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Research Article

FORMULATION OPTIMIZATION AND CHARACTERIZATION OF IRINOTECAN NANOPARTICLES

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ABSTRACT

The goal of the present investigation was to formulate, optimize and characterize poly lactide -o - glcolic acid (PLGA) nanoparticles of Irinotecan for cancer therapy. Here the nanoparticles formed are based on solvent evaporation technique by using aqueous and organic phases. The formulation optimization is also carried out optimizing its various process and formulation parameters. The analytical method development is carried out using acetonitrile and phosphate buffer saline. Different organic solvents were tried and various surfactants were used to optimize the nanoparticulate formulation. The size range and zeta potential was measured using Malvern zeta seizer. The lyophilization was carried out using two different cryoprotectants. The maximum percent drug entrapment was found out to be 37.2%. The in vitro drug release of IRN NP was also found out using dialysis method in phosphate buffer saline pH 7.4. The in vitro drug release showed sustained release of drug over 24 hours. Hence the IRN loaded PLGA nanoparticles have potential as a drug delivery system. Furthermore, they may have utility for site-specific drug delivery since the small size of the particles may allow their delivery to extra vascular target sites through the leaky endothelia of inflamed and cancerous area

Key words: Bilayered buccal tablet, buccal delivery, mucoadhesion, carvedilol.

INTRODUCTION

The substances with size ranges from 1 to 1000 nm are called nanoparticles. These materials are mainly used in oncology for early detection of malignancy and precise localisation of cancer therapeutics without or with minimal adverse effect to the somatic tissues. These carriers are used to protect drugs, vaccines, nutrients and cosmetics. Nanoparticles exerts its site specific drug delivery by avoiding the reticuloendothelial system, utilising enhanced permeability and retention effect and tumour specific targeting. The formation of nano particles and physicochemical parameters such as pH, monomer concentration, ionic strength as well as surface charge, particle size and molecular weight are important for drug delivery1,2. Further, these nanoparticles have the capability to reverse multi drug

resistance, a major problem in chemotherapy. The distinct capability of nanoparticles to provide access to virtually all cell types may be utilised for the delivery of therapeutic agents to wide array of cellular types and targets3, 4. Irinotecan is chemically1(S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1H-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate. Irinotecan Hydrochloride Trihydrate contains not less than 98.0 per cent and not more than 102.0 per cent ofC33H3sN406.HCl, calculated on the anhydrous basis. It is a camptotecan analogue. The mechanism of action of camptotecanis by stabilising the cleavable complex in which topoisomerase I is covalently bound to DNA at a single – stranded breaksite5. Conversion into lethal DNA damage follows when a DNA replication fork encounters these cleavable complexes(fork collision model). Irinotecan is a prodrug that requiresenzymatic cleavage of the C-10 side chain by an irinotecancarboxylesterase – coverting enzyme to generate the biologically active metabolite, SN-382 both irinotecan and SN-382can undergo nonezymatic hydrolysis of the lactone ring to the open – ring carboxylate species. Irinotecan can also undergo hepatic oxidation of its dipiperidino side chain to nform the inactive metabolite 7 –ethyl -10(4-N-(5 aminopentanoic acid) 1- piperidino) carbonyloxycamptothecin (APC). Elimination of irinotecan occurs by urinary excretion, biliary excretion, and hepatic metabolism. Approximate terminal half – life ofirinotecan lactone is 6.8 h and approximate clearance is 46.9L/h/m. Approximate terminal half- life of SN-382 lactone is 11.05h. Myelosuppression, predominantly neutropenia and less commonly thrombocytopenia are the main toxicity of irinotecan. Elevated hepatic transminases pulmonary toxicity (uncommon) associated with a reticulonodularinfiltrate, fever, dyspnea and eosinophilia5, 6. Irinotecanhydrochloride is an intravenous antineoplastic agent. Here the Irinotecannano particles were prepared by using PLGA as a polymer.

EXPERIMENTAL

MATERIALS

Irinotecan hydrochloride trihydrate obtained from Zydus Research Centre, Ahmadabad. Acetonitrile and acetone were from Merck specialities Pvt Ltd, Mumbai. Poly(dl-lactide-co-glycolide)(50:50)obtained from Durect corporation, Birmingham division, Pelham. Poloxomer 188 and Poly vinyl alcohol were from Sigma chemical co., St Louis, USA. Sodium sulphate and sodium chloride from Canton laboratories pvt, ltd, Baroda, India. And remaining all other chemicals was of analytical grade.

METHODS

Solvent Evaporation Technique

The drug (Irinotecan) and polymer(PLGA) were dissolved in organic phase (ACN/acetone). Then a weighed quantity of surfactant was dissolved in aqueous phase. The organic phase was added to aqueous phase in a drop wise manner. The formed suspension was kept for mechanical stirring until the complete evaporation of solvent. The residual quantity of solvent was removed by rotatory vacuum evaporator for 1 hour. Then this dispersion was passed through the Sephadex G-25 column (size exclusion chromatography) for the separation of free drug and entrapped drug[7,8 &]

Optimization parameters

1. **Process optimization**
   - 1.1. *Selection of speed of the magnetic stirrer*: Different batches were prepared at various stirring speed of 400, 600 and 800 rpm and its effect on the nanoparticles preparation was studied. The particle size, PDI and percent drug entrapment were evaluated.
   - 1.2. *Effect of rate of addition of organic phase to aqueous phase*: The speed at which the solvent is added will demonstrate its effect on the formulation3. For this purpose the

rate of addition of solvent containing drug and polymer to aqueous surfactant solution was kept at 0.25ml/min, 0.5ml/min and 1ml/min. The effect of solvent addition rate on the particle size, PDI and percent drug entrapment was evaluated.

2. Formula Optimization

2.1. *Solvent selection (organic phase):* Acetonitrile and acetone are the most common of the solvents reported for preparation of nanoparticles. Both the solvents were used initially at ratio of 1:2 ratio of nanoparticulate system in aqueous phase keeping other parameters constant. The effect of these solvents on particle size, PDI and percent drug entrapment was evaluated8. The IRN is insoluble in acetone, so it was dissolved in aqueous phase and the nanoparticles were fabricated in ACN.

2.2. *Surfactant Selection:*In order to optimize the concentration of aqueous surfactant solution in IRN NP, the nanoparticles were prepared by using PVA and poloxomer188 concentrations of 1% to 2 % each and other parameters were kept constant and their effect on particle size, PDI and percent drug entrapment was evaluated10.

2.3. *Effect of drug to polymer ratio on formulation:* The IRN NP with the different ratios of drug/PLGA i.e., 1:5, 1:10 and 1:20, were prepared keeping other parameters constant11-14. The amount of drug was kept constant while amount of polymer was varied. The particle size, PDI and percent drug entrapment were evaluated.

2.4. *Effect of aqueous to organic phase ratio on formulation*:The aqueous to organic phase ratio in the nanoparticle formation was also optimized, varying the amount of organic phase in three different formulation batches and keeping the amount of aqueous phase constant. here PLGA and drug were weighed and both drug and polymer were dissolved in the 10ml (1:1), 5ml (1:2), 3.33 (1:3), 2.5ml (1:4) acetonitrile for 4 different batches respectively.The particle size, PDI and percent drug entrapment were evaluated.

2.5. *Effect of poloxomer188 concentration on formulation*:In order to optimize the concentration of aqueous surfactant solution, the IRN NP were prepared by using poloxomer-188 at various concentrations of 1%, 2%, 3% and 4% each and other parameters were kept constant. The effect of poloxomer188 on particle size, PDI and percent drug entrapment was studied10.

2.6. *Effect of salt addition on formulation*:Two salts i.e. sodium sulphate and sodium chloride were used in various concentrations i.e. 2%, 1%, 0.5% and 0.1%. These salts were added in the aqueous phase along with the surfactant. The effect of salt addition of IRN NP on the particle size, PDI and percent drug entrapment was evaluated.

Characterization of IRN Nanoparticles

1. Percent drug entrapment

An aliquot of IRN NP dispersion was added to CAN and sonicated well to dissolve nanoparticles completely. The absorbance of the solution was measured at λmax of 256nm using U.V. visible spectrophotometer (UV-1700, Pharmaspec, Shimadzu, Japan).The percent drug entrapment was calculated using following formula.

$$\text{Drug entrapment (\% w/w)} = \frac{\text{Amount of drug in nanoparticles}}{\text{Total amount of the drug used}} \times 100$$

2. Measurement of particle size and zeta potential

The mean particle size, polydispersity index and zeta potential of prepared IRN NP was measured using Dynamic Light Scattering method. Briefly the IRN NP

dispersion was filled in the cuvette and placed in the zeta sizer (Nano 25, Malvern, UK). Analysis was performed at 25 °C with an angle of detection of 90°.Each reported value is the average of three measurements.Each measurement was performed in triplicate and particle size, PDI and zeta potential was measured8.

3. **Lyophilization of the IRN NP**
   Here two cryoprotectants i.e. Sucrose and Trehalose were used at different ratio of solid content to cryoprotectant. The ratio (w/w) of total solid content to cryoprtectant was selected from 1:3, 1:5 and 1:7. The cryoprotectants were dissolved in the IRN NP dispersion as per different ratio and the vials were lyophilized using lyophilizer Vertis Advantage, USA for about 36 hrs. After lyophilization, vials were removed and sealed immediately15-17. The lyophilized vials were reconstituted with 3ml of D.M water followed by 2 min bath sonication and particle size and PDI was measured using zeta sizer (Nano ZS, UK).
4. **Description of in vitro release process for IRN NP**
   The in vitro drug release study for IRN NP was carried out using dialysis method. Briefly the IRN NP dispersion equivalent to 1.5mg of IRN was placed in dialysis tube of Mol. wt 12,000 (Sigma Aldrich, Mumbai). The tube was sealed and dipped in a 500mlbeaker containing phosphate buffer saline pH 7.4. The buffer medium was stirred at speed of 100 rpm and the temperature of medium was kept 37±20 C18. After certain time intervals, 3ml samples were withdrawn and the media was replenished with same volume of fresh buffer using UV visible spectrophotomer (UV-1700, Pharmaspec, Shimadzu, Japan) and the percent cumulative drug release was calculated based on calibration curve of IRN in PBS pH 7.4.

RESULTS AND DISCUSSION

Selection of speed of the magnetic stirrer

The stirring speed of the mechanical stirrer for preparation of IRN NP was kept at various rpm i.e. 400, 600, 800. The effect of stirring speed on the particle size, PDI and percent drug entrapment was evaluated and are shown in table: 1.

The water miscibility of the solvent is the determining factor for nanosuspension preparation. At 600 rpm, high solubility of acetonitrile in water enables their fast diffusion from dispersed droplets into aqueous phase. Thus, as soon as the dispersed phase comes in contact with a large amount of aqueous phase during the emulsion dilution the fast diffusion of organic solvent occurs, leading to fast drug precipitation and particle formation. The finding obtained confirms that at higher speed (i.e 800 rpm) less particle aggregation occurs but the PDI observed was very high compared to batch prepared at 600 rpm, which having similar particle size with lower PDI.Further at speed of 400rpm aggregation was observed and resulted into higher particle size8. Hence the stirring speed was optimized to 600 rpm.

Effect of rate of addition of organic phase to aqueous phase

The rate of addition of solvent containing drug and polymer to aqueous surfactant solution was varied to 0.25ml/min, 0.5ml/min and 1ml/min. The effect of solvent addition speed on the nanoparticles preparation was studied and the results obtained were shown in table:2

The rate of addition of the organic phase to the aqueous phase governs the formation of nanoparticles. The speed at which the solvent is added will demonstrate its effect on the formulation. As shown in table:2, the rate of addition of organic phase at rate of 0.5 ml/min resulted into optimum particle size with lower PDI. In addition, the percent drug entrapment was higher compared to other batches.Hence from the above results it was clear that rate 0.5ml/min was the optimum

speed for the addition of the organic phase to aqueous phase.

Solvent selection

For formulating nanoparticle drug delivery system the solubility of the drug in different solvents is an essential step.So before the formulation of nanoparticles one must have to select the proper solvent in which the drug is maximum soluble in the range which is essential in the nanoparticle drug delivery system[8]. Various solvents like CAN, DMSO, DMF, ethyl acetate were used for preparation of PLGA NP's. Out of these two solvents like acetone and acetonitrile, which are commonly reported in the literature for formation of PLGA NP's, were used.

As shown in table: 3 it was observed that IRN nanoparticles prepared using acetonitrile showed lower particle size and PDI and had comparatively higher percent drug entrapment than acetone. In acetone lower drug entrapment was observed because drug asdissolved in aqueous phase for the preparation of IRN NP.Hence acetonitrile was selected was a solvent of choice for nanoparticle preparation.

Surfactant selection

In order to optimize the concentration of aqueous surfactant solution, the nanoparticles were prepared by using PVA and Poloxomer188 concentrations of 1% to 2 % each and other parameters were kept constant and results obtained are shown in table: 4. For formulating nanoparticle drug delivery system the stability of the drug in different surfactants is an essential step. So before formulation of nanoparticles one must have to select the proper surfactant. The presence of surfactant molecules stabilizes the emulsion nanodroplets and prevents them from coalescing with each other.

For effective stabilization, the surfactant molecules must cover the organic/aqueous interfacial area of all the droplets3. Hence a minimum number of surfactant molecules are required to achieve small particle size and narrow size distribution. As shown in table: 4, the batches prepared using PVA showed aggregation upon solvent evaporation.Hence 2% of poloxomer-188 was selected as the surfactant of choice for nanoparticle preparation.

Drug: Polymer (D: P) ratio

The nanoparticles of IRN with the different ratios of drug: PLGA i.e., 1:5, 1:10 and 1:20, were prepared using solvent evaporation method. The amount of drug was kept constant while amount of polymer was varied and results obtained are shown in table:5.

From the above observations, it observed that on decreasing the drug to polymer ratio the particle size increased with decrease in the percent drug entrapment.At 1:10 ratio of polymer an optimum particle size and percent drug entrapment was observed. Hence as a result the drug to polymer ratio was selected as 1:10.

Aqueous: Organic Phase Ratio

The aqueous to organic phase ratio was varied to note its effect on the particle size and percent drug entrapment. The Results obtained are shown in table: 6.

From the above results obtained, it was noted that with decrease in the volume of organic phase, resulted in the increase in particle size and PDI of IRN NP. A decrease in the percent drug entrapment was also noted.However, we observed no difference in percent drug entrapment upon changing ratio of aqueous to organic phase.At 1:2 ratios we observed less particle size with low PDI.Hence the aqueous to organic phase ratio was selected as 1:2.

Poloxomer188 Concentration

In order to optimize the concentration of aqueous surfactant solution, the nanoparticles were prepared by using poloxomer-188 at concentrations of 1%, 2%, 3% and 4% and other parameters were kept constant. Results were obtained is summarized in the table: 7.

As the Poloxomer188 concentration is increased, the mean diameter of nanoparticles increased. As shown in table: 7, it was observed that there was no any major

change in the particle size, but the PDI and percent drug entrapment was altered. The use of poloxomer188 at 3% & 4% concentration showed no benefit compared to 2% poloxomer 1888. Hence the poloxomer 188 concentration was selected as 2%.

Salt Addition

In order to increase percent drug entrapment two salts were used i.e. sodium sulphate and sodium chloride in varying concentrations i.e. 2%, 1%, 0.5% and 0.1%. Results obtained are shown in table: 8.

The use of both the salt resulted into increase in particle size with decrease in drug entrapment at 0.1% concentration, while the 1% and 2% concentration of these salts did not induced nanoprecipitation and IRN NP was not formed. Hence the use of salt i.e. NaCl and sodium sulphate showed no any role increase in drug entrapment with maintenance of particle size near to 200nm8. So no salts were added to the formulation.

In Vitro Release Profile of IRN NP

The in vitro release pattern of IRN NP is represented in table: 9 and figure: 1.

The drug release profile of IRN NP showed sustained release of drug over 24 hours.A initial burst release of 20.59 was observed at 4hour followed by sustained release. The results obtained are in similar fashion of other water-soluble drug entrapped in PLGA NP's.

Lyophilization of IRN NP

Lyophilized IRN NP with different cryoprotectants at various ratios was reconstituted with D.M. water and following data were obtained.

Trehalose as a cryoprotectant at ratio of 1:3 showed particle size after reconstitution near to the initial particle size.The trehalose at higher ratio (1:5 and 1:7) showed minor change in particle size and PDI.It was found that trehalose at 1:3 ratio showed comparatively better cryoprotective behavior to other ratio of trehalose and sucrose.Trehalose seems to be a preferable cryoprotectant for biomolecules. It has many advantages in comparison with the other sugars as: less hygroscopicity, an absence of internal hydrogen bounds which allows more flexible formation of hydrogen bonds with nanoparticles during freeze-drying, very low chemical reactivity and finally, higher glass transition temperature Tg16.

Particle Size and Zeta Potential Measurement

The particle size and zeta potential was measured using zeta sizer (Nano ZS, Malvern, UK).Zeta potential is the overall charge acquired by particles in a particular medium and its value gives the indication of potential physical stability of nanoparticles dispersion.If all the particles have large positive or negative of zeta potential they will repel each other and system is considered to be stable.Higher the value, more stable the system.The zeta potential obtained was –13.3 mV. The particle size and zeta potential are shown in figure: 2 and 3 respectively.

Table 1: Effect of rotation speed

Batch code	Rotation per minute	Particle size (nm)	Poly dispersivity index	Percent drug entrapment
IRNF024	800	211.6±10.4	0.154±0.020	30
IRNF025	600	218.3±12.5	0.116±0.023	33.82
IRNF026	400	336.5±14.7	0.302±0.019	26.082

Table 2: Effect of rate of addition

Batch code	Rate of addition	Particle size (nm)	Poly dispersivity index	Percent drug entrapment
NF027	0.25 ml/min	309.6±10.8	0.272±0.020	26
IRNF028	0.50IRml/min	213.0±9.6	0.113±0.023	35.3
IRNF029	1 ml/min	245.6±12.1	0.192±0.024	21.3

Table 3: Effect of solvent

Batch code	Solvent	Particle size (nm)	Poly dispersivity index	Percent drug entrapment
IRN F002	Acetone	336.1±12.3	0217±0.010	11.4
IRN F003	Acetonitrile	223.2±10.3	0.113±0.014	34.2

Table 4: Surfactant Selection

Batch code	Surfactant	Particle size (nm)	Poly dispersivity index	Percent drug entrapment	Remarks
IRNF004	1% PVA	-	-	-	Aggregation observed
IRNF005	2%PVA	-	-	-	Aggregation observed
IRNF006	1% Poloxomer188	445.3±11.4	0.342±0.010	25.3	Passed
IRNF007	1% Poloxomer188	218±10.2	0.108±0.013	33.82	Passed

Table 5: Effect of drug to polymer ratio

Batch code	D:P Ratio	Particle size (nm)	Poly dispersivity index	Percent drug entrapment	Remarks
IRNF008	1:5	190.3±8.3	0.271±0.013	8.476	Failed
IRNF009	1:10	223.1±8.2	0.079±0.018	37.2	Passed
IRNF010	1:20	323.2±12.6	0111±0.010	6.32	Failed

Table 6: Effect of aqueous to organic phase ratio

Batch code	Aq:Or Ratio	Particle size (nm)	Poly dispersivity index	Percent drug entrapment	Remarks
IRNF011	1:1	-	-	-	Aggregation was observed
IRNF012	1:2	232.1±4.2	0.111±0.030	32.4	Passed
IRNF013	1:3	268.7±10.2	0.116±0.025	31.7	Passed
IRNF014	1:4	281.8±11.7	0.173±0.021	30.7	Passed

Table 7: Effect of Poloxomer 188 Concentration

Batch code	Poloxomer188 concentration	Particle size (nm)	Poly dispersivity index	Percent drug entrapment
IRNF015	1%	292.4±9.2	0.372±0.014	16.62
IRNF016	2%	221.3±10.6	0.172±0.010	35.64
IRNF017	3%	239.4±13.7	0.297±0.016	27.56
IRNF018	4%	243.6±10.3	0.201±0.013	25.23

Table 8 : Effect of salt addition

Batch code	Salt concentration	Particle size (nm)	Poly dispersivity index	Percent drug entrapment	Remarks
IRNF018	Sodium sulphate 2%	-	-	-	No formation of nanoparticles took place
IRNF019	Sodium sulphate 1%	-	-	-	No formation of nanoparticles took place
IRNF020	Sodium sulphate 0.1%	247.9±20.3	0.397±0.012	23.87	Passed
IRNF021	Sodium chloride 1%	-	-	-	No formation of nanoparticles took place
IRNF022	Sodium chloride 2%	-	-	-	No formation of nanoparticles took place
IRNF023	Sodium chloride 0.1%	232.5±17.8	0.301±0.013	14.47	Passed

Table 9: In vitro profile of IRN NP

Time	Cumulative Percent drug release
15 min	0
30 min	0.4
1 hours	1.3
2 hours	4.75
4 hours	20.59
6 hours	31.85
8 hours	48.32
24 hours	87.3

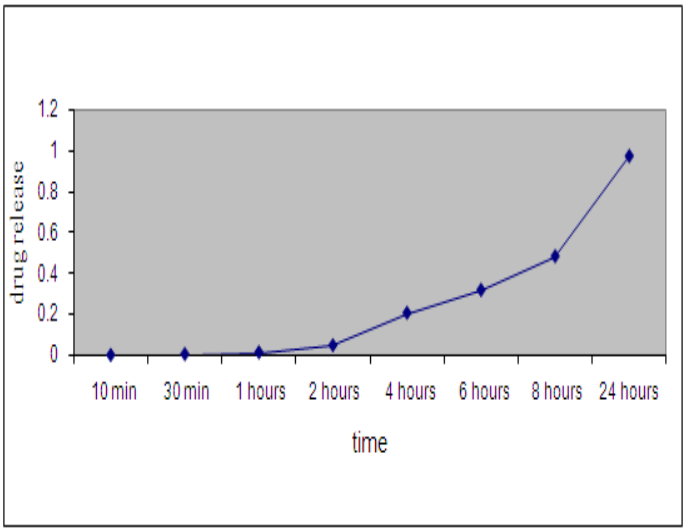

Fig. 1: In vitro release pattern of IRN NP

Table 10: Lyophilization of IRN NP

Batch code	Cryoprotectants	Particle size	PDI
IRNF030	Sucrose 1:3	367.1± 20.1	0.203±0.023
IRNF031	Sucrose 1:5	277.2± 10.2	0.227±0.025
IRNF032	Sucrose 1:7	284.2± 13.3	0.256±0.015
IRNF033	Trehalose1:3	215.5± 11.4	0.112±0.010
IRNF034	Trehalose1:5	186.1± 16.4	0.094±0.013
IRNF035	Trehalose1:7	198.1± 12.8	0.101±0.006

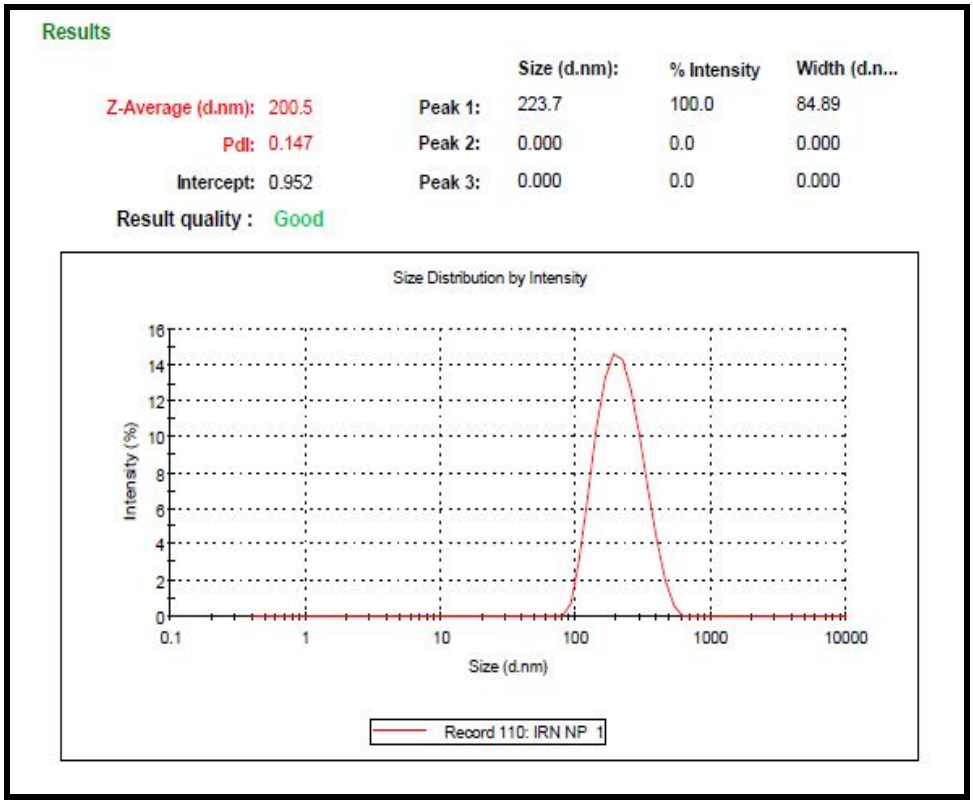

Fig. 2: Particle size of IRN NP

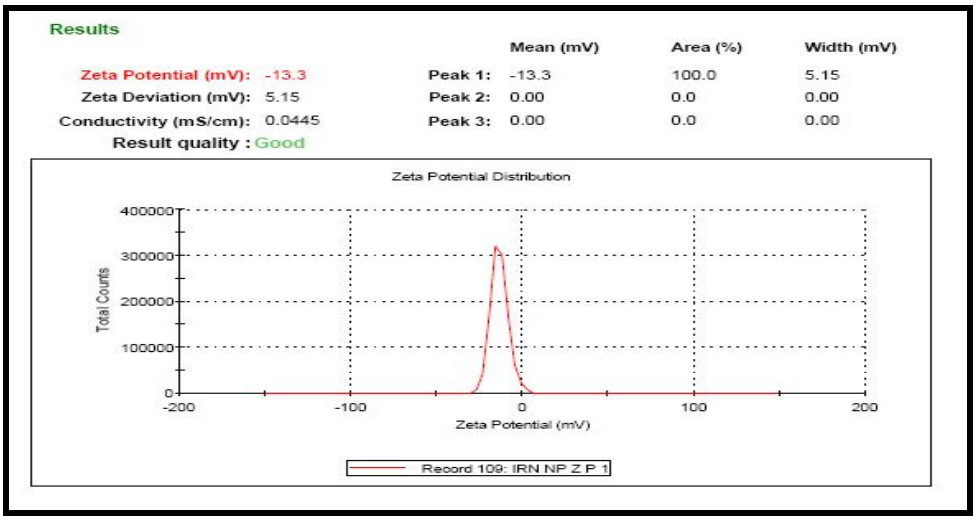

Fig. 3: Zeta potential of IRN NP

REFERENCES

1. Banker G S; Pharmaceutical applications of controlled release – an overview of the past, present and future. In Medical Applications of Controlled Release; Langer, R. S. Wise, D. L. Eds; CRC Press: Boca Raton, Florida, 1984; 11, Chapter 1.

2. Surendiran, S. Sandhiya, S.C. Pradhan& C. Adithan, Novel applications of nanotechnology in medicine, Indian J Med Res. 2009; (130), 689-701.
3. Margaret A. Phillips a, Martin L. Granb, Nicholas A. Peppas, Targeted nanodelivery of drugs and diagnostics, Nano Today. 2010; 5: 143-159.
4. Mauro Ferrari, Cancer Nanotechnology: Opportunities and challenges, Nature reviews. 2005; 5: 161-171.
5. http://www.drugbank.ca/drugs/DB00762
6. http://en.wikipedia.org/wiki/Irinotecan.
7. AkanshaTripathi, Ranjana Gupta, Shubhini A. Saraf, PLGA nanoparticles for antitubercular drug: Drug Loading and Release Studies of a Water In-Soluble Drug, International Journal of PharmTech Research. 2010; 2(3): 2116-2123.
8. AvinashBudhian, Steven J. Siegel, Karen I Winey, Haloperidol-loaded PLGA nanoparticles: Systematic study of particle size and drug content, Pharmaceutical Nanotechnology, International Journal of Pharmaceutics.2007;336:367-375.
9. AkanshaTripathi, Ranjana Gupta, Shubhini A. Saraf, PLGA nanoparticles for antitubercular drug: Drug Loading and Release Studies of a Water In-Soluble Drug, International Journal of PharmTech Research. 2010; 2(3): 2116-2123.
10. Redhead, S.S. Davis, L. Illum, Drug delivery in poly (lactide-co-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: in vitro characterisation and in vivo evaluation, Journal of Controlled Release.2001;70: 353-363.
11. Muthu, Nanoparticles based on PLGA and its copolymers: An overview, Asian journal of pharmaceutics. 2009;35-75.
12. Astete, C. E. and Sabliov, C. M. Synthesis and characterization of PLGA nanoparticles. Journal of Biomaterials Science. 2006;17(3): 247-289.
13. Karen I. AvinashBudhian, Steven J. Siegel, Winey Haloperidol-loaded PLGA nanoparticles: Systematic study of particle size and drug content, Pharmaceutical Nanotechnology, International Journal of Pharmaceutics.2007; 336: 367-375.
14. Cristina Fonsecaa, Se´rgioSimo˜esa,b , Roge´rioGaspara, Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro anti-tumoral activity, , Journal of Controlled Release.2002;83:273-286.
15. Salvatore A. Velardi, Antonello A. Barresi, Development of simplified models for the freeze-drying process and investigation of the optimal operating conditions, chemical engineering research and design.2008; 86: 9-22.
16. Crowe, D.S. Reid, J.H. Crowe, Is trehalose special for preserving dry materials. Biophys. J. 1996;71: 2087-2093.
17. WassimAbdelwahed, GhaniaDegobert, Serge Stainmesse , HatemFessi, Freeze-drying of nanoparticles: Formulation, process and storage considerations, Advanced Drug Delivery Reviews. 2006;58:1688-1713.
18. Ali Mohammadi, FarnazEsmaeili, Simultaneous Determination of Irinotecan Hydrochloride and its Related Compounds by High Performance Liquid Chromatography Using Ultraviolet Detection, Asian Journal of Chemistry. 2010;22(5):3966-3972.