prepared three formulations were characterized for drug-polymer interactions using FT-IR

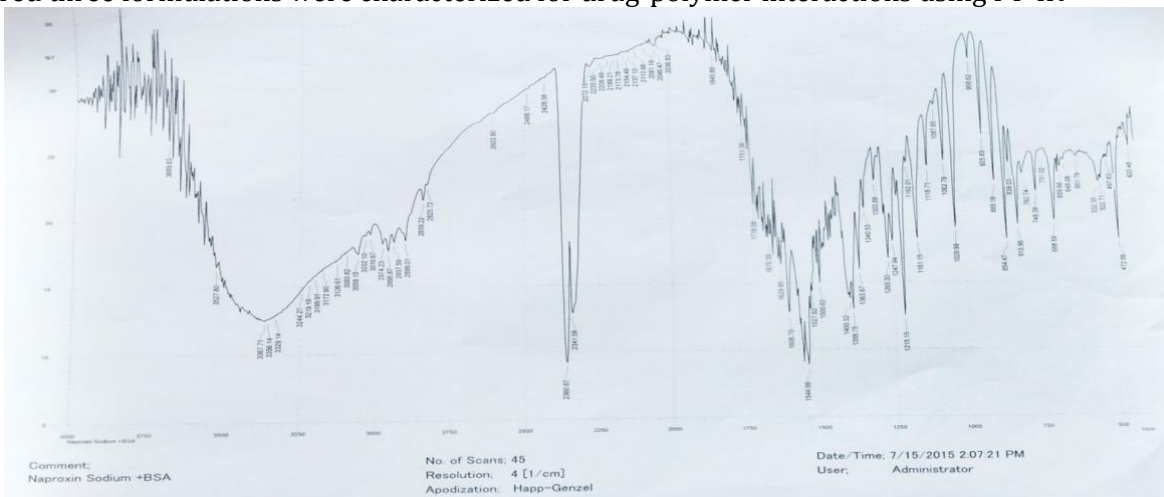


Figure 5: FTIR spectrum of P2 formulations of Naproxen sodium loaded Bovine serum albumin nanoparticles.

In the FTIR spectrum C-H stretching vibration at 3153-3059 cm<sup>-1</sup>, C- O stretching vibrations at 1300-1000 cm<sup>-1</sup>, aromatic C=C stretching vibration at 1631 cm<sup>-1</sup> and CH<sub>3</sub> bending vibration at 1363 cm<sup>-1</sup> indicating the significant peaks of Naproxen sodium. Thus no drug-polymer interactions observed.

**MEAN PARTICLE SIZE:-**

The prepared three formulations were characterized for mean particle diameter using Zeta size.

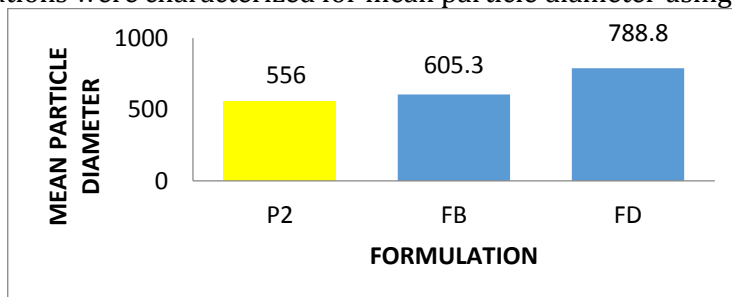


Figure 6: comparison of particle size values of three formulations of Naproxen sodium- bovine serum albumin nanoparticles (intermittent addition method).

All the formulations were within nano range. Among all these P2 formulation was showing small particle size when compared to other formulation.

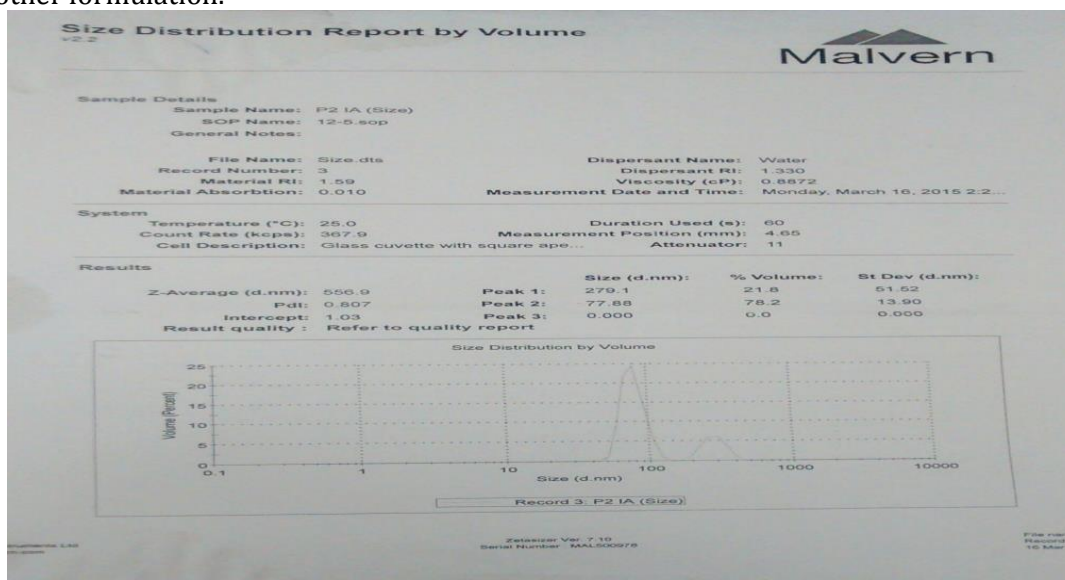


FIG 7: Particle size distribution report of P2 formulations of Naproxen sodium loaded BSA (Intermittent addition method) nanoparticles.

**ZETA POTENTIAL:-**The prepared three formulations were characterized for zeta potential value using Zeta sizer. The analysis was performed at a temperature of 25°C with double distilled water as dispersion medium.

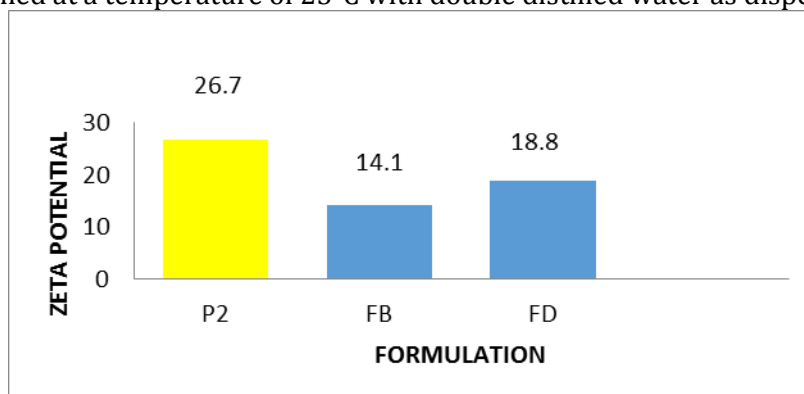


Figure 8: comparison of zeta potential values of three formulations of Naproxen sodium- bovinserum albumin nanoparticles (intermittent addition method). Among all these P2 formulation was showing better stability when compared to other formulation.

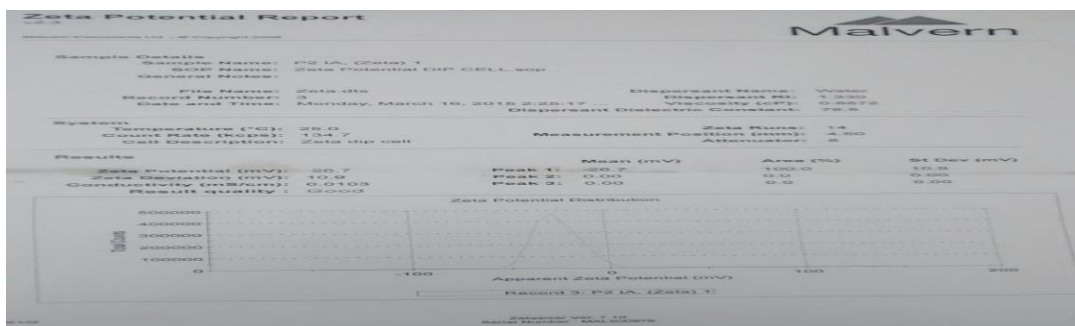


FIG 9: Zeta potential of P2 formulations of Naproxen sodium loaded BSA (intermittent addition method) nanoparticles.

**PRODUCT YIELD:-**

The prepared three formulation were evaluated for product yield

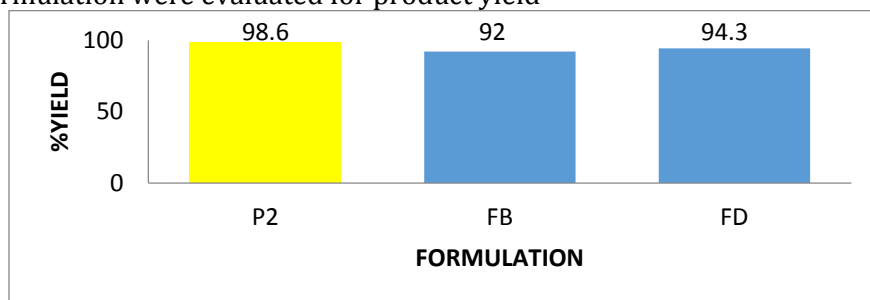


Figure 10 : comparison of product yield among the three formulations of Naproxen sodium loaded BSA (intermittent addition method) nanoparticles.

Among all these P2 formulation was showing higher product yield when compared to other formulation.

**DRUG CONTENT:-**The prepared nanoparticles were evaluated for drug content.

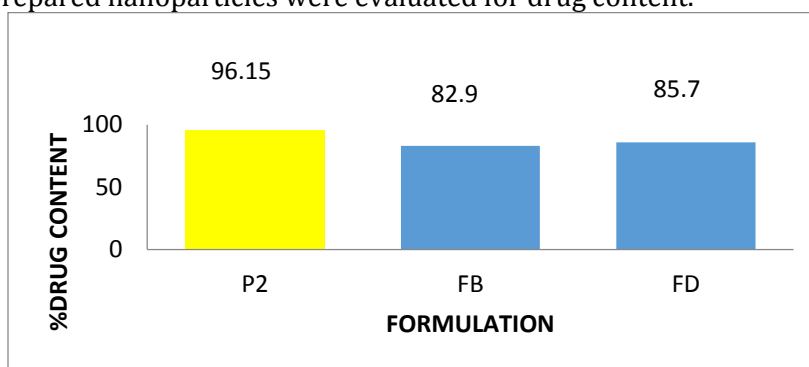


Figure 11 : comparison of drug content among the three formulations of Naproxen sodium loaded BSA (intermittent addition method) nanoparticles.

Comparison of three formulation of intermittent addition method. Among all these P2 formulation showing higher drug content when compared to other formulation.

**ENTRAPMENT EFFICIENT:-**The three formulations were evaluated for drug entrapment efficiency using cooling ultra-centrifuge.

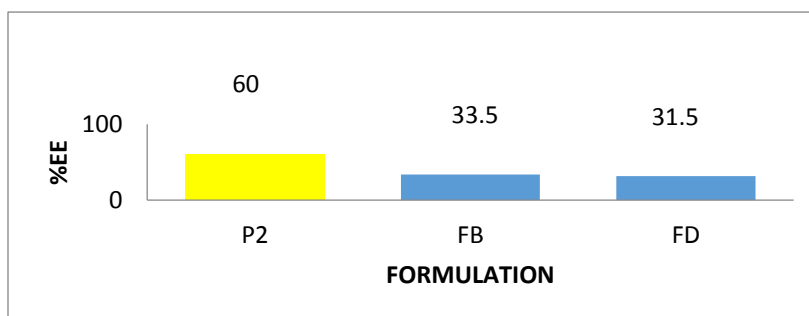


Figure 12: comparison of drug entrapment efficiency of the three formulations of Naproxen sodium loaded BSA (intermittent addition method) nanoparticles.

Among all these P2 formulation was showing higher drug entrapment efficiency when compared to other formulation.

**LOADING CAPACITY:-**The nanoparticles prepared by intermittent addition method were evaluated for loading Capacity.



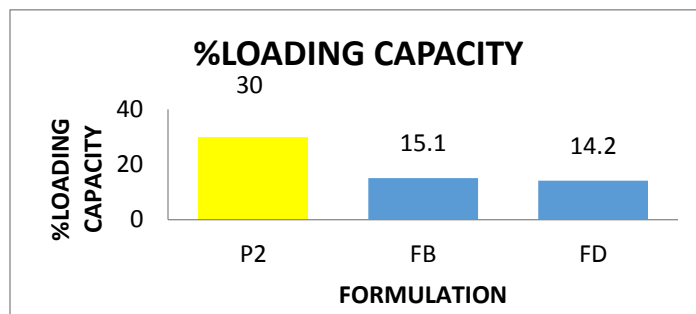


Figure 10 : comparison of drug loading capacity of the three formulations of Naproxen sodium loaded BSA (intermittent addition method) nanoparticles

Among all these P2 formulation was showing higher drug entrapment efficiency when compared to other formulation.

**Comparative Study of *In Vitro* Drug Release Profile Of The Best Formulation Of All The Three Desolative Agent:-**

All the formulations were evaluated for *In-vitro* drug release study.

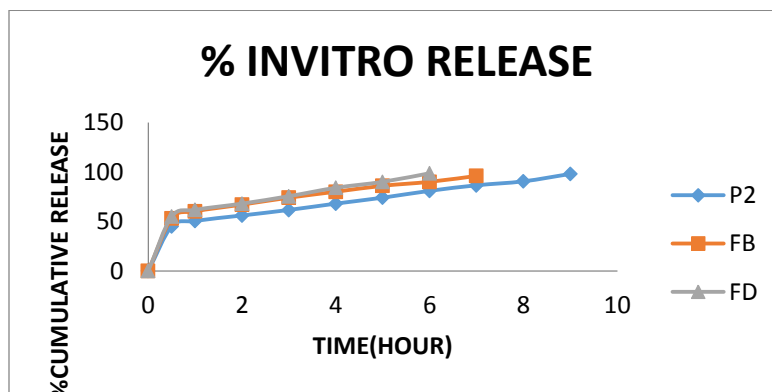
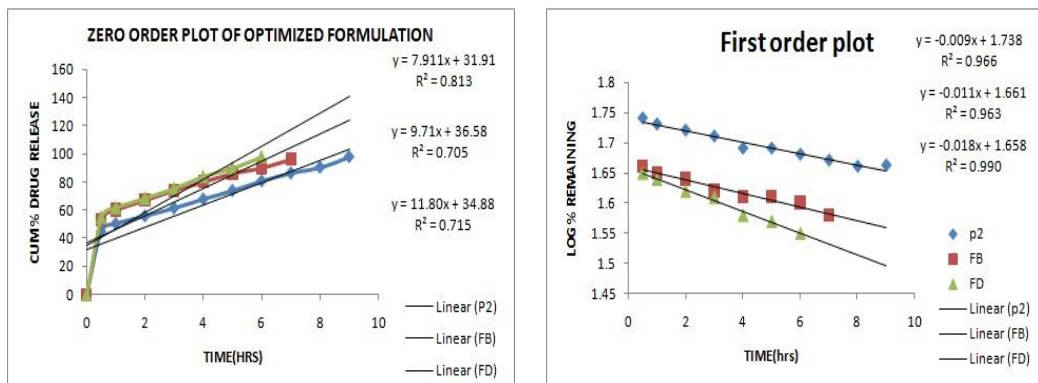


Figure 13: comparison of drug release study among the three formulations of Naproxen sodium loaded BSA (intermittent addition method) nanoparticles.

From the results it was observed that FB and FD formulations showed 96% and 98.7% of drug release within 7 hrs, 6 hrs time period respectively. P2 formulation showed 98% drug release within 9 hrs.

Several plots (Zero order plot, First Order Plot, Higuchi Plot and Peppas Plots) were drawn in order to know the release kinetics and drug release mechanism.



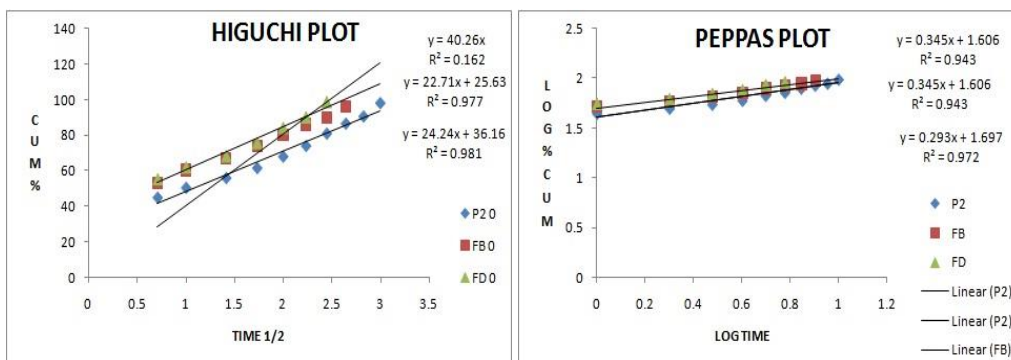


Figure 14: comparison of drug release plots (zero order plot, first order plot, higuchi plot & peppas plot) among the three formulations of Naproxen sodium loaded BSA (Intermittent addition method) nanoparticles

Table 2: Data of *In vitro* plots of best three formulation

Formulation	Zero order plot (R <sup>2</sup> )	First order plot (R <sup>2</sup> )	Higuchi plot (R <sup>2</sup> )	Peppas plot (n)
P2	<b>0.813</b>	0.966	0.162	<b>0.345</b>
FB	0.705	0.963	0.981	0.345
FD	0.715	0.990	0.977	0.293

From the results it was found that the drug release was following first order kinetics and fitted into Korsmeyer Peppas equation revealing Fickian diffusion mechanism.

### Conclusion:-

Attempts have been made to prepare Naproxen sodium nanoparticles by desolvation technique using various Desolvating agents such as Acetone, Ethanol, Isopropanol. Two methods were adapted for the addition of desolvating agent, namely continuous addition method and intermittent addition method. In continuous addition method the desolvating agent was added at a rate of 1ml per min. In intermittent addition method the desolvating agent was added at a rate of 1ml for every 5min time interval.

All the formulation prepared by continuous and intermittent addition of Acetone, Ethanol, Isopropyl alcohol were compared for mean particle diameter, stability, drug content, entrapment efficiency, loading capacity and drug release studies.

The Naproxen sodium nanoparticles were prepared by desolvation technique at three different RPM. Three formulations were prepared at 400, 600, 800 rpm respectively. All the three formulation were evaluated for particle size, zeta potential, drug content, loading capacity. From the result it was observed that for P1 formulation formulated at 800rpm was showing better stability with sustain release up to 7 hrs. Hence 800rpm was selected to further experiment.

From the result it was observed that intermittent addition method and Acetone were considered as best method and desolvating agent for the preparation of naproxen sodium nanoparticle respectively.

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