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Preparation and characterization of mefenamic acid loaded bovine serum albumin nanoparticles by desolvation technique using acetone as desolvating agent

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ABSTRACT

The objective of the present work is to prepare Mefenamic acid loaded bovine serum albumin nanoparticles by Desolvation technique. Drug-polymer dispersion was prepared under continuous mechanical stirring. Desolvating agent was added by means of two methods. In continuous addition method desolvating agent was added at the rate of 1ml/1min whereas in Intermittent addition method the desolvating agent was added at the rate of 1ml/5mins. Appearance of turbidity was considered as the end point. A cross-linking agent was added to stabilize the nanoparticles and stirring was continued for 12hrs.The obtained nano formulations were studied for characterization and evaluation parameters.Among the five formulations of continuous addition method and intermittent addition method the F3 formulations was found to be the best formulation with entrapment efficiency of 93.13% and 95.05% respectively, loading capacity of 32.04% and 32.3% respectively, mean particle diameter of 211.6nm and 208.7nm respectively and zeta potential value of -49.8mVand -52.8mV respectively. On comparison the intermittent addition method was concluded as the best method for the preparation of mefenamic acid nanoparticles because of its small mean particle diameter (208.7nm), zeta potential value of -52.8mV, higher drug entrapment efficiency (95.05%) and sustained drug release profile.

Keywords: Mefenamic acid, Desolvation, Bovine Serum Albumin, Particle size, Zeta potential.

INTRODUCTION

Nanoparticles

Nanotechnology has achieved breakthrough in therapeutics, bioengineering, diagnostics, imaging, and optics in recent vintage. The development of nanosystems by tailoring the macromolecules is the recent topic of interest[1]. As nanoparticles possess extraordinary, often tunable properties dramatically different from the bulk materials, such as high surface to volume ratio, particle size and so forth there is an enormous demand for the tailor made functional nanoparticle systems [2]. Inorganic, organic or hybrid nanoparticular materials are used in various applications fields as medicine, pharmaceuticals, analytics, catalysis, coating, and several others[3].

Nanoparticles are efficient and versatile devices for drug delivery as they can improve crucial properties of a drug entity such as solubility, pharmacokinetic, biodistribution and in vivo stability[4]. Due to their tailoring properties they can overcome physiological barriers and can help to guide their payload to specific cells or intercellular compartments. By which side effects can be minimized and therapeutic benefits of a drug can be increased. By virtue of their small size and by functionalizing their surface with polymers and appropriate ligands, polymeric nanoparticles can also be targeted to specific cells and locations in the body. Depending on the polymer characteristics, polymeric nanocarriers can also be engineered in such a way that they can be activated by changes in the environmental pH, chemical stimuli, or temperature[5-8].

Macrophages are well recognized phagocytic cells of the reticuloendothelial system (RES) and one of the main cells responsible for the uptake and clearance of administered drug-loaded nanoparticles[6]. In general, once nanoparticles are opsonised, endocytosis/phagocytosis occurs and the nanoparticles are incorporated in an endolysosome/phagolysosome and degrade. However, the ability of various nanoparticles to escape the endolysomal compartment allows incorporated drugs to be delivered to the cytoplasm and finally to the nucleus. Thus this property of the nanoparticles to be easily taken up by phagocytic cells makes them feasible to carry proteins, genes and other biological macromolecules as well. Other applications include cytoplasmic release of plasmid vectors and therapeutic agents (e.g. for cytoplasmic infections and for slow cytoplasmic release of drugs that act on nuclear receptors) [7-11].

Depending on the preparation methods used, two different types ofnanoparticles can be obtained, namely nanospheres and nanocapsules. Nanoparticles are drug loaded particles with diameter ranging from 1 to 1000nm[9-13]. 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 nanocapsules. Nanospheres have a matrix type structure and the drug may be adsorbed at the sphere surface or encapsulated within the particle. Nanocapsules are the vesicular system in which the drug is confined to a cavity consisting of an inner liquid core surrounded by a polymeric membrane. In this case the drug is usually dissolved in the inner core but may also be adsorbed to the capsule surface[10].

Advantages
By formulating drug entities as nano particulate systems, increased stability of any volatile pharmaceutical agents is achieved. They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.Less toxicity and good control over size and size distribution. Stable dosage forms of drug which are either unstable or have unacceptably low bioavailability can be formulated as nanoparticles. When formulated as nano systems an improved drug bioavailability through enhancing aqueous solubility is seen. It increases the resistance time in the body (increasing the half-life for the clearance/ increasing specificity for its cognate receptor).Relatively higher intercellular uptake is observed because of their small size and can penetrate through smaller capillaries and is taken up by cells, which allow efficient drug accumulation at the target sites.The use of biodegradable materials for nanoparticle preparation allows sustained drug release within the target site over a period of days or even weeks[11-15].

Limitations
Their small size and large surface area can lead to particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms.In addition, small particles size and large surface areas readily result in limited drug loading and burst release.The major threat to safety question is yet to be revealed[13].

Types of nanoparticles:
Nanoparticles are broadly classified as polymeric nanoparticles and solid lipid nanoparticles. Polymeric nanoparticles are made from biodegradable and biocompatible polymers such as polymers, either natural polymer (e.g., gelatin, chitosan etc.) or synthetic polymers (e.g., polylactides, polyacrylcyanoacrylates etc.).

Carrier (Polymer):
Polymeric nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides & synthetic polymers[14]. The selection of matrix materials is dependent on many factors including[15-20]:

(a) Size of nanoparticles required;
(b) Inherent properties of the drug, e.g., aqueous solubility and stability;
(c) Surface characteristics such as charge and permeability;
(d) Degree of biodegradability, biocompatibility & toxicity;
(e) Drug release profile desired; and
(f) Antigenecity of the final product.

Generally the properties of the resultant polymeric nanoparticles depend evidently on the method and polymer carrier chosen. Natural polymers are most commonly used polymers due to their bio-compatibility and bio-degradability. They are Gelatin, Sodium alginate, Albumin, Chitosan, Fibroin, Lectins, Legumin and so forth. The synthetic polymers that can be used are Poly lactic acid(PLA), Ethyl cellulose, Eudragit® S100, Poly (lactide co-glycolides) (PLGA), Poly epsilon caprolactone, Poly (ethylene glycol) (PEG) and so forth.

Bovine Serum AlbuminS(BSA) is a macromolecular carrier and is widely used to prepare nanoparticles, due to its biodegradability, nontoxicity and nonimmmunogenicity. As a major plasma protein, albumin has a distinct edge

over other materials for nanoparticle preparation. On the other hand, albumin nanoparticles are biodegradable, easy to prepare in defined sizes, and carry drug entities on their surfaces by covalent linkage that can be used for ligand binding. Drugs entrapped in albumin nanoparticles can be digested by proteases and drug loading can be quantified. A number of studies have shown that albumin accumulates in solid tumors making it a potential macromolecular carrier for the site-directed delivery of antitumor drugs[23-26].

In this study Mefenamic acid was formulated as nanoparticle drug delivery system using natural polymer (Bovine serum albumin). Mefenamic acid is a widely prescribed NSAID and used as first line therapy for the treatment of ailments such as Arthritis and Dysmonorrhoea. Mefenamic acid is a Non-steroidal anti-inflammatory drug (NSAID), with analgesic, and anti-pyretic properties. It is considered to be a BCS Class II drug (low soluble and high permeable). Mefenamic acid binds the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase. As these receptors have a role as a major mediator of inflammation, the symptoms of pain are temporarily reduced.

Mefenamic acid has less biological half life (t1/2) of 2hrs and being an NSAID has a major side effect of gastric irritation. Formulating such drug into nanoparticles using biodegradable and biocompatible polymers is expected to increase the sustain release action and patient compliance with fewer side effects.

MATERIALS AND METHODS

2.1 Materials: Mefenamic acid (purchased from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore), Bovine serum albumin (HiMedia Laboratories Pvt. Ltd., Mumbai), Potassium Dihydrogen phosphate, Sodium hydroxide, Glutaraldehyde 25% and Acetone from SD Fine Chemical Limited, Mumbai.

2.2 Methodology: Preparation of Mefenamic acid loaded BSA nanoparticles was carried out by Desolvation technique. Desolvation is a thermodynamically driven self –assembly process for polymeric materials to prepare nanoparticles. Aqueous drug polymer dispersion was prepared and pH was adjusted (away from iso-electric point). The desolvating agent (Acetone) was added under continuous mechanical stirring. 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 appearance of turbidity was observed as the end point of the reaction. A cross-linking agent (glutaraldehyde 25%) was added and stirring was continued. The solvent was removed by rotary evaporation at a vacuum pressure of 760mmHg. Free flowing amorphous nanoparticles were obtained.

Five formulations were prepared by varying the concentration of polymer and drug for each method (Continuous addition method and intermittent addition method).

Table1: parameters optimized for the preparation of Mefenamic acid nanoparticles by desolvation technique

Optimized parameter	Formulations	Variables	Constant parameters
pH	A1	5	Stirring speed=700rpm
	A2	**7**	Stirring time=9hrs
	A3	9	
Stirring speed (rpm)	B1	500	pH=7
	B2	**700**	Stirring time=9hrs
	B3	900	
Stirring time (hours)	C1	6	pH=7
(after the addition of	C2	9	Stirring speed=700rpm
Cross-linking agent)	**C3**	**12**	

Table 2: Optimized formulations for the preparation of Mefenamic acid-Bovine serum albumin nanoparticles

Formulation	Drug: polymer ratio	Optimized parameters
F1	1:1	pH=7
F2	1:1.5	Stirring speed = 700rpm
F3	1:2	Stirring Time = 12hrs
F4	1.5:1	
F5	2:1	

2.3 Characterization andEvaluation of Mefenamic acid nanoparticles

Study of surface morphology of nanoparticles by scanning electron microscope (SEM)

The prepared amorphous nanoparticles were dispersed in deionised 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 for morphology using S-3700N, Hitachi, Japan

Determination of size distribution and zeta potential

The prepared nanoparticles were dispersed in deionised water and sonicated for 30 minutes. The resultant dispersion was diluted and observed for particle size and zeta values using Zetasizer (Horiba Instruments Ltd).Zeta potential reflects the electrical potential of the particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above (+/-) 25 mV have been shown to be stable in suspension, as the surface charge prevents particle aggregation[3, 11]. The Zeta sizer calculates the particle size in a sample by means of Stokes-Einstein Equation (refer Eq.1).

$D=KT/6\pi\eta R_H$ Eq.1

Where D= Diffusion coefficient
K= Boltzmann Constant
T= Temperature
η= Viscosity
R_H= Hydrodynamic radius.

Study of interaction between the drug and the excipients using FTIR spectroscopy

Mefenamic acid, Bovine serum albumin and 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^{-1} in a FTIR 7000[9, 10](Horiba scientific, Mumbai.).

Drug content

The nanoparticle formulations were examined for drug content. Prepared nanoparticle were added to equivalent quantity of methanol and kept for magnetic stirring at 600 rpm for 3hrs separately. The amount of drug present in the supernatant was analyzed under UV spectrophotometer.

Entrapment efficiency study

The prepared formulations were examined for Entrapment Efficiency. 50mg of the prepared formulation was taken in equivalent quantity of 7.2 pH phosphate buffer. The suspension is ultra centrifuged at 17000rpm and temperature of -4^oC for 40 minutes. The entrapment efficiency (EE) and Loading Capacity (LC) can be expressed as follow[10, 11] (refer Eq2 & 3);

%EE= Total amount of the drug entrapped ×100/ Total amount of drug initially taken Eq.2

%LC =Total amount of the drug entrapped × 100/ Total weight of nanoparticles taken Eq.3

***In vitro* drug release study of 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 Arbitary shaker in 7.2 pH buffer at a temperature of 37 (+/-) 0.5^oc 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 285nm.

RESULTS AND DISCUSSION

3.1 Mefenamic Acid Loaded BSA Nanoparticles by Continuous Addition Method and Intermittent addition method.

The pH is the most important factor to control the coagulation of BSA molecule during desolvation process. The isoelectric point (pI) of BSA is about 4.7. When the pH of solution was close to pI, enhanced protein-protein reactions might occur resulting in increased coagulation among BSA. This is due to the higher electrostatic repulsion resulting in larger particles. At pH 7 BSA possess a negative charge at which coagulation results in formation of smaller particles. With increase in pH beyond 7 the solubility of BSA in aqueous medium is decreased[29-31].

As reported by the previous studies for the desolvated albumin particles, the lowest required glutaraldehyde concentration for the production of stable nanoparticles appear to be 40%and 50% [33]. In the present study 25% of glutaraldehyde was found to result in stable particles.

Previous study support the use of acetone as desolvating agent may yield nanoparticles with particle size<200nm compared to ethanol as desolvating agent[34]. This is because acetone being a better non-solvent to BSA than other organic solvents.

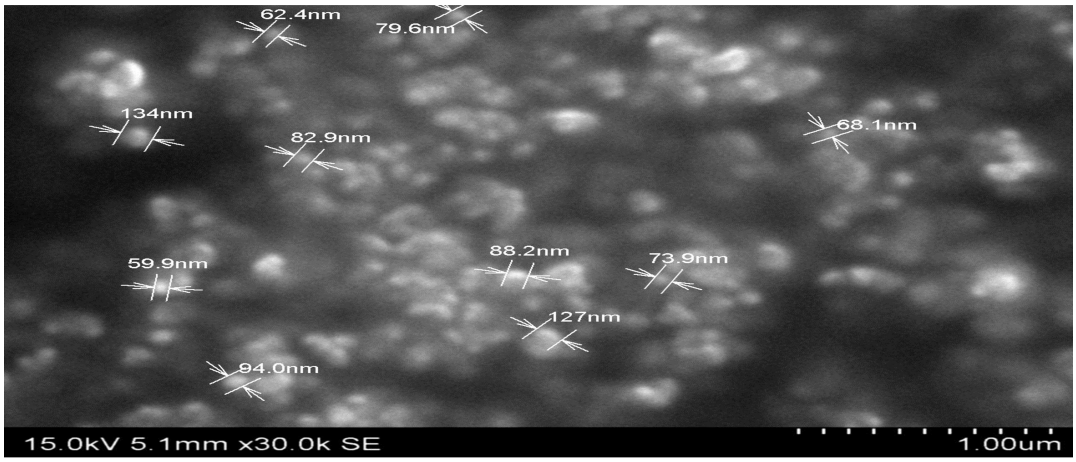

Fig. 1: SEM images of F3 formulation of continuous addition method

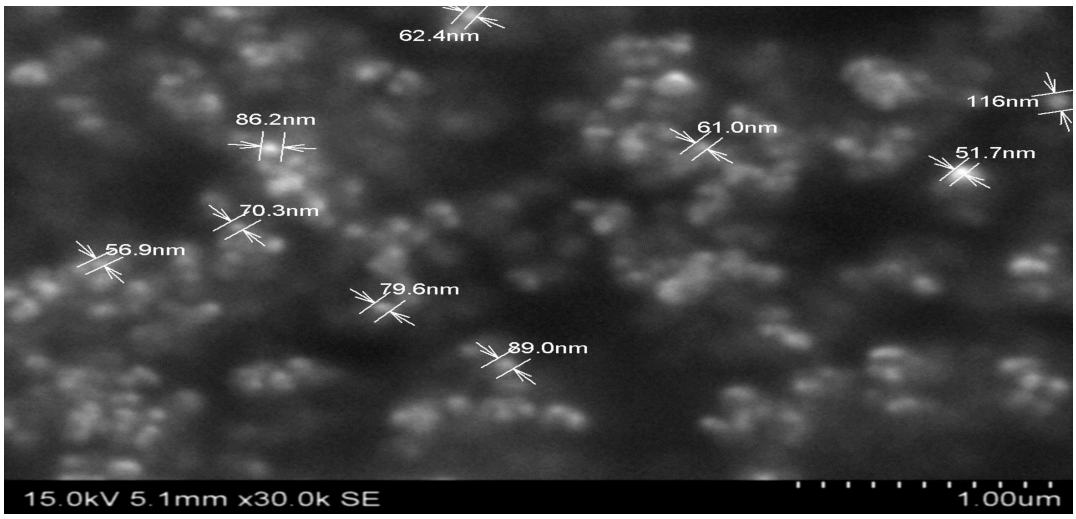

Fig.2: SEM pictures of F3 formulations of intermittent addition method
From the resultant images all the five formulations show spherical surface in nano meters.

Effect of Drug-polymer concentration on optimized formulation

The effect of drug-polymer concentration on the optimized formulation was studied. By varying drug-polymer concentration five formulations were prepared for each method. The prepared formulations were characterized and evaluated for following parameters.

CHARACTERIZATION PARAMETERS

3.1.1 Scanning electron microscopic

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

3.1.2 Particle Size Distribution

The prepared formulations were characterized for particle size distribution using Zeta sizer (Horiba Scientifics, Mumbai). The analysis was performed at a temperature of 25^{o}C with double distilled water as dispersion medium.

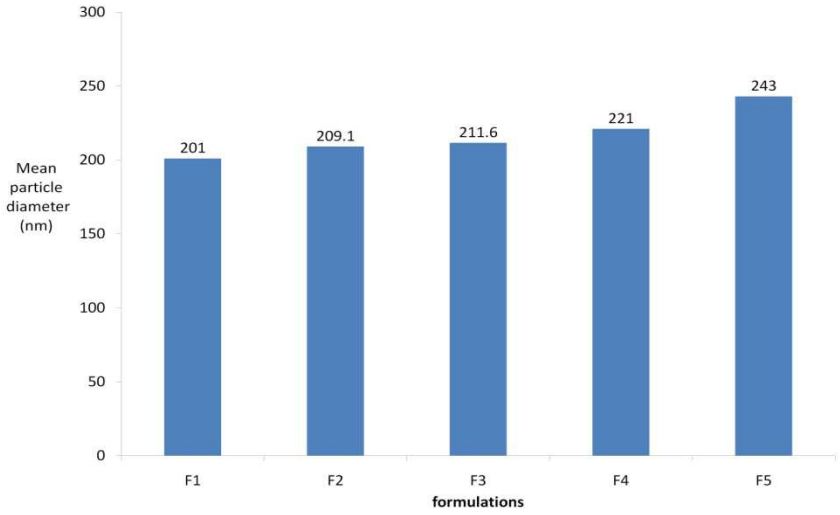

Fig.3: comparison of mean particle diameter of five formulations of continuous addition method

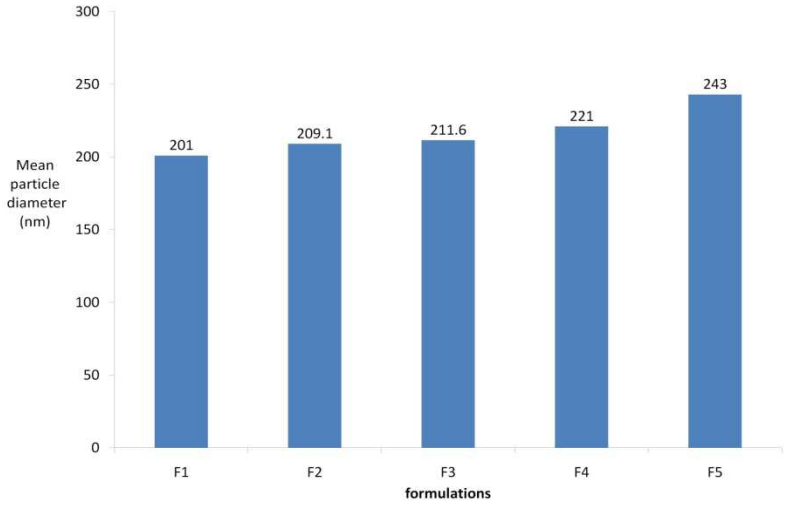

Fig.4: comparison of mean particle diameter of five formulations of intermittent addition method

All formulations were within nano range. The mean particle diameter of five formulations F1, F2, F3, F4 and F5 of continuous addition method was found to be 201, 209.1, 211.6, 221 and 243 nm respectively. The mean particle diameter of five formulations F1, F2, F3, F4 and F5 of intermittent addition method was found to be 193.2, 191, 208.7, 220 and 227 nm respectively.

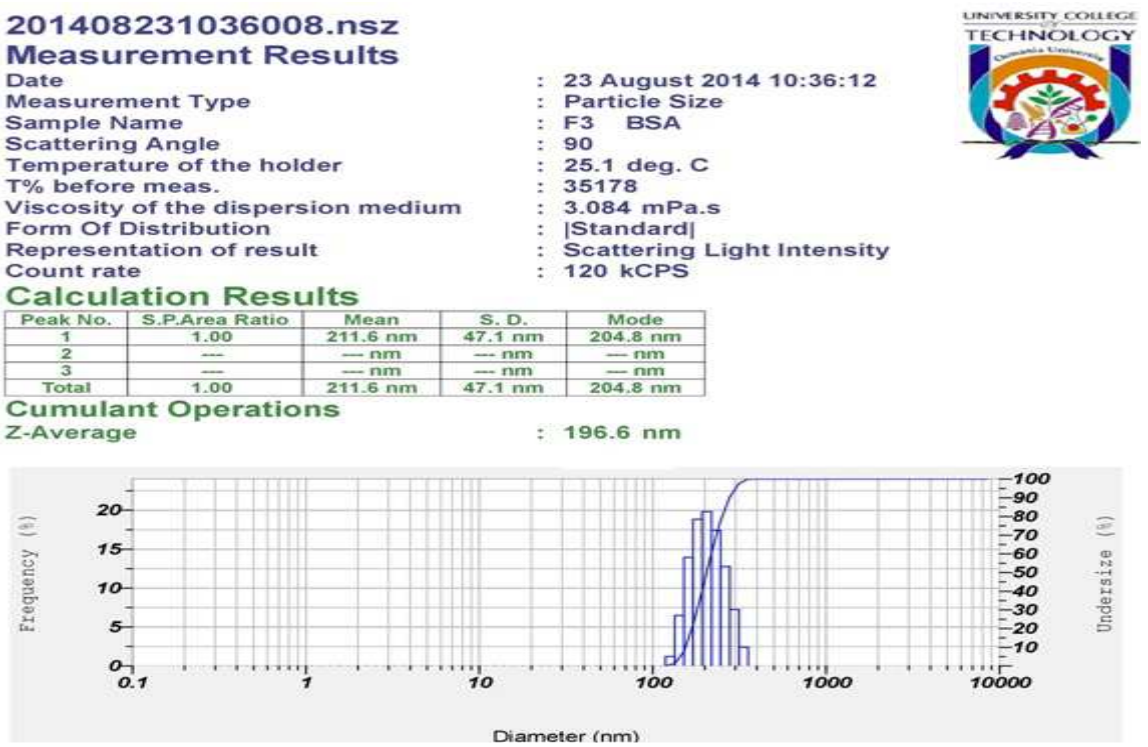

Fig.5: Particle size distribution report of F3formulations of continuous addition method

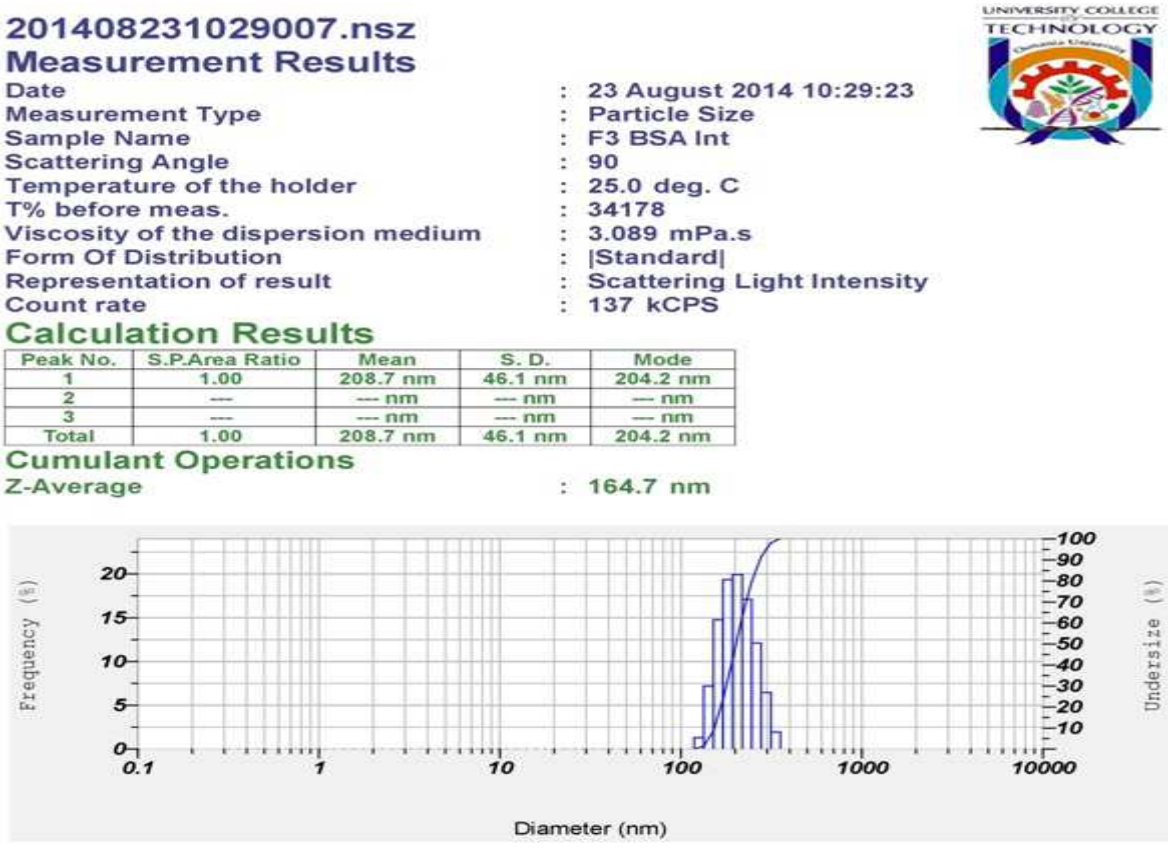

Fig.6: Particle size distribution report of F3 formulations of intermittent addition method

The results show that polymer concentration has an effect on particle size of the nanoparticles. With Increase in the polymer concentration an increase in particle size was observed[41].This might be explained by the fact that Increased BSA concentration during the desolvation process presumably led to increased nucleation of BSA particles. Thus resultingin the formation of larger BSA nanoparticles [35, 40].

3.1.3 Zeta potential

The nanoparticles prepared by continuous addition method were characterized for zeta potential using Zeta sizer (Horiba Scientific, Mumbai). The analysis was performed at a temperature of 25^{o}C with double distilled water as dispersion medium.

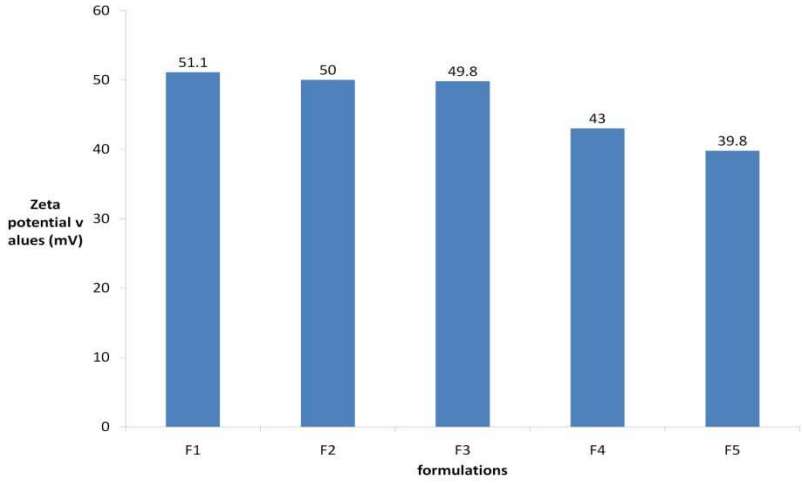

Fig.7: comparison of zeta potential values of five formulations of continuous addition method

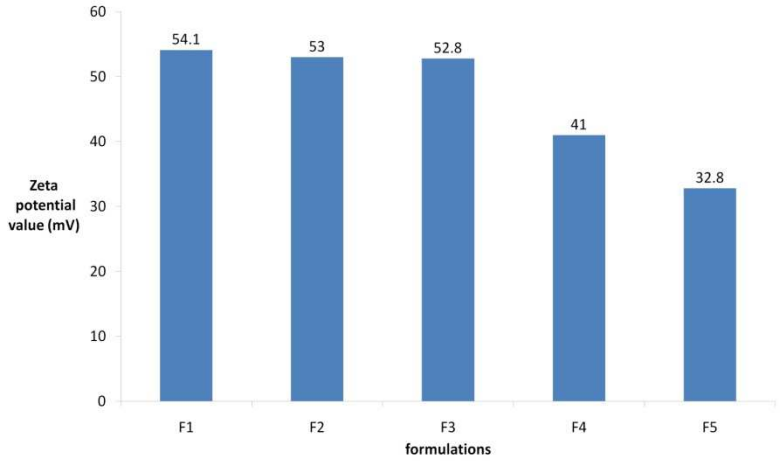

Fig.8: comparison of zeta potential values of five formulations of intermittent addition method

From the results all the formulations were found to be stable. The zeta potential values of five formulations of continuous addition method F1, F2, F3, F4 and F5 was found to be 51.1, 50, 49.8, 43 and 39.8 mV respectively. The zeta potential values of five formulations of intermittent addition method F1, F2, F3, F4 and F5 was found to be 54.1, 53, 52.8, 41, 32.8 mV respectively.

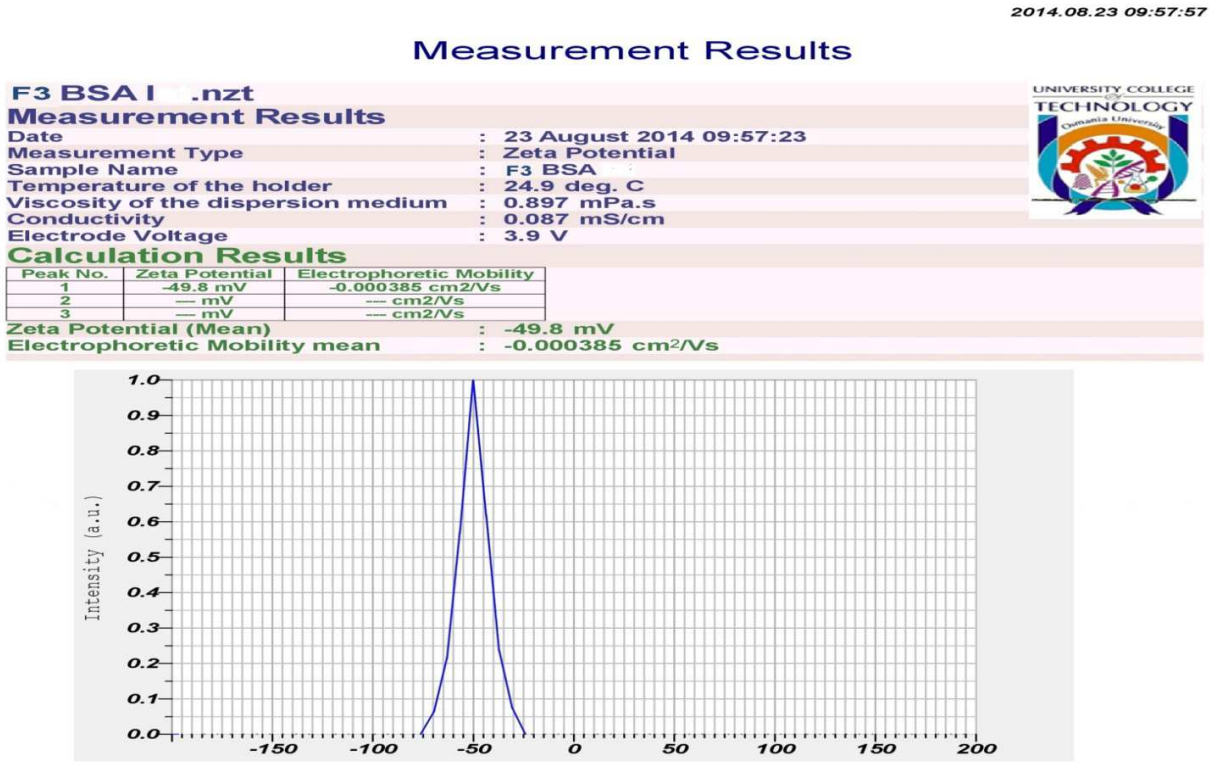

Fig.9: Zeta potential report of F3 formulations of continuous addition method

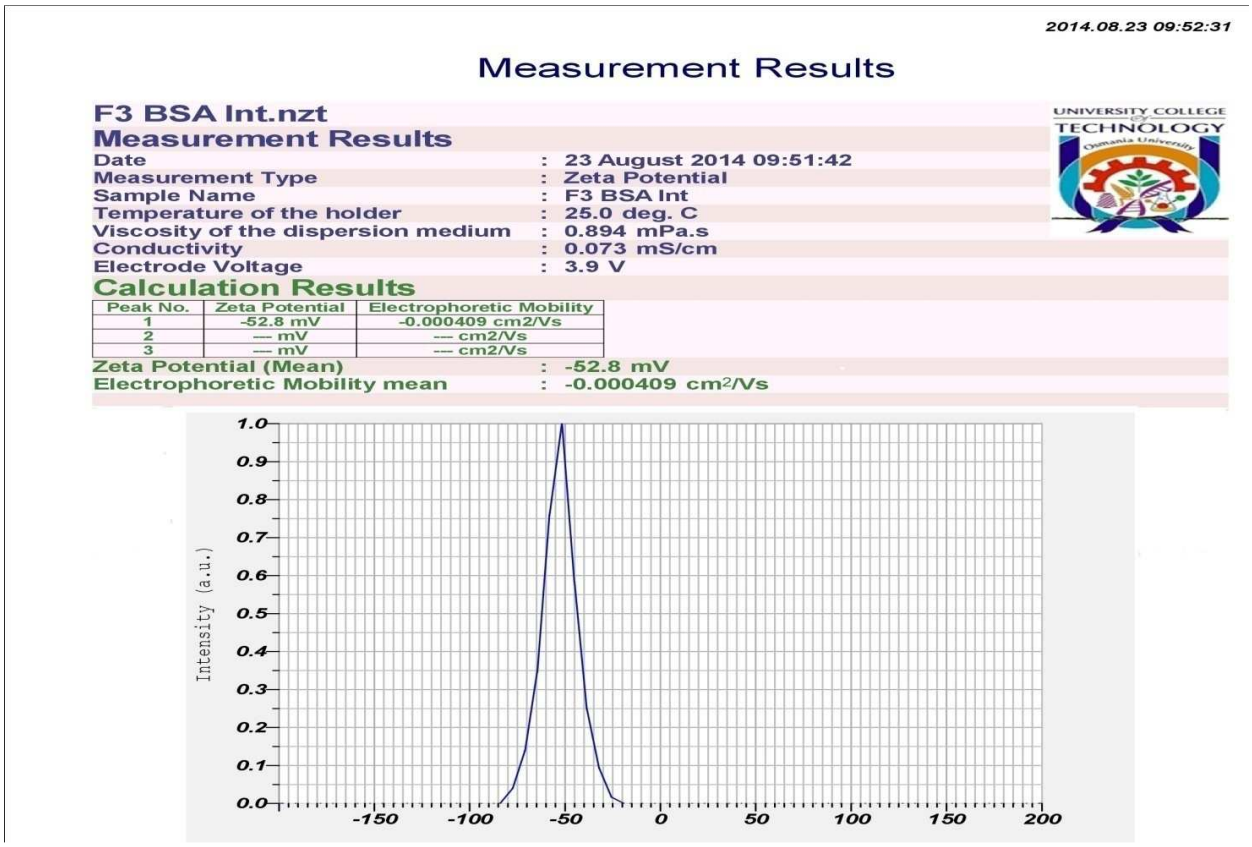

Fig.10: Zeta potential report of F3 formulations of intermittent addition method

From the results it was observed that increase in polymer concentration decreased the stability of the particles. This can be explained as increase in particle size decreases the stability as the rate of aggregation increases[37, 41, 42].

3.1.4 FTIR Spectrum

The prepared formulations were characterized for drug polymer interactions using FTIR.

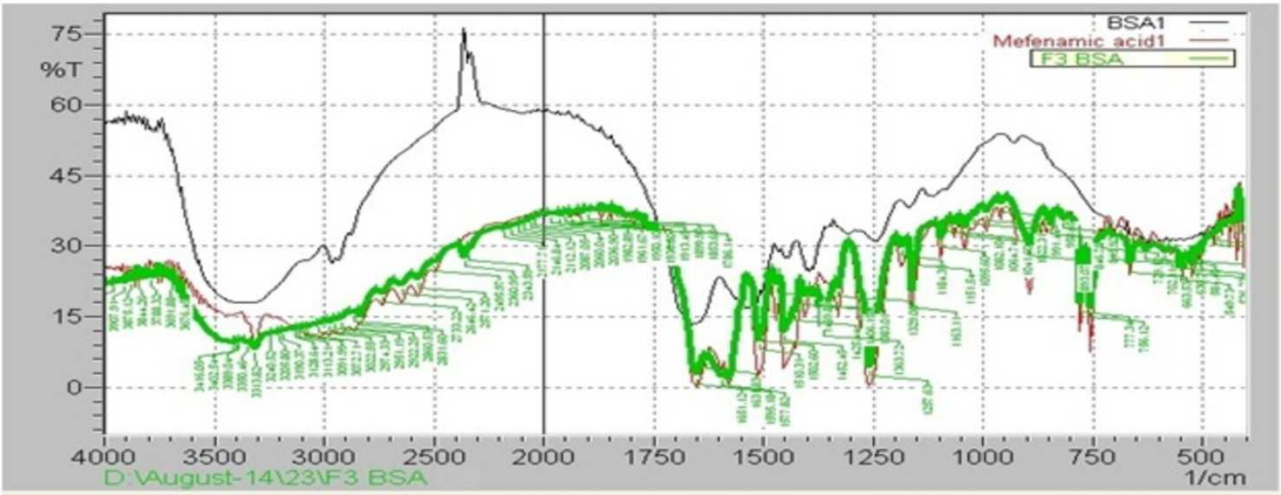

Fig.11: FTIR spectrum of F3 formulations of continuous addition method

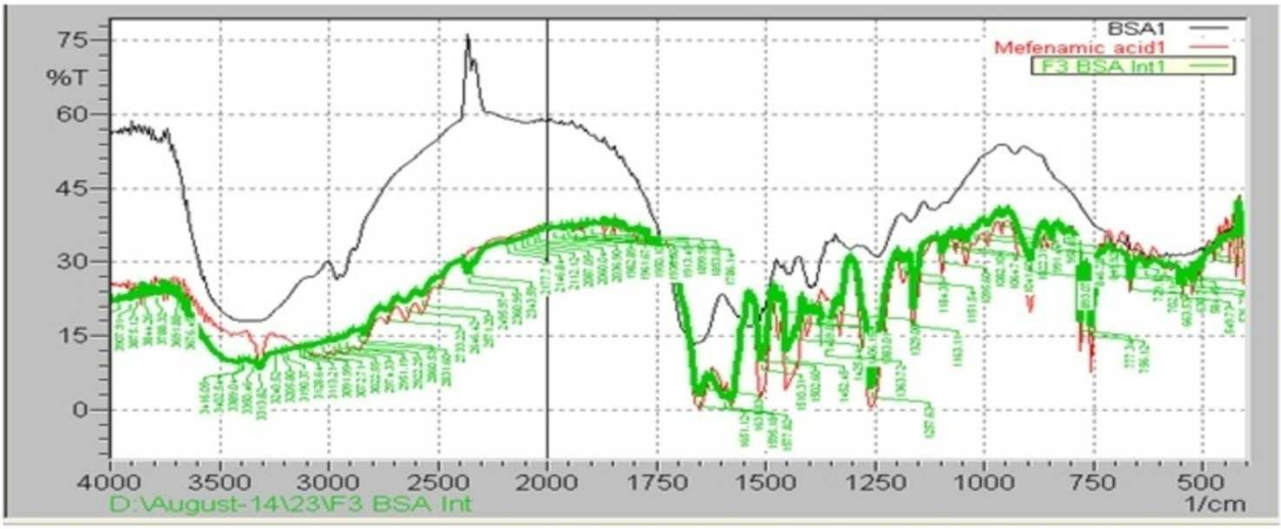

Fig.12: FTIR spectrum of F3 formulations of intermittent addition method

In the FTIR spectrum NH-stretching vibration at 3310.23 cm^{-1}, NH-bending vibrations at 1647 cm^{-1}, C=0 stretching vibration at 1572 cm^{-1}, C=C stretching vibration at1570.08 $cm^{-1}$and aromatic O-CH_3 stretching vibration at 1163 cm^{-1} indicating the significant peaks of Mefenamic acid. Thus no drug-polymer interactions observed.

EVALUATION PARAMETERS

3.1.5 Product yield

The product yield was estimated for all the prepared formulations.

The product yield of five formulations of continuous addition method F1, F2, F3, F4 and F5 was found to be 90.3%, 98.84%, 93.3%, 98.7% and 93.3% respectively. Out of all five formulations the F2 formulation showed higher product yield. The product yield of prepared five formulations of intermittent addition method F1, F2, F3, F4 and F5 was found to be 92.8%, 91.83%, 94%, 86.98% and 98.6% respectively. Out of all five formulations the F5 formulation showed higher product yield.

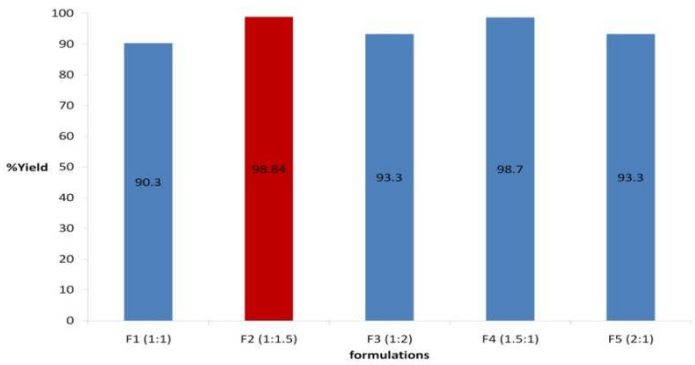

Fig.13: comparison of product yield among the five formulations of continuous addition method

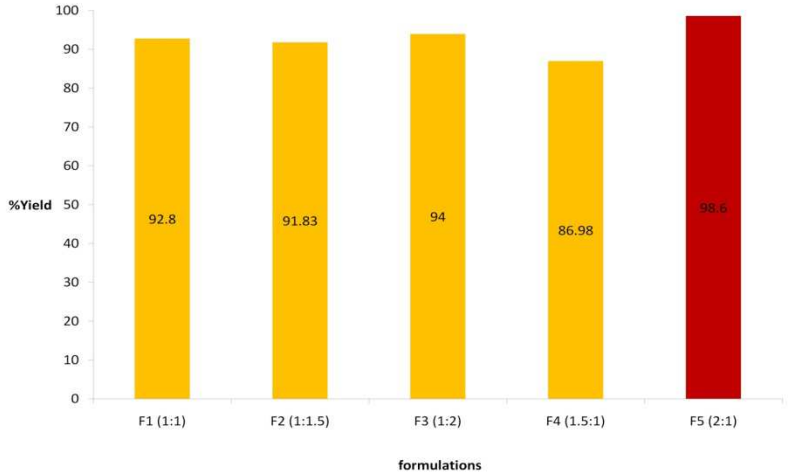

Fig.14: comparison of product yield among the five formulations of intermittent addition method

3.1.6 Drug content

The prepared formulations were evaluated for drug content.

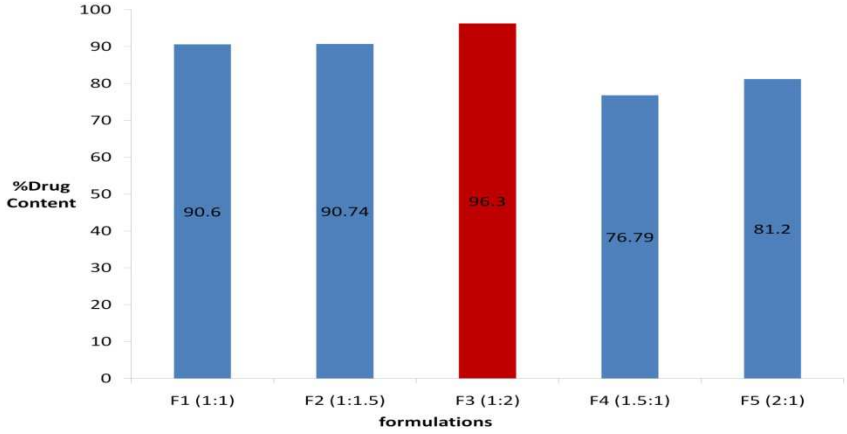

Fig.15: comparison of % drug content among the five formulations of continuous addition method

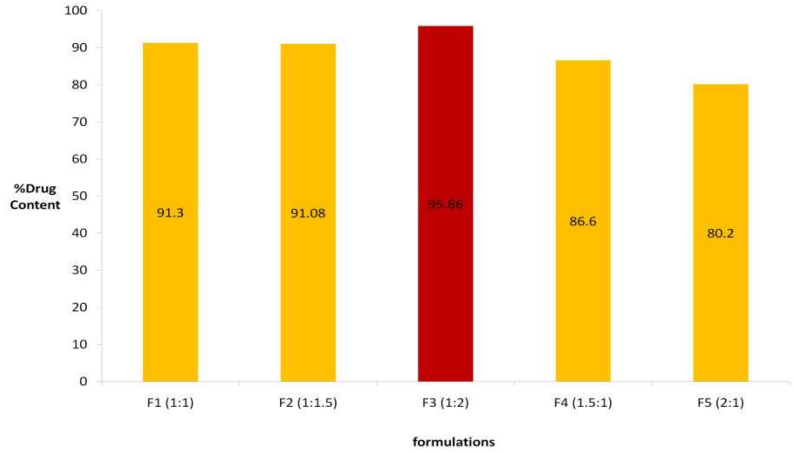

Fig.16: comparison of drug content among the five formulations of intermittent addition method

The drug content of five formulations of continuous addition methods F1, F2, F3, F4 and F5 was found to be 90.6%, 90.74%, 96.3%, 76.79% & 81.2% respectively. From the results the F3 formulation showed higher drug content. The drug content of all the five formulations of intermittent addition method F1, F2, F3, F4 and F5 was found to be 91.3%, 91.08%, 95.86%, 86.6% and 80.2%. From the results the higher % drug content was observed for F3 formulation.

3.1.7 Encapsulation efficiency

The prepared formulations were evaluated for drug entrapment efficiency.

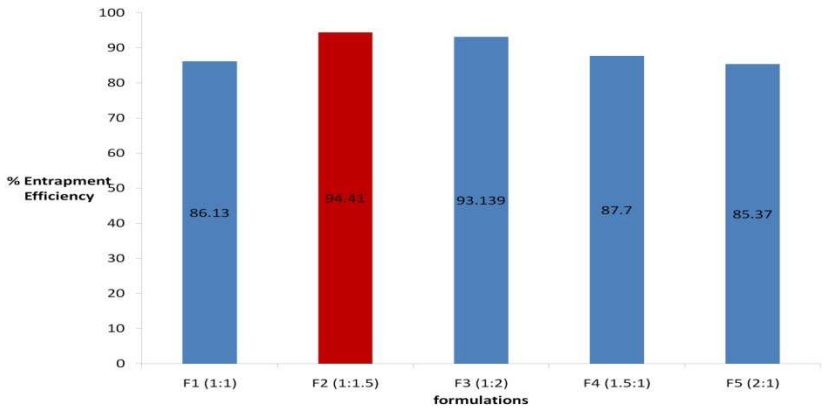

Fig.17: comparison of drug entrapment efficiency among the five formulations of continuous addition method

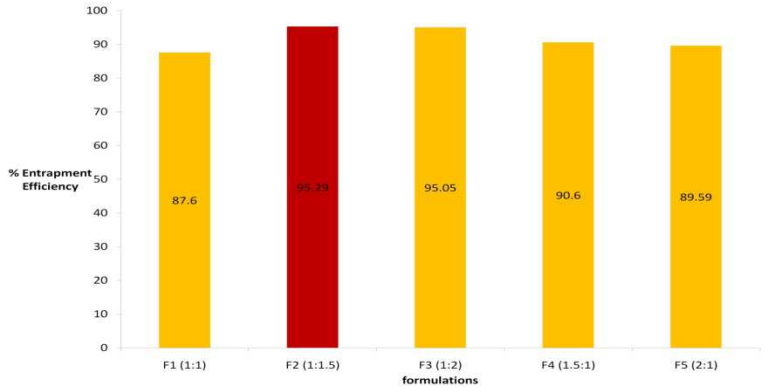

Fig.18: comparison of drug entrapment efficiency of the five formulations ofintermittent addition method

For all the five formulations F1, F2, F3, F4 and F5 prepared by continuous addition method, the drug entrapment efficiency was found to be 86.13%, 94.41%, 93.13%, 87.7% and 85.37% respectively. From the results the F2 formulation was showing higher percentage of drug entrapment efficiency. Among all the five formulations F1, F2, F3, F4 & F5 prepared by intermittent addition method, the drug entrapment efficiency was found to be 87.6%, 95.29%, 95.05%, 90.6% and 89.59% respectively. Higher drug entrapment efficiency was observed for F2 formulation.

With increase in polymer concentration the percentage of drug entrapment efficiency was found to be increased. This can be related to the availability of higher amount of polymer for entrapment[40].

3.1.8 Drug loading capacity

The nanoparticles prepared were evaluated for drug loading capacity.

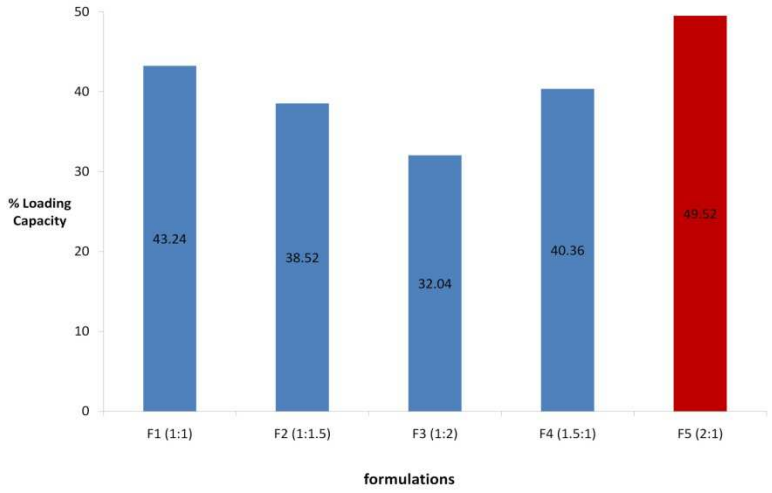

Fig.19: comparison of drug loading capacity among the five formulations of continuous addition method

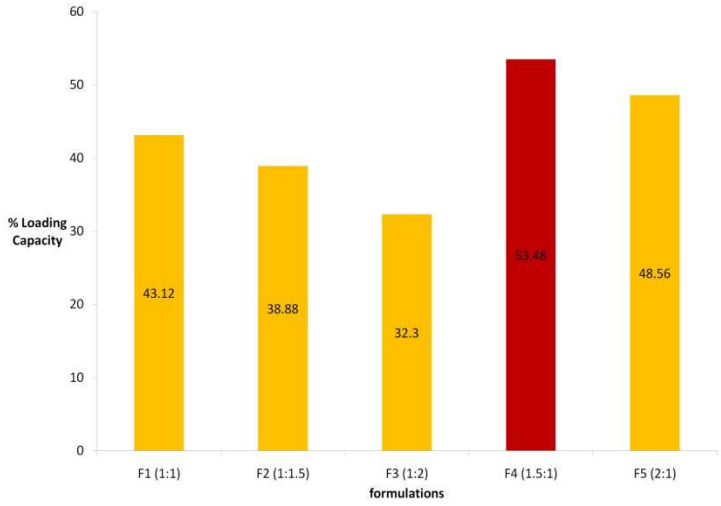

Fig.20: comparison of drug loading capacity of the five formulations of intermittent addition method

For all the five formulations F1, F2, F3, F4 and F5 prepared by continuous addition method, the drug loading capacity was found to be 43.24%, 38.52%, 32.04%, 40.36% and 49.52% respectively. From the results the F5 formulation showed higher drug loading capacity.Among all the five formulations F1, F2, F3, F4 and F5 prepared by intermittent addition method, the drug loading capacity was found to be 43.12%, 38.88%, 32.3%, 53.48% and 48.56% respectively. Higher drug loading capacity was observed for F4 formulation.

From the results it was found that the drug loading capacity was observed to have a direct linear relationship with the drug concentration. It can be said that the saturation capacity of the polymer with respect to the selected drug occurred at a relatively lower concentration and at a faster rate[36-41].

3.1.9 Comparison of *In vitro* drug release data of five formulations of Continuous addition method.
All the formulations were evaluated for *In-vitro* drug release study conducted for a time period of 24 hrs.

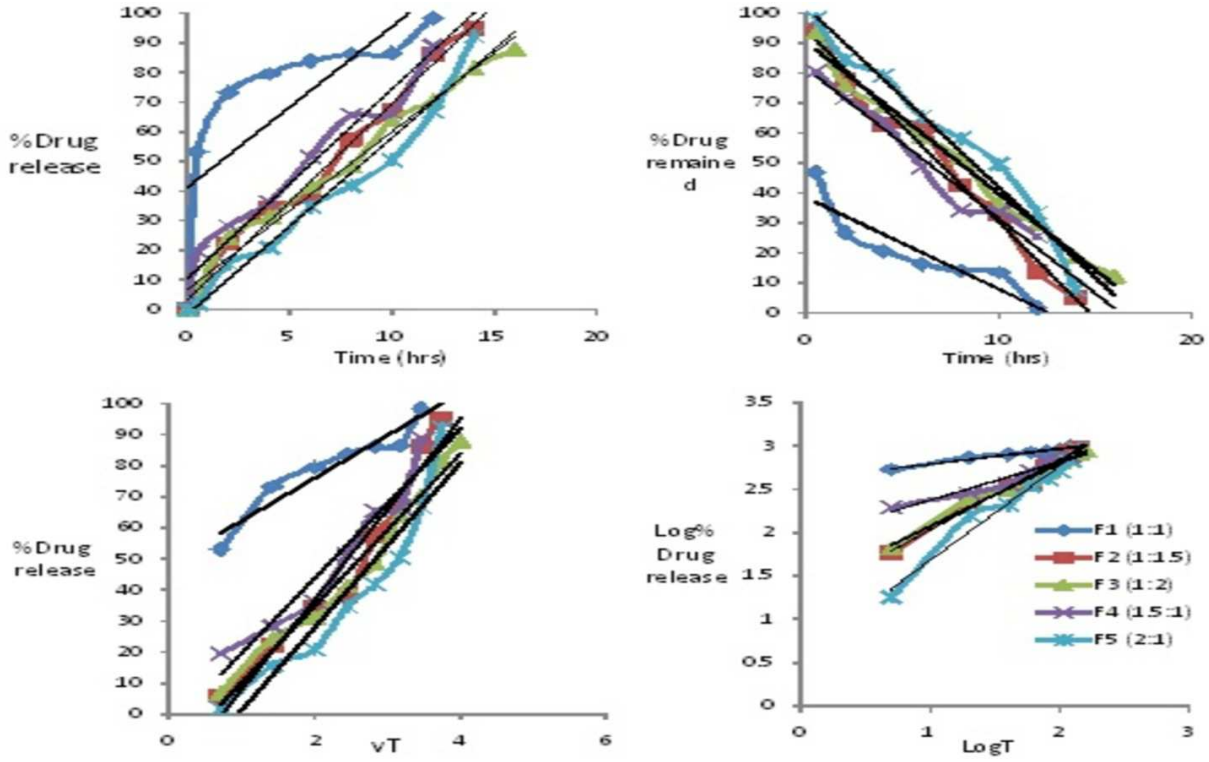

Fig.21: comparison of drug release plots (zero order plot, first order plot, higuchi plot &peppas plot) among the five formulations of continuous addition method

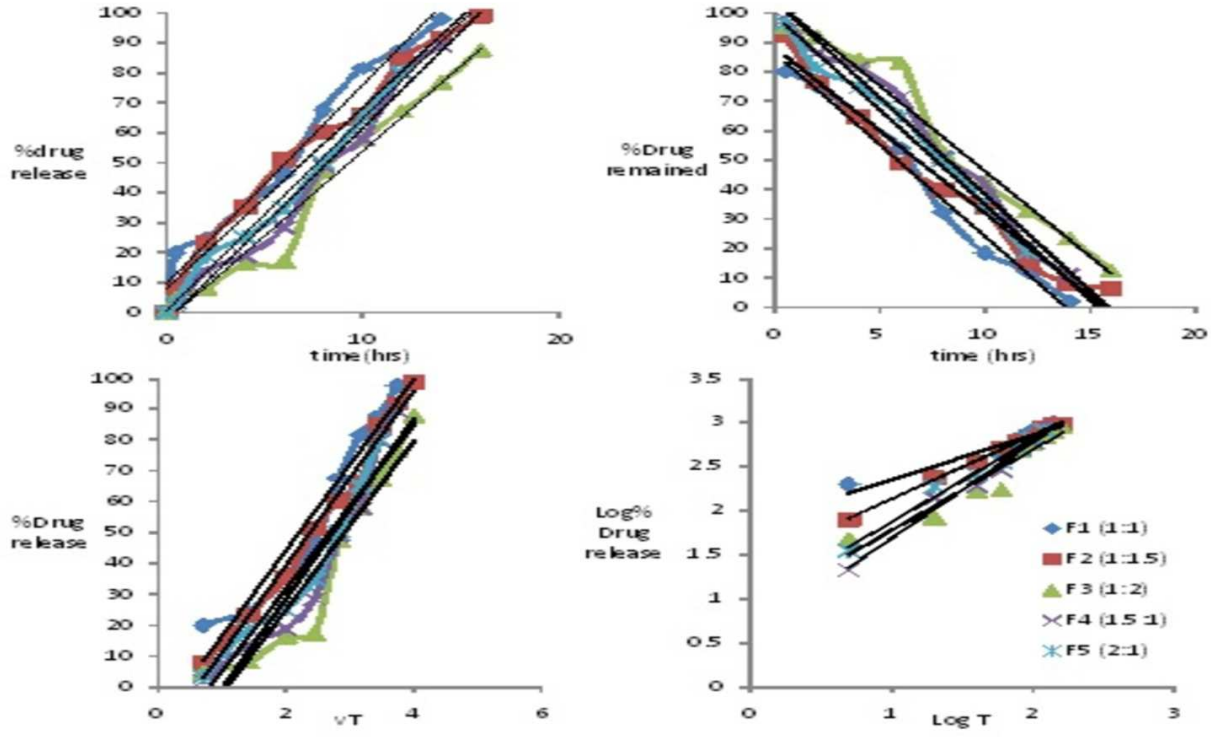

Fig.22: comparison of drug release plots (zero order plot, first order plot, higuchi plot &peppas plot) among the five formulations of intermittent addition method

From the results it was observed that F1 and F4 formulations showed 98.38% and 89.02% of drug release within12 hrs time period respectively. For F2 and F5 formulations 94.95% and 92.17% of drug release was observed within 14 hrs respectively and F3 formulation showed 87.88% drug release within 16 hrs.

From the data it was observed that F1 and F4 formulations showed 98% and 89.11% of drug release within 14 hrs respectively. From F2 formulation 99.96% of drug release was observed within 16hrs. From F3 formulation 93.20% of drug release was observed within a time period of 16.5 hrs. F5 formulation showed 80.23% of drug release within 12hrs.

From the In-vitro drug release study it was found that polymer concentration has an effect on formulation degradation and drug release rate. With increase in polymer concentration the sustain release profile of the formulation was found to be increased for all the prepared formulations. This is because increase in polymer concentration decreases the diffusivity of solvent through the formulation resulting in decreased drug release rate. The slow diffusion of surrounding medium into the formulation by means of water filled pores results in degradation of polymer.

Table 3: parameters determined from the *In vitro* drug release plots

Formulations (Drug:polymer)	zero order plot (R^2)	First order plot (R^2)	Higuchi plot (R^2)	Peppas plot (n)
F1 (1:1)	0.5920	0.8260	0.9130	0.1770
F2 (1:1.5)	0.9870	0.9820	0.9520	0.8100
F3 (1:2)	0.9820	0.9860	0.9770	0.7200
F4 (1.5:1)	0.9550	0.9680	0.9310	0.4400
F5 (2:1)	0.9690	0.9620	0.8970	0.9910

Table 4: parameters determined from the *In vitro* drug release plots (intermittent addition method)

Formulations (Drug:polymer)	zero order plot (R^2)	First order plot (R^2)	Higuchi plot (R^2)	Peppas plot (n)
F1 (1:1)	0.9740	0.9830	0.9370	0.513
F2 (1:1.5)	0.9800	0.9740	0.9850	0.723
F3 (1:2)	0.9860	0.9820	0.9400	0.930
F4 (1.5:1)	0.9910	0.9890	0.9380	0.981
F5 (2:1)	0.9840	0.9530	0.9370	0.941

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. From the results it was found that the F3 formulation of continuous addition method was following first order drug release kinetics and fitted into korsemeyerpeppas equation revealing non fickian diffusion mechanism. The F3 formulation of intermittent addition method was following zero order kinetics and fitted into korsemeyerpeppas equation revealing non fickian diffusion mechanism.

3.2 Comparative study between the best formulations of Continuous addition method and intermittent addition method.

Mefenamic acid loaded Bovine serum albumin nanoparticles were prepared by continuous addition method and intermittent addition method. The obtained nano formulations were studied for characterization parameters like particle size, zeta potential, surface morphology and drug-polymer interactions and evaluated for drug content, entrapment efficiency, loading capacity.

After evaluating the parameters the F3 formulation of continuous addition method was found be the best formulation because of the mean particle diameter of 211.6nm, zeta potential value of -49.8mV and higher drug entrapment efficiency of 93.13%. The F3 formulation of intermittent addition method was found to be the best formulation

because of the mean particle diameter of 208.7nm, zeta potential value of-52.8mV and higher drug entrapment efficiency of 95.05%.

A comparative study was conducted between the F3 formulations of continuous addition method and intermittent addition method. All the characterization and evaluation parameters were compared in order to know the better method for the fabrication of Mefenamic acid nanoparticles.

3.2.1 Particle Size Distribution

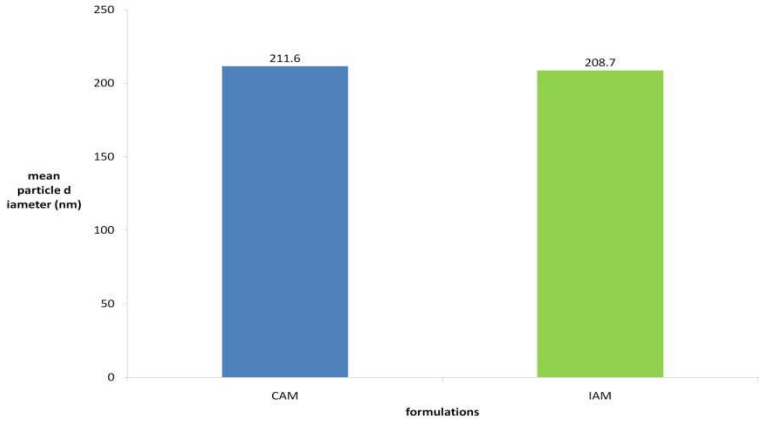

Fig.23: Comparison of mean particle diameter of F3 formulations of continuous addition method and intermittent addition method

The mean particle diameter of the F3 formulationsprepared by continuous addition method and intermittent addition method was found to be 211.6nm and 208.7nm respectively. On comparison smaller mean particle diameter was observed for the F3 formulation of intermittent addition method.

3.2.2 Zeta potential

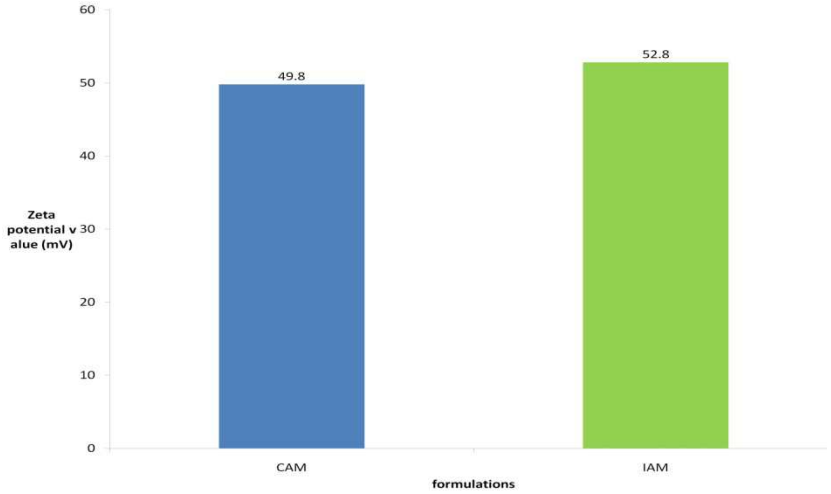

Fig.24: Comparison of zeta potential value of the F3 formulations ofcontinuous addition method and intermittent addition method

The zeta potential value of the F3 formulations prepared by continuous addition method and intermittent addition method was found to be -49.8mV and -52.8mV respectively. On comparison the F3 formulation of intermittent addition method showed better stability.

3.2.3 Product yield

The product yield of the F3 formulations by continuous addition method and intermittent addition method was found to be 93.3% and 94%. On comparison the F3 formulation of intermittent addition method revealed better product yield.

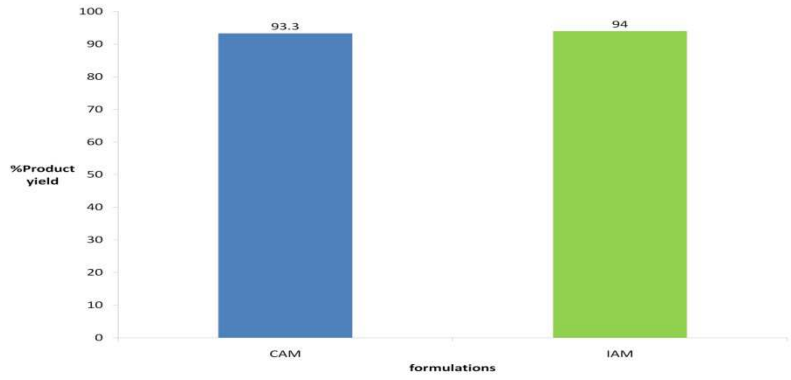

Fig.25: Comparison of product yield of the F3 formulations of continuous addition method and intermittent addition method

3.2.4 Drug content

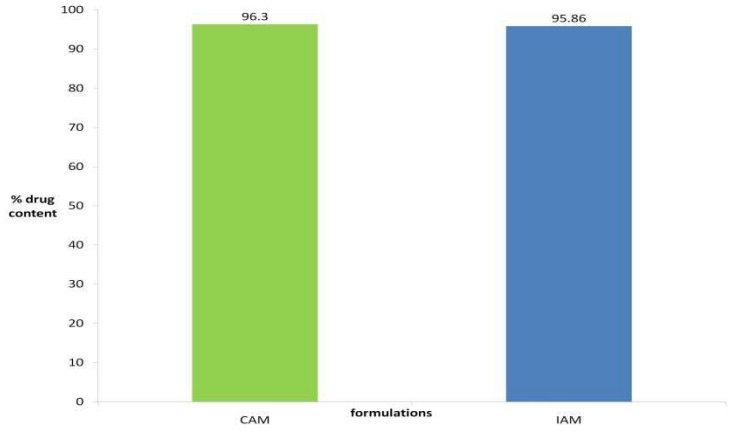

Fig.26: Comparison of drug content of the F3 formulations of continuous addition method and intermittent addition method

The drug content of the F3 formulations by continuous addition method and intermittent addition method was found to be 96.3% and 95.86% respectively. On comparison the F3 formulation of intermittent addition method showed better % drug content.

3.2.5 Encapsulation efficiency

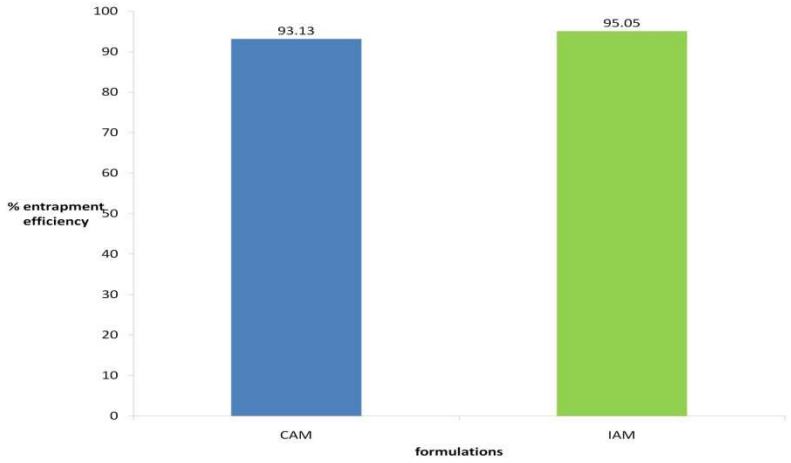

Fig.27: Comparison of drug entrapment efficiency of the F3 formulations of continuous addition method and intermittent addition method

The drug entrapment efficiency of the F3 formulations by continuous addition method and intermittent addition method was found to be 93.12% and 95.05% respectively. On comparison the F3 formulation of intermittent addition method revealed better drug entrapment efficiency.

3.2.6 Drug loading capacity

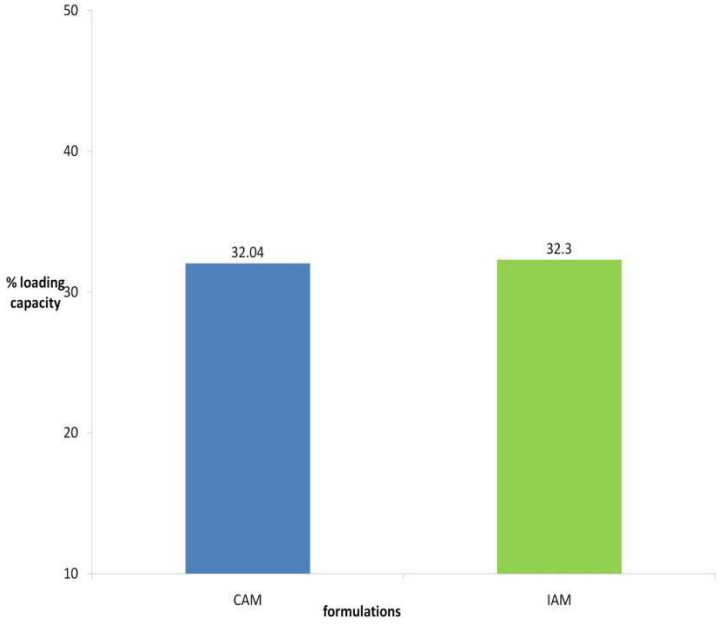

Fig.28: Comparison of drug loading capacity of the F3 formulations of continuous addition method and intermittent addition method

The drug loading capacity of the F3 formulations by continuous addition method and intermittent addition method was found to be 32.04% and 32.3% respectively. On comparison the F3 formulation of intermittent addition method was found to have better drug loading capacity.

CONCLUSION

Mefenamic acid nanoparticles were prepared by desolvation technique. In this technique a natural polymer such as Bovine Serum Albumin was selected for the study. Two methods were opted 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 5 mins time interval. The process parameters including pH, stirring speed and stirring time were optimized. For each method five formulations were prepared by varying drug-polymer concentration.

The obtained nano formulations were studied for characterization parameters like particle size, zeta potential, surface morphology and drug-polymer interactions and evaluated for drug content, entrapment efficiency, loading capacity, invitro drug release.

From the results it was concluded that increase in polymer concentration resulted in increase in mean particle diameter, zeta potential value and drug entrapment efficiency and increase in drug concentration led to increased drug loading capacity. A best formulation was selected from each method and compared for all the parameters.

On comparison the intermittent addition method was concluded as the best method for the preparation of mefenamic acid nanoparticles over continuous addition method because of its small particle size (208.7nm), Stability (zeta potential value of -52.8mV), higher drug entrapment efficiency (95.05%) and sustain drug release profile.This can be explained by the fact that intermittent addition method gives more time for desolvation process to form more stable particles.

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REFERENCES

[1] Anil Mahapatro and Dinesh K Singh,*Journal of bio technology*, **2011**, 9-15.
[2] ManivannanRangasamy and KugalurGanesanParthiban, *International Journal of Research in Ayurveda and Pharmacy*, Nov-Dec **2010**, 1(2), 316-326.
[3] VJ Mohanraj and Y Chen.*Tropical Journal of Pharmaceutical Research,* June **2006**, 5(1), 561-573.
[4] Majeti N.V. Ravi Kumar,*Journal of pharma and pharmaceutical sciences*, **2000**, 3(2), 234-258.
[5] Martins OchubiojoEmeje, IfeomaChinwudeObidike, EkaeteIbangaAkpabio and SabinusIfianyiOfoefule,*Recent Advances in Novel Drug Carrier Systems*, **2012**, (1), 69- 106.
[6] Frank Alexis, Eric Pridgon, Linda K. Molnar, and Omid C. Farokhzad,*Molecular Pharmaceutics*, **2008**, VOL. 5(4), 505-515.
[7] Anne des Rieux , VirginieFievez , Marie Garinot ,Yves-Jacques Schneider , VéroniquePréat,*Journal of controlled drug release,* **2006**, 116, 1-27.
[8] Donald E. Owens III, Nicholas A. Peppas,*International journal of pharmaceutics*, **2006**, 307, 93-102.
[9] Abhilash M,*International Journal of Pharma and Bio Sciences*, **2011**, Vol.1 (1), 1-12.
[10] Rakesh Bagul, Vijay Mahajan, AvinashDhake,*International Journal of Current Pharmaceutical Research*, **2012**, Vol. 4(3), 29-38.
[11] Vyas S.P, Khar R.K, Targeted & Controlled drug delivey, Novel carrier systems, CBS publication,**2002, 331-381**.
[12] Nilesh Jain, Ruchi Jain, Navneet Thakur, Brham Prakash Gupta, Deepak Kumar Jain, JeetendraBanveer, Surendra Jain,*Journal of Pharmaceutical and Clinical Research*, **2010**, Vol. 3(3), 159-165.
[13] Kumaresh S. Soppomath, TejrajM.Aminabhavi, Anandrao R. Kulkarni, Waiter E. Rudzinski,*Journal of controlled release*,**2001**, vol.7, 1-20.
[14] NimishShah, R.K.Mewada and Tejal Shah, *Application of Biodegradable polymers in controlled drug delivery*, **2011**, vol.3, 8-10.
[15] S. Karthik Raja Namasivayam, A.T. George Robin, R.S. Arvind Bharani, L.Vigneshwaraprakash and J.M.Vivek,*World Journal of Pharmacy and Pharmaceutical Sciences*, **2013**, Vol. 2 (5), 3094-3106.
[16] Basanta Kumar Behera, Sunit Kumar Sahoo, RanjitMohapatra, VaddadiAnkita, AdarshMahapatra,*World journal of pharmaceutical research*, **2012**, Vol. 1(3), 591-625.
[17] Joachim Allouche, R. Brayner, Nanomaterials: A Danger or a Promise? : Chapter 2, Synthesis of Organic and Bioorganic Nanoparticles: An Overview of the Preparation Methods, Springer-Verlag London **2013,** 27-57.
[18] Ugo Bilati, Eric Allemann and Eric Dorelker,*AAPS PharmSciTech*, **2005**, vol. 6(4), Article 74, E594-604.
[19] Nagavarma B V N, Hemant K.S. Yadav, Ayaz A, Vasudha L.S, Shivakumar H.G,*Asian Journal of Pharmaceutical and Clinical Reasearch*, **2012**, Vol.5(3), 16-23.
[20] Krishna Sailaja, P. Amareswar, P.Chakravarthy, *International Journal of Pharmacy and Pharmaceutics sciences*, **2011**, Vol.3(2), 1643-1647.
[21] Kumar Ganesh, DhyaniArchana, KothiyalPreeti,International Journal of Pharmaceutical and Life Sciences, **2013**,Volume 2(5),202-229.
[22] Merodio M,.Arnedo A, Renedo M.J. Irache J.M, *European J of Pharmceutical Sciences*, **2001**,12, 251-256.
[23] Gallo J.M., Hung C.T., Perrier D.G., 1984,*Int. J.Pharm.*, **1984**,22, 63–74.
[24] Lin W., Coombes A.G.A., Davies M.C., Davis S.S., Illum L., *J. Drug Target*, **1993**,1, 237–243.
[25] Sripriyalakshmi. S, Anjali. C. H, George Priya Doss. C, Rajith B, AswathyRavindran,*PLOS ONE*, **2014**, Volume 9(2), 1-10.
[26] Ramdhan Yadav, Dharmesh Kumar, AvneshKumari, Sudesh Kumar Yadav,*EXCLI Journal*, **2014**, 13, 331-346.
[27] Saikat Das, Rinti Banerjee and JayeshBellare.Saikat Das, Rinti Banerjee and JayeshBellare,*Trends Biomater*,**2005**, Vol 18(2), 203-212.
[28] S Karthick Raja Namasivayam, LFA Anand Raj, AT George Robin, J Helan Chandra, and RS Arvind Bharani, Research Journal of Pharmaceutical Biological and Chemical Science, **2014**, 5(3), 47-60.
[29] Zhan Yu, Min Yu, Zhibao Zhang, Ge Hong and QingqingXiong,*Nanoscale Research Letters***2014**, 9, 343(1-7).
[30] Sae-Yeol-Rim Paik, Hoang Hai Nguyen, JinaRyu, Jeong-Hwan Che, Tae Seok Kang, Jong Kwon Lee, Chi Won Song, SanghoonKo,*Food Chemistry*, Elsevier, **2013**, 141, 695–701.
[31] K. Sailaja, P. Amareswar, asian journal Pharma clinical research,**2012,** volume5(2),132-134.
[32] Yong Li, Xiuhua Zhao, YuangangZu , Xue Han, Yunlong Ge, Weiguo Wang and Xinyang Yu,*Materials***2012**, 5, 2403-2422.
[33] Dongmei Zhao, Xiuhua Zhao, YuangangZu, Jialei Li, Yu Zhang, Ru Jiang, Zhonghua Zhang,*International Journal of Nanomedicine*, **2010**, (5), 669-677.
[34] Guilin Wang, Kevin Siggers, Sufeng Zhang, Hongxing Jiang, Zhenghe Xu, Ronald F. Zernicke,JohnMatyas, and Hasan Uludag, Preparation of BMP-2 Containing Bovine Serum Albumin (BSA) Nanoparticles Stabilized by Polymer Coating, Pharmaceutical Research, Springer Science,**2008**, 1-14.

[35] Swathi A. Guhagarkari, Vinod C.Malshe, Padma V. Devarajan,*AAPS. PharmaSciTech*, Sep**2009**, Vol.10, No.3, 1-24.
[36] ShanmugasundaramSangeetha, DhandapaniNagasamyVenkatesh, RajendranAdhiyaman, KumareswamySathi, Bhojrtaj Suresh,*Tropical journal of pharmaceutical research*, March **2007**, 6(11), 653-657.
[37] Y.Thomas He, Jiamin Wan Testu Tokunaga, J. Nanopart Res, Kinetic stability of Hematite nanoparticles: the effect of particle size, **2008**, 10, 321-332.
[38] KunalPagar, Pradeep Vavia ,*Sci Pharm*, **2013**, 81, 865–885.
[39] Meenu Chopra, Pawan Kaur, ManjuBernela, Rajesh Thakur,*International journal of scientific and technology research*, **2012**, 1(10), 1-7.
[40] SovanLal Pal, Utpal Jana. G. P. Mohanta and P. K. Manna, Pelagia Research Library *Der Pharmacia Sinica*, **2013**, 4(1), 40-46.
[41] S. Karthick Raja Namasivayam,Sasikumar&Shaleesha Stanley,*Global Journal of Science Frontier Research*: G *Bio-Tech & Genetics*, Volume 14 (1), **2014**, 478-485.
[42] Hoang Hai Nguyen and SanghoonKo, Preparation of Size Controlled BSA Nanoparticles by Intermittent Addition of Desolvating Agent, ICDBME in Victman IFMBE proceedings, **2010**, 27, 231-234.