Journal of Pharma Creations (JPC)

JPC |Vol.11 | Issue 3 | Jul - Sept -2024

www.pharmacreations.com

DOI : https://doi.org/10.61096/jpc.v11.iss3.2024.230-237

ISSN: 2348-6295

Research

Preparation Characterization of Irinotecan Gum Ghatti Nano Particles

Saju Rajappanm*[1], NS. Surendiran[2]

[1]*Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, India*
[2]*Nirmala college of Health sciences, meloor, chalakudy, Kerala, India*

*Author for Correspondence: Saju Rajappanm
Email: saivaishnavyp@gmail.com

Published on: 04 Aug 2024

Published by:
DrSriram Publications

Abstract

The aim of the present investigation was to formulate, optimize, and characterize Gum Ghatti (GG) nanoparticles containing Irinotecan for cancer therapy. The nanoparticles were formed using a solvent evaporation technique involving aqueous and organic phases. The formulation was optimized by adjusting various process and formulation parameters. The analytical method was developed using acetonitrile and phosphate buffer saline. Different organic solvents and various surfactants were tested to optimize the nanoparticulate formulation. The size range and zeta potential were measured using a Malvern zetasizer. Lyophilization was carried out using two different methods, with the maximum drug entrapment percentage found to be 35.2%. The in vitro drug release of Irinotecan nanoparticles was assessed using the dialysis method in phosphate buffer saline at pH 7.4. The in vitro drug release demonstrated sustained drug release over 24 hours. Therefore, Irinotecan-loaded GG nanoparticles have potential as a drug delivery system. Furthermore, they may be useful for site-specific drug delivery, as their small size may allow them to reach extravascular target sites through the leaky endothelium of inflamed and cancerous areas.

Keywords: Irinotecan**,** Malvern zetasizer**,** phosphate buffer**,** Gum Ghatti, extravascular target endothelium

INTRODUCTION

Substances with sizes ranging from 1 to 1000 nm are called nanoparticles. These materials are primarily used in oncology for early detection of malignancies and precise localization of cancer therapeutics with minimal adverse effects on somatic tissues. Nanoparticles are utilized to protect drugs, vaccines, nutrients, and cosmetics. They achieve site-specific drug delivery by avoiding the reticuloendothelial system, utilizing enhanced permeability and retention effects, and targeting tumors specifically. The formation of nanoparticles and their physicochemical parameters, such as pH, monomer concentration, ionic strength, surface charge, particle size, and molecular weight, are crucial for effective drug delivery. Furthermore, these nanoparticles can reverse multidrug resistance, a significant problem in chemotherapy. Their unique ability to access virtually all cell types can be exploited to deliver therapeutic agents to a wide array of cellular targets.

Irinotecan, chemically known as 1(S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo-1H-pyrano [3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'-bipiperidine]-1'-carboxylate, is a camptothecin analogue.

Irinotecan Hydrochloride Trihydrate contains not less than 98.0 percent and not more than 102.0 percent of C33H38N4O6·HCl, calculated on an anhydrous basis. Camptothecins work by stabilizing the cleavable complex where topoisomerase I is covalently bound to DNA at a single-stranded break site. Conversion into lethal DNA damage occurs when a DNA replication fork encounters these cleavable complexes (fork collision model). Irinotecan is a prodrug that requires

MATERIALS AND METHODS

Irinotecan hydrochloride trihydrate was obtained from Zydus Research Centre, Ahmadabad. Acetonitrile and acetone were sourced from Merck Specialities Pvt Ltd, Mumbai. Poly(dl-lactide-co-glycolide) (50:50) was obtained from Durect Corporation, Birmingham Division, Pelham. Poloxamer 188 and polyvinyl alcohol were sourced from Sigma Chemical Co., St. Louis, USA. Sodium sulfate and sodium chloride were procured from Canton Laboratories Pvt Ltd, Baroda, India. All other chemicals used were of analytical grade. Different batches were prepared at stirring speeds of 400, 600, and 800 rpm, and their effects on nanoparticle preparation were studied. The particle size, PDI (polydispersity index), and percent drug entrapment were evaluated.

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 formulation. For this purpose, the Effect of Addition Rate of Solvent Containing Drug and Polymer to Aqueous Surfactant Solution The rate of addition of the solvent containing drug and polymer to the aqueous surfactant solution was maintained at 0.25 ml/min, 0.5 ml/min, and 1 ml/min. The effect of the solvent addition rate on particle size, PDI, and percent drug entrapment was evaluated.

Formula Optimization

Solvent Selection (Organic Phase)

Acetonitrile and acetone are the most commonly reported solvents for nanoparticle preparation. Both solvents were initially used in a 1:2 ratio of the nanoparticulate system in the aqueous phase, keeping other parameters constant. The effect of these solvents on particle size, PDI, and percent drug entrapment was evaluated. Since IRN is insoluble in acetone, it was dissolved in the aqueous phase, and the nanoparticles were fabricated in ACN.

Surfactant Selection

To optimize the concentration of aqueous surfactant solution in IRN NP, nanoparticles were prepared using PVA and poloxamer 188 concentrations ranging from 1% to 2%, with other parameters kept constant. Their effects on particle size, PDI, and percent drug entrapment were evaluated.

Effect of Drug to Polymer Ratio on Formulation

IRN NP with different ratios of drug to PLGA (1:5, 1:10, and 1:20) were prepared, keeping other parameters constant. The amount of drug was kept constant while the amount of polymer was varied. The particle size, PDI, and percent drug entrapment were evaluated

Effect of Aqueous to Organic Phase Ratio on Formulation

The aqueous to organic phase ratio in nanoparticle formation was optimized by varying the amount of organic phase in three different formulation batches, keeping other parameters constant. The particle size, PDI, and percent drug entrapment were evaluated to determine the optimal ratio.

Effect of Aqueous to Organic Phase Ratio on Formulation

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

Effect of Poloxamer 188 Concentration on Formulation

To optimize the concentration of aqueous surfactant solution, IRN nanoparticles were prepared using Poloxamer 188 at various concentrations of 1%, 2%, 3%, and 4%, while keeping other parameters constant. The effect of Poloxamer 188 on particle size, PDI, and percent drug entrapment was studied.

Effect of Salt Addition on Formulation

Two salts, sodium sulfate and sodium chloride, were used in various concentrations (2%, 1%, 0.5%, and 0.1%). These salts were added to the aqueous phase along with the surfactant. The effect of salt addition on IRN nanoparticles regarding particle size, PDI, and percent drug entrapment was evaluated.

Characterization of IRN Nanoparticles

Percent Drug Entrapment

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

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

Measurement of Particle Size and Zeta Potential

The mean particle size, polydispersity index, and zeta potential of the prepared IRN nanoparticles were measured using the Dynamic Light Scattering method. The dispersion was filled in the cuvette and placed in the Zetasizer (Nano 25, Malvern, UK). Analysis was performed at 25°C with a detection angle of 90°. Each reported value is the average of three measurements. Each measurement was performed in triplicate, and particle size, PDI, and zeta potential were measured.

Lyophilization of the IRN Nanoparticles

Two cryoprotectants, sucrose and trehalose, were used at different ratios of solid content to cryoprotectant. The ratio (w/w) of total solid content to cryoprotectant was selected as 1:3, 1:5, and 1:7. The cryoprotectants were dissolved in the IRN nanoparticle dispersion according to the different ratios, and the vials were lyophilized using a lyophilizer (Vertis Advantage, USA) for about 36 hours. After lyophilization, the vials were removed and sealed immediately. The lyophilized vials were reconstituted with 3 ml of DM water, followed by 2 minutes of bath sonication, and the particle size and PDI were measured using the Zetasizer (Nano ZS, UK).

Description of In Vitro Release Process for IRN Nanoparticles

The in vitro drug release study for IRN nanoparticles was carried out using the dialysis method. Briefly, IRN nanoparticle dispersion equivalent to 1.5 mg of IRN was placed in a dialysis tube with a molecular weight cutoff of 12,000 (Sigma Aldrich, Mumbai). The tube was sealed and immersed in a 500 ml beaker containing phosphate-buffered saline (PBS) pH 7.4. The buffer medium was stirred at a speed of 100 rpm, and the temperature was maintained at 37 ± 2°C. At specific time intervals, 3 ml samples were withdrawn, and the medium was replenished with the same volume of fresh buffer. The percent cumulative drug release was calculated based on the calibration curve of IRN in PBS pH 7.4, using a UV-visible spectrophotometer (UV-1700, Pharmaspec, Shimadzu, Japan).

RESULTS AND DISCUSSION

Selection of Speed of the Magnetic Stirrer

The stirring speed of the mechanical stirrer for the preparation of IRN nanoparticles (NP) was varied at 400, 600, and 800 rpm. The effects of stirring speed on particle size, PDI, and percent drug entrapment were evaluated and are shown in Table 1. The water miscibility of the solvent is a determining factor for nanosuspension preparation. At 600 rpm, the high solubility of acetonitrile in water enables fast diffusion from dispersed droplets into the aqueous phase. Thus, when the dispersed phase contacts a large amount of aqueous phase during emulsion dilution, rapid diffusion of the organic solvent occurs, leading to fast drug precipitation and particle formation. The findings confirm that at higher speeds (800 rpm), less particle aggregation occurs, but the PDI observed was higher compared to the batch prepared at 600 rpm, which had a similar particle size with a lower PDI. Furthermore, at 400 rpm, aggregation was observed, resulting in larger particle size. Therefore, the stirring speed was optimized to 600 rpm.

Effect of Rate of Addition of Organic Phase to Aqueous Phase

The rate of addition of the solvent containing drug and polymer to the aqueous surfactant solution was varied to 0.25 ml/min, 0.5 ml/min, and 1 ml/min. The effect of solvent addition speed on nanoparticle preparation was studied, and the results are shown in Table 2.The rate of addition of the organic phase to the aqueous phase governs nanoparticle formation. The speed at which the solvent is added demonstrates its effect on the formulation. As shown in Table 2, the rate of addition of the organic phase at 0.5 ml/min resulted in optimal particle size with

lower PDI. Additionally, the percent drug entrapment was higher compared to other batches. Hence, 0.5 ml/min was determined to be the optimal speed for adding the organic phase to the aqueous phase.

Solvent Selection

For formulating a nanoparticle drug delivery system, the solubility of the drug in different solvents is an essential step. Before formulating nanoparticles, one must select a solvent in which the drug is maximally soluble, which is crucial in the nanoparticle drug delivery system. Various solvents like CAN, DMSO, DMF, and ethyl acetate were used for the preparation of PLGA nanoparticles. Acetone and acetonitrile, commonly reported in the literature for the formation of PLGA nanoparticles, were used. As shown in Table 3, IRN nanoparticles prepared using acetonitrile exhibited lower particle size and PDI and had comparatively higher percent drug entrapment than those prepared with acetone. Lower drug entrapment was observed with acetone because the drug dissolved in the aqueous phase during the preparation of IRN nanoparticles. Hence, acetonitrile was selected as the solvent of choice for nanoparticle preparation.

Surfactant Selection

To optimize the concentration of the aqueous surfactant solution, nanoparticles were prepared using PVA and Poloxamer 188 at concentrations of 1% to 2%, while keeping other parameters constant. The results are shown in Table 4. For formulating a nanoparticle drug delivery system, the stability of the drug in different surfactants is essential. Before formulating nanoparticles, one must select the proper surfactant. Surfactant molecules stabilize emulsion nanodroplets and prevent them from coalescing. Effective stabilization requires surfactant molecules to cover the organic/aqueous interfacial area of all droplets. A minimum number of surfactant molecules are needed to achieve small particle size and narrow size distribution. As shown in Table 4, batches prepared using PVA showed aggregation upon solvent evaporation. Hence, 2% of Poloxamer 188 was selected as the surfactant of choice for nanoparticle preparation.

Drug: Polymer (D) Ratio

Nanoparticles of IRN with different ratios of drug (1:5, 1:10, and 1:20) were prepared using the solvent evaporation method. The amount of drug was kept constant while the amount of polymer was varied, and the results are shown in Table 5. Observations indicated that decreasing the drug-to-polymer ratio increased the particle size and decreased the percent drug entrapment. At a 1:10 polymer ratio, optimal particle size and percent drug entrapment were observed. Therefore, the drug-to-polymer ratio was selected as 1:10.

Aqueous: Organic Phase Ratio

The aqueous to organic phase ratio was varied to observe its effect on particle size and percent drug entrapment. The results are shown in Table 6. From the results, it was noted that decreasing the volume of the organic phase increased the particle size and PDI of IRN nanoparticles. A decrease in the percent drug entrapment was also noted. However, no difference in percent drug entrapment was observed when changing the ratio of the aqueous to organic phase. At a 1:2 ratio, less particle size with low PDI was observed. Hence, the aqueous to organic phase ratio was selected as 1:2.

Poloxamer 188 Concentration

To optimize the concentration of the aqueous surfactant solution, nanoparticles were prepared using Poloxamer 188 at concentrations of 1%, 2%, 3%, and 4%, while keeping other parameters constant. The results are summarized in Table 7. As the Poloxamer 188 concentration increased, the mean diameter of nanoparticles also increased. As shown in Table 7, it was observed that increasing Poloxamer 188 concentration... (Text cuts off here; please let me know if you need more details on the concentration effects.)

Poloxamer 188 Concentration

Increasing the concentration of Poloxamer 188 did not significantly change the particle size, but it did alter the PDI and percent drug entrapment. The use of Poloxamer 188 at 3% and 4% concentrations showed no benefit compared to 2% Poloxamer 188. Hence, the Poloxamer 188 concentration was selected as 2%.

Salt Addition

To increase percent drug entrapment, two salts—sodium sulfate and sodium chloride—were used at varying concentrations of 2%, 1%, 0.5%, and 0.1%. The results obtained are shown in Table 8. The use of both salts resulted in increased particle size and decreased drug entrapment at 0.1% concentration, while the 1% and 2% concentrations did not induce nanoprecipitation, and IRN nanoparticles were not formed. Therefore, the use of NaCl and sodium sulfate showed no role in increasing drug entrapment while maintaining a particle size near 200 nm. Consequently, 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 drug release over 24 hours. An initial burst release of 20.59% was observed at 4 hours, followed by sustained release. The results obtained were similar to those of other water-soluble drugs entrapped in PLGA nanoparticles.

Lyophilization of IRN NP

Lyophilized IRN NP with different cryoprotectants at various ratios were reconstituted with distilled water, and the following data were obtained. Trehalose as a cryoprotectant at a ratio of 1:3 showed particle size after reconstitution close to the initial particle size. Trehalose at higher ratios (1:5 and 1:7) showed minor changes in particle size and PDI. It was found that trehalose at a 1:3 ratio showed comparatively better cryoprotective behavior than other ratios of trehalose and sucrose. Trehalose seems to be a preferable cryoprotectant for biomolecules. It has many advantages compared to other sugars, such as less hygroscopicity, an absence of internal hydrogen bonds, allowing more flexible formation of hydrogen bonds with nanoparticles during freeze-drying, very low chemical reactivity, and a higher glass transition temperature (Tg).

Particle Size and Zeta Potential Measurement

The particle size and zeta potential were measured using a zeta sizer (Nano ZS, Malvern, UK). Zeta potential indicates the overall charge acquired by particles in a particular medium and gives an indication of the potential physical stability of nanoparticle dispersions. If all the particles have a large positive or negative zeta potential, they will repel each other, and the system is considered stable. The higher the value, the more stable the system. The zeta potential obtained was -13.3 mV. The particle size and zeta potential are shown in Figures 2 and 3, respectively.

Table 1: characters of actual security

Appearance	Transparent quantifiable
Odor	odorless
Color	White
Solubility	9.64±0.27mg/ml
Melting point	40-45ºC
Absorbance	205 nm

Table 2: Rotation per min

S. No	Rotation per min	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1	600	212.7±10.5	0.15±0.02	29.9
2	800	219.4±12.6	0.11±0.02	34.82

Table 3: Rate of addition

S. No	Rate of addition	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1	0.25 ml/min	308.6±10.87	0.272±0.020	27
2	0.50 ml/min	212.0±9.7	0.113±0.023	36.3
3	1 ml/min	246.6±12.2	0.192±0.024	22

Table 4: Effect of solvent

S. No	Effect of solvent	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1	Acetone	336.1±12.3	0.217±0.010	11.4
2	Acetonitrile	223.2±10.3	0.113±0.014	34.2

Table 5: Surfactant

S. No	Surfactant	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1	1% PVA	-	-	-
2	2% PVA	-	-	-
3	1% Poloxamer 188	445.3±11.4	0.342±0.010	25.3
4	2% Poloxamer 188	218±10.2	0.108±0.013	33.82

Table 6: Drug polymer ratio

S. No	Drug polymer ratio	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1	1:5	190.3±8.3	0.271±0.013	8.476
2	1:10	223.1±8.2	0.079±0.018	37.2
3	1:20	323.2±12.6	0.111±0.010	6.32

Table 7: Polymer

polymer	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1%	292.4±9.2	0.372±0.014	16.62
2%	221.3±10.6	0.172±0.010	35.64
3%	239.4±13.7	0.297±0.016	27.56
1%	292.4±9.2	0.372±0.014	16.62

Table 8: Aqueous: organic

Aqueous: organic	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1:1	-	-	-
1:2	232.1±4.2	0.111±0.030	32.4
1:3	268.7±10.2	0.116±0.025	31.7
1:4	281.8±11.7	0.173±0.021	30.7

Table 9: Poloxamer 188 conc

Poloxamer 188 conc	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
1%	292.4±9.2	0.372±0.014	16.62
2%	221.3±10.6	0.172±0.010	35.64
3%	239.4±13.7	0.297±0.016	27.56

Table 10: Salt

Salt	Particle size (nm)	Polydispersity Index	Percept of Drug Entrapment
Sodium sulfate 2%	-	-	-
Sodium sulfate 1%	-	-	-
Sodium sulfate 0.1%	247.9±20.3	0.397±0.012	23.87
Sodium chloride 2%	-	-	-
Sodium chloride 0.1%	232.5±17.8	0.301±0.013	14.47
Sodium chloride 2%	-	-	-

Table 11: In vitro drug release

S. No	Time	Percentage
1	15 Mins	0±0.0
2	30 Mins	0.45±0.01
3	45 Mins	1.5±0.01
4	60 Mins	2.8±0.1
5	2 Hrs	8.6±0.2
6	4 Hrs	17.2±0.4
7	8 Hrs	35.7±0.4
8	12 Hrs	60.4±0.2
9	24 Hrs	84.7±0.7

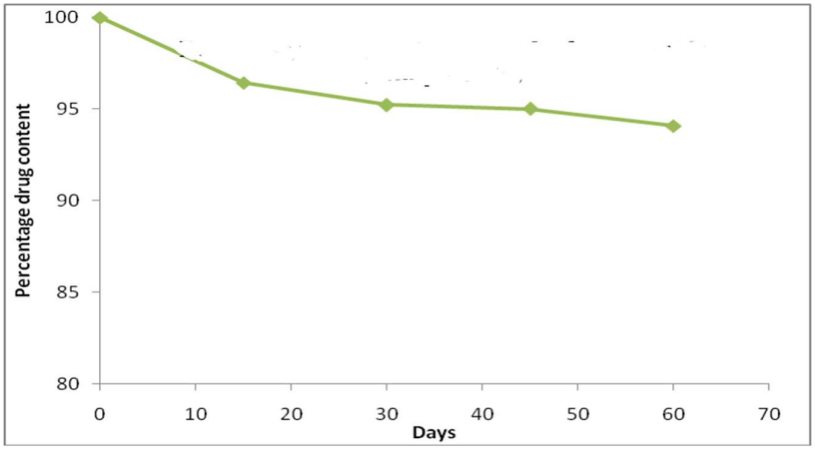

Fig 1: Prepared ITC 5 ºC ± 3 ºC (Long-term stability)

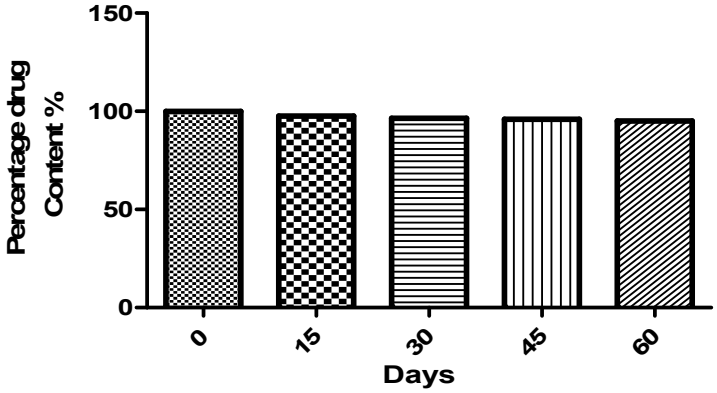

Fig 2: Proportion drug release prepared ITC 5ºC±3 ºC

SUMMARY AND CONCLUSION

The science of drug delivery can be described as the application of chemical and biological principles to control the temporal and spatial location of drug molecules in the body for therapeutic benefit. Generally, the objective of targeted systems is to increase the efficacy and reduce the toxicity of drugs. This is achieved by optimizing the amount and duration of the drug delivered to the target cells. To obtain the optimum therapeutic effect of a drug, a proper delivery system should be fabricated for maximum benefit. Numerous strategies are available for drug targeting; however, the development of a complete target drug delivery system still faces challenges. As a result, the industry is now focusing on miniaturizing drug delivery devices from the microscale to the nanoscale and combining nanotechnology with therapeutic molecules for various diseases. Biodegradable nanoparticles are frequently used to enhance the therapeutic potential of medicinal drugs and bioactive molecules by improving their bioavailability, solubility, and retention time. Desired nanomedicines are generally achieved through a hit-and-trial method regarding controlled release, targeted delivery, and therapeutic impact. Pharmaceutical companies' scientific efforts primarily focus on potential therapeutic interests and developing new drug delivery systems. They deal with novel formulations of existing drug molecules that can increase efficacy and reduce associated toxicity. Currently, several conventional formulations have been developed and marketed. Extensive advancements in science have resulted in innovative ideas in nanoscience and nanotechnology, opening several new possibilities in formulation development and addressing issues related to conventional dosage forms. The present study aimed to investigate the potential of ITC in different types of nanoparticles. It also explored the physical properties and formulation variables that impact the stability and efficacy of GGITC. To achieve this aim, three different types of nanoparticles were prepared: chitosan-based polymeric nanoparticles using the ionic gelation method, GG-based nanoparticles using the emulsification-sonication-solvent evaporation method, and solid lipid nanoparticles using the hot homogenization method . Attempts can be made to understand the properties of nanoparticles which have not been studied. The parameters are to be optimized to make them suitable for

biomedical applications. Safe and effective drug delivery system This work will be useful for further nanoparticles development to the treatment of cancer growth.

REFERENCES

1. Bdelwahed, W., Degobert, G., Stainmesse, S. and Fessi, H. (2006), Freeze-dryingof nanoparticles: formulation, process and storage considerations. Advanced drug delivery reviews 58:1688- 1713.
2. Abhilash, M. (2010). Potential applications of Nanoparticles . International Journel of Pharmacy and Bio Sciences l:l-12.
3. Abouelmagd, S.A., Sun, B., Chang, A.C., Ku, Y.J. and Yeo, Y. (2015). Release kinetics study of poorly water-soluble drugs lrom nanoparticles: are we doing it right? Moleculqr Pharmarmaceutics 12:997 -1003.
4. Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S.W., Zarghami, N., Hanifehpour, Y. and Nejati-Koshki, K. (2013). Liposome: classification, preparation and applications . Nanoscale Research Lelters 8:102.
5. Alavi, M., Karimi, N. and Safaei, M. (2017). Application of Various Types of Liposomes inDrug Delivery Systems. ldvarced pharmaceutical bullelin 7:3- 9.
6. Albanese, A., Tang, P.S. and Chan, W.C. (2012). The effect of nanoparticle size, shape and surface chemistry on biological systems. Annual Review of Biomedical Engineering 14: l-16.
7. Alex, R. and Bodmeier, R. (1990). Encapsulation of water-soluble drugs by a modified solvent evaporation method. I. Effect of process and formulation variables on drug entrapment. Journal of Microencapsulation 7:347 -55.
8. Alfarouk, K.O., Stock, C.M., Taylor, S., Walsh, M., Muddathir, A.K., Verduzco, D., Bashir, A.H., Mohammed, O.Y., Elhassan, G.O., Harguindey, S., Reshkin, S. J., Ibrahim, M.E. Rauch, C. (2015). Resistance to cancer chemotherapy: failure in drug respons from ADME to P-gp. Cancer Cell International 75:71.
9. Allemann, E., Leroux, J.C., Gumy, R. and Doelker, E. (1993). In vitro extended- release properties of drugJoaded poly(Dl-lactic acid) nanoparticles produced by a salting-out procedure. Pharmaceutical Research 10:1732-37
10. Bannwarth, B., pehourcq, F., Schaeverbeke, T. and Dehais, J. (1996). Clinical pharmacokinetics of low-dose pulse methotrexate in rheumatoid arthritis. C linical P harmacokinetics 30:194-210.
11. Baoum, A., Dhillon, N., Buch, S. and Berkland, C. (2010). Cationic surface modification of PLG nanoparticles offers sustained gene delivery to pulmonary epithelial cells. Journal of Pharmaceutical Sciences 99:2413-)An1
12. Barichello, J.M., Morishita, M., Takayama, K. and Nagai, T. (1999). Encapsulation of hydrophilic and lipophilic drugs in PLGA nanoparticles by thenanoprecipitation method. Drug development and Industrial pharmacy 25:471-76.
13. Calvo, P., Remunan-Lopez, C., Vila-Jato, J.L. and Alonso, M.J. (1997). Chitosan and chitosan/ethylene oxide-propylene as novel carriers for proteins and l4:1431-1436.
14. Calvo, P., Remufiiin-L6pez, C., Vila-Jato, J.L. and Alonso, M.J. (1997). Novelhydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. Journal ofApplied Polymer Science 63:125-32.
15. De Smedt, S.C., Demeester, J. and Hennink, W.E. (2000). Cationic polymer based gene delivery systems. Pharmaceutical Research 17 :1 13-126.
16. De Waard, H., Frijlink, H.W. and Hinrichs, W.L. (2011). Bottom-up preparation techniques for nanocrystals of lipophilic drugs. Pharmaceutical Research 28:1220-2
17. Diab,K.A.,Gwu,S.K.,Bhushan,S.andSaxena,A.K'(2015).InVitroAnticancerActivities of Anogeissus latifolia, Terminalia bellerica, Acacia catechu and Moringa oleifema Indian Plants. Asian Pacific Journal of cancer Prevention 16:6423-6428.
18. Diaspro, A., Krol, S., Cavalleri, O., Silvano, D. and Gliozzi' A' (2002)' Microscopical characterization of nanocapsules templated on ionic crystals and biological cells toward biomedical applications. IEEE Transactions on Nanobioscience 1:1 10- 15