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Preparation, optimization and characterization of PLGA nanoparticle

R. S. Dangi[1]*and S. Shakya[2]
1, Central India Institute of Pharmacy, Indore, (M.P.) - India
2, Dr. H. S. Gour Central University, Sagar, (M.P.) - India

Abstract

Aim of present study is to prepare PLGA nanoparticle. Nanoparticles were prepared by double emulsification solvent evaporation method. Various formulation and process variable which could affect the preparation and properties of nanoparticles. These formulation variables were identified and optimized to get uniform preparation with highest encapsulation efficiency. Formulation variables include amount of drug, polymer concentration and stabilizer concentration and process variables include stirring speed, stirring time and sonication time. All these parameter were optimized by taking the effect of variable on particle size, polydispersity index and encapsulation efficiency.

Key-Words: Nanoparticles, Formulation, Characterization, PGLA

Introduction

Nanoparticles are subnanosized colloidal carrier system composed of synthetic, semisynthetic or natural polymers in the size range of about 10-1000 nm (Vyas and Khar, 2001).Two types of nanoparticulate systems are possible, one is matrix type in which drug is dispersed homogenously into the entanglement of oligomers or polymeric units, ex. nanospheres and second is reservoir type in which oily core of drug is surrounded by the embryonic polymeric shell, ex. nanocapsules.There are many natural, synthetic, and semisynthetic polymer can be used for preparation of nanocarrier. Natural polymers include proteins such as gelatin, albumin, lecithin, legumin, vicilin. Polysaccarides such as alginates, dextran, chitosan, agarose, pullulan etc. Various synthetic polymers used for the preparation of nanoparticles ex- Polyalkylcyanoacrylate (PACA), Poly(isobutylcyanoacrylates) (PBCA), Poly(methylmethacrylates)(PMMA), Poly(hexylcyanoacrylates) (PHCA) etc. (Vyas *and Khar*, 2001). Nanoparticles have various applications in novel drug delivery system for treatment of diseases (Vyas *and Khar*, 2001).

*** Corresponding Author**
E.mail: ruchidangi3@gmail.com

Nanoparticles have advantages over conventional dosage form. Some advantages of naoparticles are: Increased bioavailability, dose proportionality, decreased toxicity, smaller dosage form (i.e., smaller tablet), stable dosage forms of drugs which are either unstable or have unacceptably low bioavailability in non-nanoparticulate dosage forms etc (Vyas and Khar, 2001). In present study PLGA (poly lactide co glycolic acid) was used for the preparation of NPs because of its biodegradability and biocompatibility. It degrades by hydrolysis of ester linkages in the presence of water in to two monomers lactic acid and glycolic acid, which under normal physiological condition, are by – products of various metabolic pathways in the body. PLGA with 50: 50 monomers and molecular weight 17000 was used in this work. It has very good mechanical properties and long shelf-life.

Material and Methods

PLGA Poly (d,l – lactide – co – glycolide) (50:50, mol wt.17000) (Sun Pharma, Vadodra) and Acyclovir was obtained as a gift sample from Biochem Pharmaceuticals industries ltd. Daman. Dichloromethane (DCM), acetone, polyvinyl alcohol (PVA), Triton X-100, dialysis membrane (MWCO 6,000 – 7,000 Da) were purchased from Himedia, Mumbai, India. All other reagents and solvents used were of analytical grade unless stated otherwise double distilled water (DDW) was used throughout the study.

Method of preparation (Tewas *et al* 2007).

PLGA was dissolved in a mixture of dichloromethane (DCM) and acetone then polyvinyl alcohol (PVA) solution was added. Further mixture was emulsified by sonication for 60 sec in an ice bath and w/o emulsion was formed. Again PVA solution was added and sonicated for 60 sec in an ice bath and w/o/w emulsion was formed. This resulting w/o/w emulsion was again diluted with 20 ml of PVA solution and slow speed stirring was continued for 5 hrs to facilitate evaporation of the solvent. The nanoparticle so formed were collected washed and freeze dried the scheme of preparation of nanoparticle is shown in scheme 1.0.

Preparation drug loaded nanoparticles

The drug was loaded at the initial step of nanoparticles preparation. The drug was dissolved in 1.5 % PVA solution then that was added to dissolved PLGA solution (prepared in a mixture of DCM and acetone) by syringe and further steps were followed as in case of plain nanoparticle. The schematic presentation of prepared drug loaded nanoparticle is showen in scheme 2.0

Optimization of nanoparticles:-

There are various formulation and process variable, which could affect the preparation and properties of nanoparticles. These formulation variables were identified and optimized to get uniform preparation with highest encapsulation efficiency. Formulation variables include amount of drug, polymer concentration and stabilizer concentration and process variables include stirring speed, stirring time and sonication time. All these parameter were optimized by taking the effect of variable on particle size, polydispersity index and encapsulation efficiency.

Effect of polymer concentration

Polymer (PLGA) concentration was optimized using their different concentration of PLGA (0.5%, 1.0%, 1.5%) while other parameters remained constant and the optimum value was identified on the basis of average particle size and polydispersity index (PDI) of nanoparticles, which were determined using Malvern zetasizer. Results are shown in table 1.1.

Table 1: Application of nanoparticles

S /No.	POLYMER	ADVANTAGE	APPLICATION
1.	Poly(alkylcyanoacrylate)nanoparticles with anticancer agents and, oligonucleotides	Reduced toxicity, enhanced uptake of antitumour agents, targeting, improve in-vitro in-vivo drug stability.	Cancer therapy
2.	Poly(alkylcyanoacrylate)polyesternanoparticles with antiparasitic/antiviral agents.	Targeting RES for intracellular targeting.	Intracellular targeting
3.	Polyester with adsorbed PEG	Prolong systemic drug effect, avoid uptake by RES.	Prolonged systemic circulation
4.	Poly (methylmethacrylate) nanoparticles with Vaccines.	Enhanced immune response.	Vaccine adjuvants
5.	Poly (methylmethacrylate) nanoparticles with Proteins and therapeutic agents	Enhanced bioavilability, protection from GIT enzymes.	Peroral absorption
6.	Poly (alkylcyanoacrylate) nanoparticles with steroids and antibacterial agents	Improve retention of drug /reduced washout.	Occular delivery
7.	DNA gelatin nanoparticles, DNA chitosan nanoparticles, DNA poly (dl-lactidecoglycolide) nanoparticles	Enhanced delivery and significant higher expression level.	DNA delivery.
8.	PACA nanoparticles with peptides.	Cross BBB.	Brain delivery
9.	PACA nanoparticles for transdermal application	Improve absorption /permeation.	Transdermal application
10.	Nanoparticles with adsorbed antigens	Enzyme immune assay.	Immunoassay
11.	Nanoparticlesd with radioactive or contrast agents.	Oral delivery of peptides.	Oral delivery of peptides

Effect of stabilizer concentration

For the optimization of stabilizer concentration, nanoparticle formulation NP-2 was selected and different nanoparticle formulations were prepared with varying concentration of stabilizer PVA (viz. 0.5%. 1.0%, 1.5%, 2.0%) keeping the other parameters constant. Optimization was done on the basis of average particle size of nanoparticles. Results are shown in table 1.2

Optimization of concentration of drug

Encapsulation efficiency is the major parameter for nanoparticle formulation so it was optimized by varying the drug concentration (5, 10 and 15 mg) in the above selected formulation NP2S-3 and keeping the other parameters constant. Optimization was done on the basis of average particle size of nanoparticle and percent drug entrapment. Results are shown in table 1.3.

Optimization of stirring speed

The size of nanoparticles depends on the stirring speed and it is optimized by preparing different formulation with varying stirring speed (2000, 3000 and 4000 rpm.) Results are shown in table 1.4

Optimization of stirring time

Stirring time optimization for selected formulation (NP2S3D2S-2) was performed by varying stirring time, during formulations. Further the particle size and percent drug entrapment were determined. Results are shown in table 1.5

Optimization of sonication time

Particle size reduction to nanometric size and their uniformity is very important parameter for nanocarrier and it can be achieved by sonication process, their size reduction depend on the time of sonication. It is optimized by varying sonication time (30, 60, 90 sec.) during formulation. Further the particle size and percent drug entrapment were determined. Results are shown in table 1.6.

Characterization of nanoparticle

Particle size

Particle size is an important aspect of developing a formulation. The average particle size and polydispersity index of the nanoparticle were determined by photon correlation spectroscopy using zetasizer (DTS ver 4.10) Malvern instrument England. The particle size distribution is represented by the average size diameter.

Polydispersity index

The polydispersdeity index PDI is a diamensionless measure for the broadness of a particle size distribution and can be used for the nanoparticle dispersion. PDI between 0.03 and 0.06 can be denoted as monodisperse, between 0.1and 0.2 as narrowely distributed and between 0.25-0.5 as broadely distributed and value above 0.5 indicated extremely broad size distribution that can not be described by means of PDI **(Mullar R.H. et al 1996).**

Surface morphology (TEM)

Transmission electron microscopy was performed using a Philips CM 10 electron microscope, with an accelerating voltage of 100 kv. A drop of the sample was placed on a carbon coated copper grid to leave a thin film on the grid. Before the film dried on the grid, the film was negatively stained with 1% phosphotungstic acid (PTA). A drop of the staining solutions was added on to the film and the excess of the solution was drained off with a filter paper. The grid was allowed to air dry thoroughly and samples were viewed under a transmission electron microscope and photographs were taken at suitable magnification (Photomicrograph No.1.0).

Particle morphology (SEM)

Particle morphology was determined by Scanning Electron Microscope (SEM) at AIIMS, New Delhi. The samples for SEM were prepared by lightly sprinkling the freeze dried nanoparticles on a double adhesive tape, which was stuck on an aluminium stub. The stubs were then coated with gold to a thickness of about 300°A by using a sputter coater. All samples were examined under a scanning electron microscope (LEO 435 VP, Eindhoven Netherlands) at an acceleration voltage of 30 kV, and photomicrographs were taken, which are shown in (Photomicrograph No. 2.0).

Entrapment Efficiency

Entrapment efficiency of the drug acyclovir in NPs was determined by using Sephadex G–50 mini column (Fry *et al*., 1978). For the preparation Sephadex G – 50 mini column, firstly 500mg of Sephadex G – 50 was allowed to swell in 0.9 % NaCl aqueous solution for 8hr and then the hydrated gel was filled in the barrel of 2ml disposable syringe plugged with filter pad and glass wool. The barrel was centrifuged (REMI, Mumbai, India) at 2000 rpm for 2 minutes to remove excess of saline solution from the Sephadex column.

For the separation of free drug from NPs formulation 2 ml of NPs dispersion was added drop wise on the top of the Sephadex column and then centrifuged at 2000 rpm for 2 min., to expel and remove void volume containing NPs in to the centrifuged tubes. This eluted NPs dispersion was collected and lysed by disrupting with 0.1% Triton X -100 and then the amount of entrapped drug was analyzed using spectrophotometric method (Schimadzu uv-1800).

% Entrapment efficiency = Weight of total drug - Weight of free drug / Weight of total drug × 100

Drug Release

The *in-vitro* drug release of entrapped drug from NPs formulation was determined using dialysis tube method (Gupta *et al*., 2005). The NPs formulation was first separated from free drug by passing through Sephadex column and then subjected to centrifugation. Separated NPs formulation was taken in to the dialysis tube (molecular weight cut off 13 KDa, Hi-media, India) and placed in a beaker containing PBS (pH 7.4) .The beaker was placed over a magnetic stirrer and the temperature was maintained at 37±1ºC throughout the procedure. Samples were withdrawn at definite time intervals and replaced with same volume of fresh phosphate buffer solution pH 7.4. It was then analyzed for drug content spectrophotometrically.

Results and Discussion

Optimization of nanoparticles

The nanoparticles were prepared using double emulsification solvent evaporation method reported by **Tewas *et al* 2007**. Particle size of the nanoparticles depends on various formulation variables include amount of drug, polymer concentration and stabilizer concentration and process variables include stirring speed, stirring time and sonication time. Optimized value of various parameters are given table 1.7.

Particle size

Average particle size and surface charge potential of NPs were determined by using a Zetasizer (DTS ver. 4.10, Malvern Instruments, England). It was observed that the size of particles increased on increasing polymer concentration and it was found to be 115.21±1.1 at 1% polymer concentration where as the polydispersity index was 0.356 at same concentration of polymer. Hence, it was inferenced that with increase in polymer concentration viscosity of the solution increases, which in turn results bigger size nanoparticles (Thiaune *et al.,* 1997). The polydispersity index is reduced on increasing its concentration from 0.5 to 1.0 while PDI increases on further increase in the polymer concentration. This caused due to increase in viscosity on increasing the polymer to 1.5% that produce hinderance in the movement or diffusion of solvent and produced bigger sized particles. It was observed that the particle size decreased when concentration of stabilizer increases, this may be due to decrease in surface tension and development of charge over the particles in the system because of presence of stabilizer (Ahlin *et al.,* 2002). At 1.5% stabilizer concentration PDI is 0.326. If the concentration of stabilizer increases beyond 1.5% the gradual increase in particle size was observed which could be formation of micellar structure of PVA and increases the PDI. Hence, 1.5% PVA concentration was taken as optimized parameter. On increasing amount of drug particle size is increase. On increasing sonication time particle size decreased up to 60 sec, but increased after it due to aggregation of particles and development of charge over the particles. Same thing is happen with stiring speed and stirring time. 3000 rpm for 3hrs. are optimized process variables, after that particle size of nanoparticles increased.

Particle morphology

Shape and surface morphology were determined by TEM and SEM analysis. Transmission electron microscopic image of nanoparticle showed that particles are spherical in shape and do not show considerable variation in shape. SEM image of nanoparticles was found showing smooth surfaces

Encapsulation Efficiency

Entrapment efficiency of the drug Acyclovir in NPs was determined by using Sephadex G – 50 mini columns (Fry *et al*., 1978). Entrapment efficiency is depends on amount of drug, stirring speed, stirring time and sonication time. It was observed that on increasing the amount of drug, the entrapment efficiency increased up to 10mg of drug while on further increasing the amount of drug, the entrapment is not increase. This could be due to saturation of drug with the polymer.

It was observed that as on increasing the stirring speed from 2000 to 3000 rpm, and increasing the stirring time 4 hr to 5 hr the size of naoparticles was decrease and drug entrapment efficiency was increased. This decrease in size of nanoparticles could be due to high shear force applied to the dispersion. Due to decrease in size the surface area of NPs was increased which in turn increased the drug entrapment efficiency. But beyond 3000 rpm and 5 hr of stirring speed, the size of NPs is reduced which are unstable and form aggregates which results increase in size. Similar effect was observed on varying the sonication time, on increasing sonication time, decrease in entrapment efficiency was observed due to leaching of entrapped drug during higher sonication.

Drug release

It was anticipated from the study of *in vitro* drug release profile that the formulation gives an initial burst release followed by a controlled release than sustained release. In vitro release studies of DNPs showed a % cumulative drug release of DNPs was 87.14±0.7 after 168 hrs.

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Table 1.1: Effect of polymer concentration

S/No.	Formulation code	Polymer concentration	Particle size (nm)	PDI
1.	NP-1	0.5%	110.34±1.3	0.513
2.	**NP-2**	**1.0%**	**115.21±1.1**	**0.356**
3.	NP-3	1.5%	168.24±2.1	0.467

Values represent mean ± SD n =3

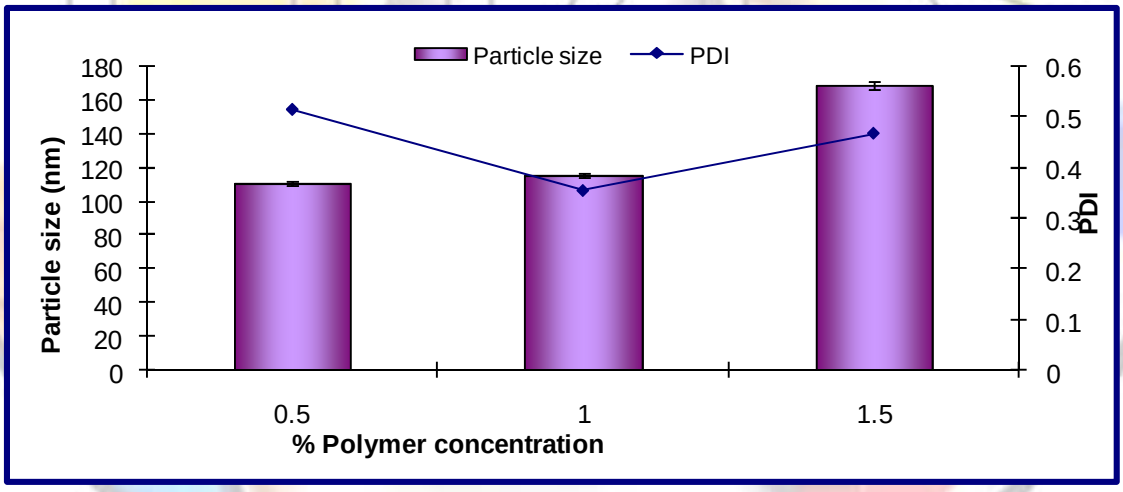


Fig 1.1: Effect of polymer concentratio

Table 1.2: Effect of stabilizer concentration

S/No.	Formulation code	Stabilizer concentration	Particle size (nm)	PDI
1.	NP2S-1	0.5%	125.83±1.2	0.389
2.	NP2S-2	1.0%	118.23±1.8	0.481
3.	**NP2S-3**	**1.5%**	**117.56±2.1**	**0.326**
4.	NP2S-4	2.0%	129.72±2.3	0.572

Values represent mean ± SD n= 3

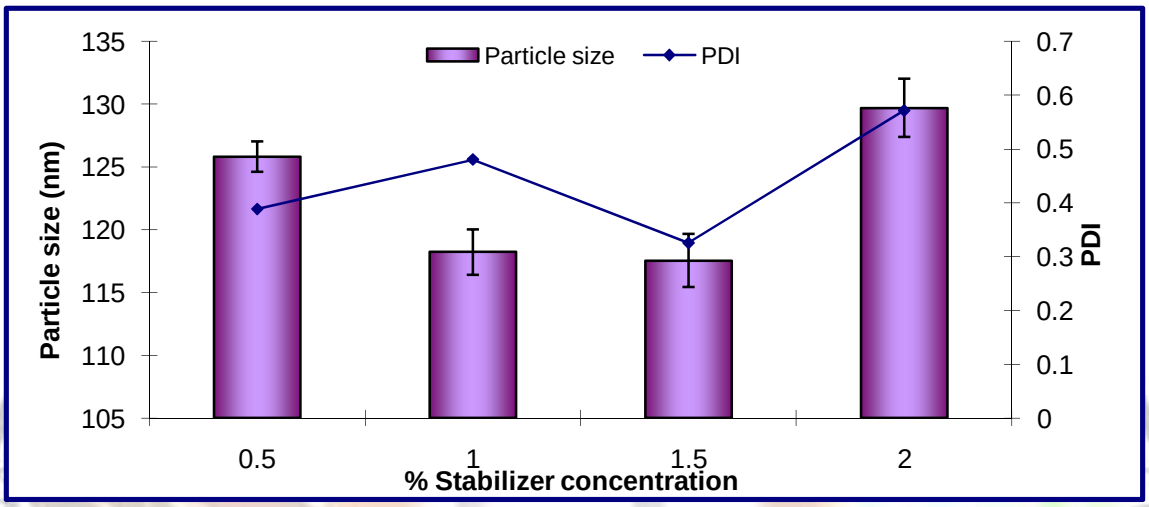


Fig 1.2: Effect of stabilizer concentration

Table 1.3: Optimization of concentration of drug

S/No.	Formulation code	Concentration of drug (mg)	Particle size (nm)	% Entrapment efficiency
1.	NP2S3D-1	5	110.36±2.3	51.23±1.2
2.	**NP2S3D-2**	**10**	**114.25±3.4**	**63.89±1.8**
3.	NP2S3D-3	15	118.21±2.1	63.90±2.1

Values represent mean ± SD n= 3

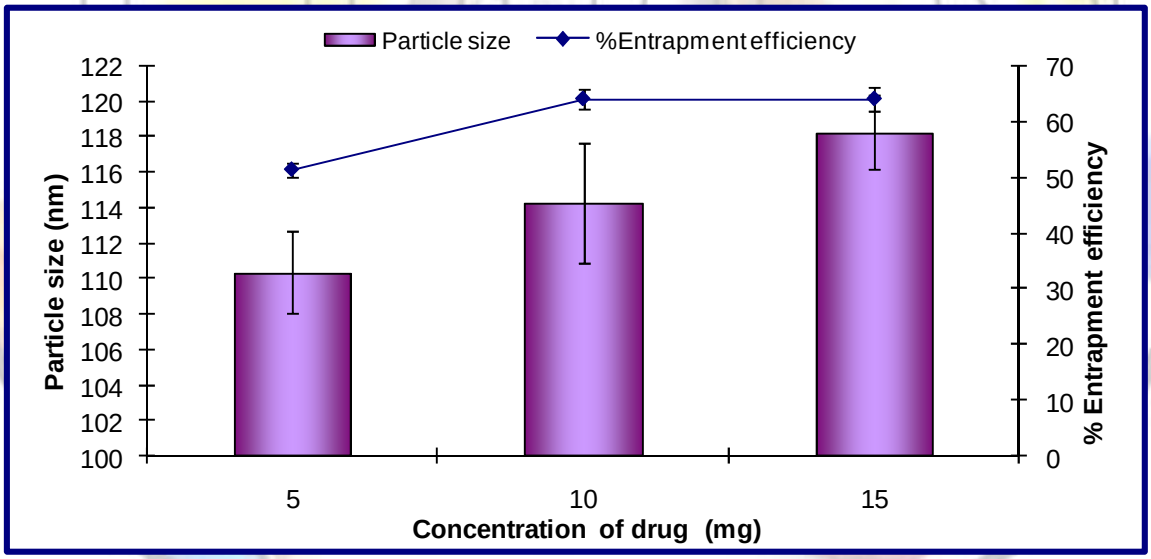


Fig 1.4: Optimization of concentration of drug

Table 1.4: Optimization of stirring speed

S/No.	Formulation code	Stirring speed (rpm)	Particle size (nm)	%Entrapment efficiency
1.	NP2S3D2S-1	2000	130.24±2.3	62.78±2.1
2.	**NP2S3D2S-2**	**3000**	**121.12±1.8**	**63.64±1.2**
3.	NP2S3D2S-3	4000	126.19±2.1	63.98±1.8

Values represent mean ± SD n=3

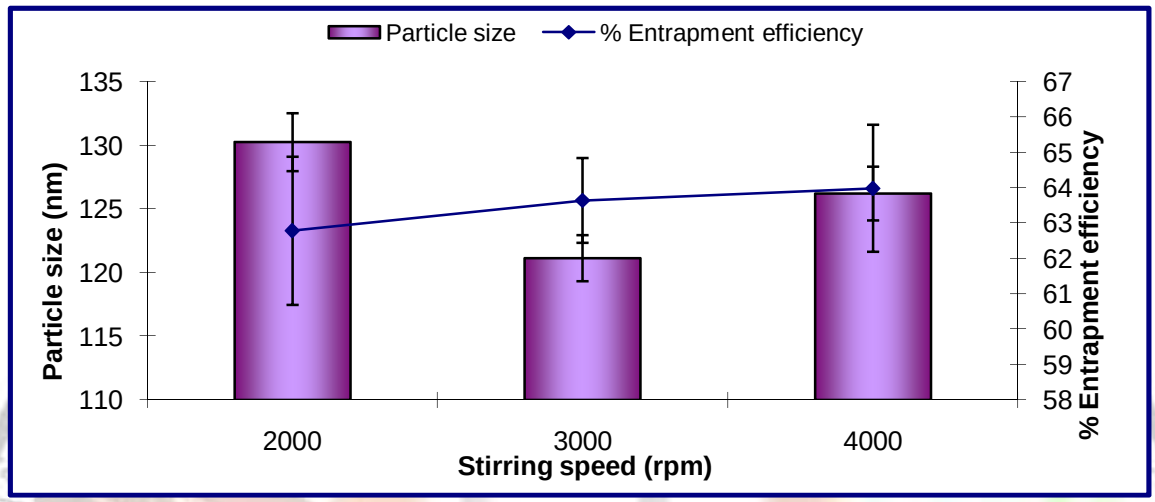


Fig 1.4: Optimization of stirring speed

Table 1.5: Optimization of stirring time

S/No.	Formulation code	Stirring time (hrs.)	Particle Size (nm)	%Entrapment Efficiency
1.	NP2S3D2St-1	4	133.87±1.8	60.98±1.9
2.	NP2S3D2St-2	5	125.79±2.1	63.72±1.5
3.	NP2S3D2St-3	6	128.95±2.3	66.78±2.1

Values represent mean ± SD n=3

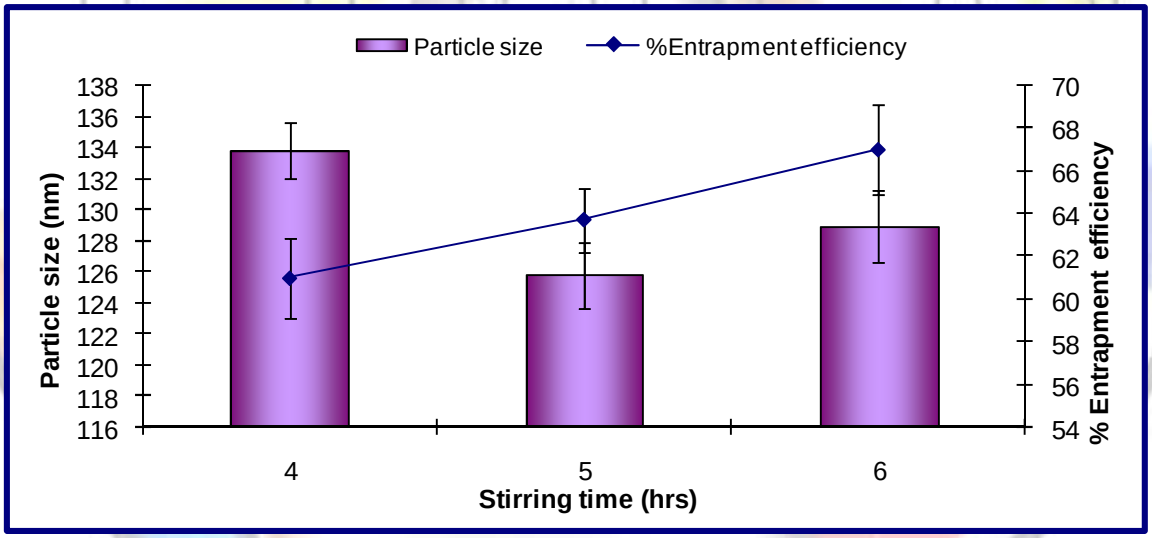


Fig 1.5: Optimization of stirring time

Table 1.6: Optimization of sonication time

S/No.	Formulation code	Sonication time (sec.)	Particle size (nm)	%Entrapment efficiency
1.	NP2S3D2S2S-1	30	148.65±2.1	63.29±1.8
2.	**NP2S3D2S2S-2**	**60**	**126.37±2.4**	**62.98±2.3**
3.	NP2S3D2S2S-3	90	131.31±1.8	51.62±1.3

Values represent mean ± SD n= 3

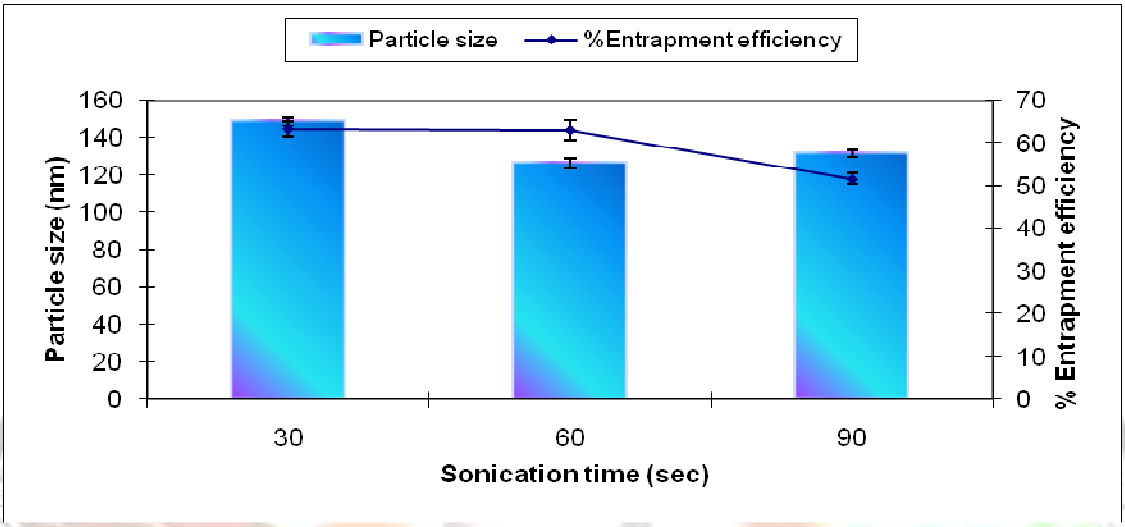
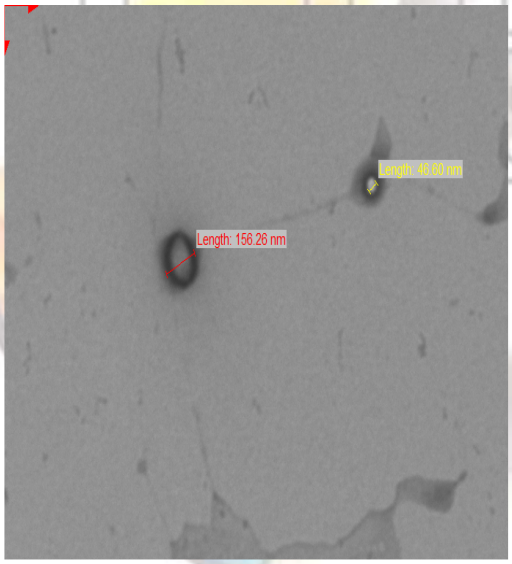


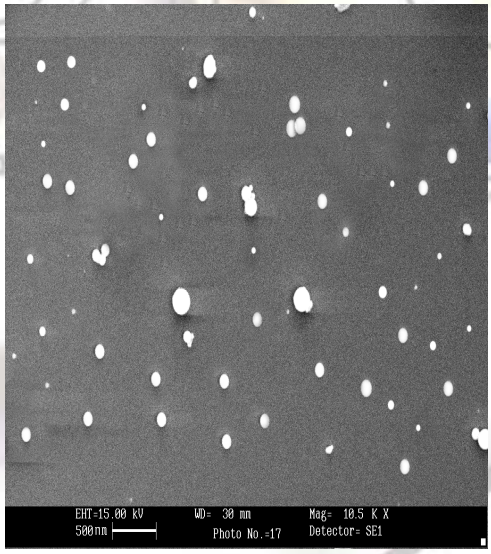
Fig1.6: Optimization of sonication time

Table 1.7: Optimized parameters for PLGA nanoparticles

S/No.	Parameter	Optimized value
1.	Polymer concentration	1%
2.	Stabilizer concentration	1.5%
3.	Amount of drug	10mg
4.	Stirring speed	3000rpm
5.	Stirring time	5hrs
6.	Sonication time	60 sec



Photomicrograph No. 1.0: TEM of LDNPs



Photomicrograph No. 2.0: SEM of LDNPs

Table 1.9: % Drug release profile from nanoparticles in PBS (pH 7.4)

S/No.	Time (hrs.)	% Drug release of DNPs in phosphate buffer pH 7.4
1.	1	2.36±0.4
2.	2	4.32±0.3
3.	4	8.22±0.7
4.	8	16.44±0.2
5.	24	30.14±0.6
6.	48	39.96±2.3
7.	72	50.07±1.2
8.	96	68.24±.0.7
9.	120	80.02±0.8
10.	144	85.08±2.3
11.	168	87.14±0.7

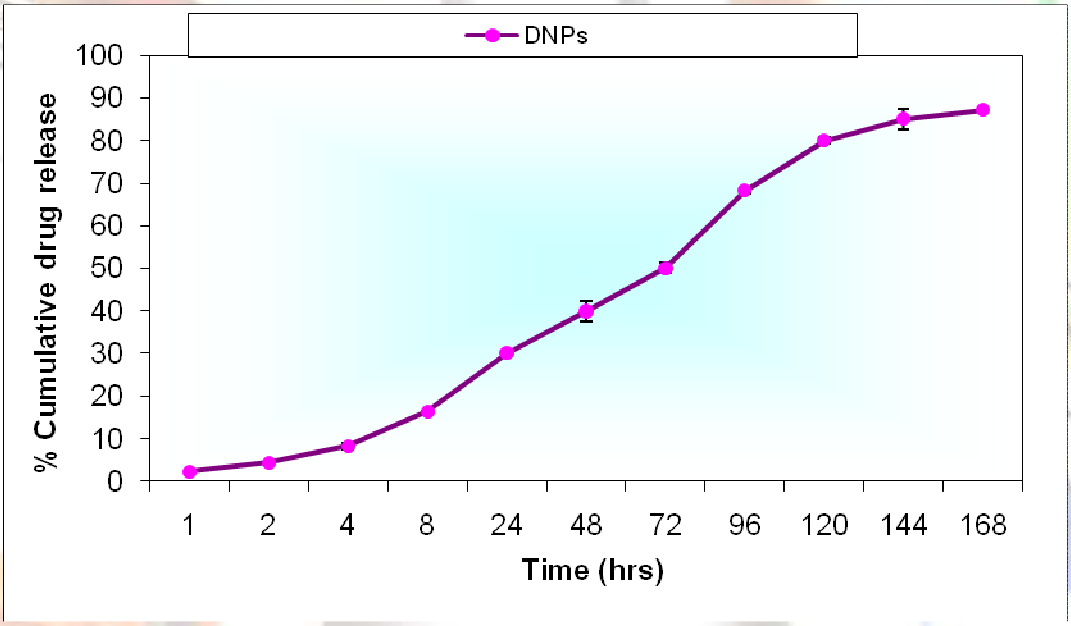


Fig 1.8: % Drug release profile from nanoparticles in PBS pH(7.4)