International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 10, 2016

Original Article

SOLID LIPID NANOPARTICLE: FABRICATED THROUGH NANOPRECIPITATION AND THEIR PHYSICOCHEMICAL CHARACTERIZATION

ARUN SHARMA, VINEET MEHTA, ARUN PARASHAR, RIDDHI PATRWAL, UDAYABANU MALAIRAMAN*

Department of Pharmacy, Jaypee University of Information Technology, Waknaghat, Himachal Pradesh, India 173234
Email: m_udayabanu@rediffmail.com

Received: 02 Jun 2016 Revised and Accepted: 12 Aug 2016

ABSTRACT

Objective: Aim of the present study was to formulate solid lipid nanoparticles (SLNs) and to determine their physicochemical parameters when stored at cold temperature in aqueous solution (D-SLNs) prior to biological application.

Methods: SLNs were formulated though nanoprecipitation technique which comprised of stearic acid (lipid), poloxamer 188 and lecithin (surfactant). Physicochemical parameters were estimated though particle size analysis, polydispersity index, surface morphology analysis (Scanning electron microscopy and Transmission electron microscopy**)** and cytotoxicity studies followed by live-dead staining through acridine orange and ethidium bromide.

Results: SLNs with spherical morphology were successfully fabricated as revealed though SEM and TEM investigations. Fabricated SLNs had the mean particle size ranging from 188 nm (SLNs) to 327 nm (D-SLNs). Zeta potential was found to be±14mV to±6mV and polydispersity index was 0.297±0.18 for SLNs without incubation and 0.538±0.07 for SLNs after incubation. No cytotoxicity was observed for SLNs.

Conclusion: SEM and TEM investigations showed morphological variation in SLNs and D-SLNs. Dissimilarity in mean particle size, zeta potential and polydispersity index indicates the increase in size and aggregation of nanoparticles. No cytotoxic effects of SLNs were observed in normal cells, suggesting storage of nanoformulation in the aqueous state has no effect in context to cytotoxicity. Hence we conclude that prolonged storage of formulation at cold temperature causes the deterioration of polymeric formulation.

Keywords: Solid lipid nanoparticles, Scanning electron microscopy, Transmission electron microscopy, Nanoprecipitation

DOI: http://dx.doi.org/10.22159/ijpps.2016v8i10.13207

INTRODUCTION

In recent past, biological applications in nanotechnology have gained much consideration from scientific world to develop new advanced nanoparticle system for better therapeutic applications [1]. The lipid-based system is the best instance of nano-technological advancements in this perspective, which has undergone extensive research in past decade [2, 3]. These lipid-based formulations are well acknowledged as solid lipid nanoparticles (SLNs) which in general are solid lipid-based matrix systems. SLNs have some prodigious benefits over conventional carrier systems such as microspheres, lipospheres, emulsions and colloidal systems in context to better controlled drug delivery[4]. SLNs are broadly known for their biocompatible and biodegradable assets and have been considered harmless to the human body as per GRAS (Generally Recognized as Safe) status given by FDA [5-8].

Among existing preferences, stearic acid (SA), an 18-carbon chain saturated fatty acid, is the most appropriate and widely employed lipid-based polymer for nano-formulations [9, 10]. Nano-precipitation is an efficient technique to fabricate nanoparticles at small-scale laboratory conditions and has been comprehensively used to develop SLNs [11]. Lipid-based nano-formulations are known for their proficiency to tackle solubility problems related to numerous chemical and biological compounds. Drug release in case of SLNs occurs either through diffusion mechanism or by degradation of lipid matrix due to enzymatically driven degradation process though lipase enzyme [12]. Enzymatic degradation was well studied in a report published by C. Olbrich et. al where they have used lipase and colipase enzymes. The hydrolytic process is also responsible for the degradation of the lipid-based formulation but to a little extent [12]. The rate of hydrolytic degradation process was found to be faster at room temperature but significantly reduced at cold storage conditions. Further, prolonged storage of nanoformulation in aqueous solution at cold storage (4 °C) leads to deformity or development of cracks on the surface of the nanosphere, which changes physicochemical characteristics and leads to failure of the formulation [13]. This problem can be eliminated successfully by lyophilisation, but its higher cost and increased chances of getting contaminated limits its use [14].

The associated problem of formulation failure can be efficiently eliminated with a proper understanding of the changes which are continuously taking place at a physicochemical level under aqueous storage conditions (4 °C). Keeping this in mind, we made an attempt to investigate the morphological changes in SLNs when stored in aqueous solutions (4 °C). The aim of the present study was to investigate the effect of stearic acid on the stability of nanoformulation fabricated though nanoprecipitation technique and to evaluate its effect on cytotoxicity.

MATERIALS AND METHODS

Materials

Poloxamer 188 was received as gift sample from BASF (India). Stearic acid and lecithin were purchased from Himedia (India). Unless and otherwise specified all chemical were procured from Merck chemicals (India).

Preparation of SLNs

Solid lipid nanoparticles were formulated in accordance with earlier reported and well-recognised nanoprecipitation method with slight modification at laboratory conditions [15]. Stearic acid (SA) 100 mg was melted at 80 °C over a water bath and 500 µl of ethanol was injected into melted SA solution under sonication. Poloxamer 188 (75 mg) and lecithin (75 mg) were dispensed into 3 ml of distilled water and sonicated for 15 min at 80 °C over a water bath. Further, they were added into previously melted SA solution under sonication. The solution was then dispersed in 2 ml of distilled water at 4 °C and sonicated for 15 more min. Afterward, the formulation was washed twice with triple distilled water in order to remove traces of impurities (surfactants or other excipients).

Samples were processed for filtration prior to a further investigation by diluting the SLNs dispersion four times in PBS (7.4 pH) making the final volume up to 30 ml and passing through 0.22 µm sterile syringe filter. This step is essential to remove bigger sized NPs from the nanoformulation and sidewise to attain homogeneous dispersion, as the most desirable size of NPs for biological application is in the range of 200 nm [16, 17].

SLNs incubation at cold temperature

Fabricated nanoformulation was incubated at 4 °C in laboratory refrigerator for three weeks in PBS (7.4 pH) and further analyzed for morphological changes. Solid lipid nanoparticles before incubation are designated as SLNs and after degradation as D-SLNs

Scanning electron microscopy investigation

Surface morphology was characterized by scanning electron microscopy (S-3400N, Hitachi, Japan). Briefly, the colloidal suspensions were deposited on a metallic probe and placed in liquid nitrogen for 10 min followed by vacuum evaporation. SLNs were metallized by gold/palladium using a cathodic pulverizer technics hummer II (6 V–10 mA). Images were captured and processed for morphological data acquisition.

Transmission electron microscopy (TEM) examination

TEM is efficient technique to examine the interior lipid matrix arrangement of SLNs which is an important feature for better homing and localization of drug molecules [6]. We employed this technique to understand the uniformity in the internal rearrangement of lipid molecule. For TEM analysis, 0.2% (w/v) SLNs dispersion after appropriate dilution was gently scattered on 3 mmol forman-coated copper grid 300 mesh size, stained with 4% uranyl acetate for 30s, dried and analyzed at an accelerating voltage of 100 kV. Images were captured and examined for the geometrical arrangement of particles by using transmission electron microscope (TEM, JEM-1400, Jeol-Japan).

Measurement of Particle size distribution, zeta potential, and polydispersity index

Particle size, polydispersity index (PDI) and zeta potential were estimated in solution by using light scattering instrument Beckman coulter Zeta-sizer, as it quantifies the hydrodynamic radius, surface potential and dispersity of a nanoparticle population. All samples were appropriately diluted with deionized water, and pH was adjusted within the range of 6.8-7 followed by sonication for 5 min before analysis.

Cell line and culture

MDCK normal epithelial cell lines were obtained from the NCCS, Pune. Cells were grown in T-25 culture flasks containing DMEM supplemented with 10% FBS and 1% antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Upon reaching confluency, the cells were trypsinized and passaged.

Cytotoxicity studies

Both the SLNs and D-SLNs were studied for the possible cytotoxic effects. Briefly, MDCK cells were seeded at a density of 1×10^4cells/well in 96 well plates in 0.2 ml of growth medium consisting of DMEM with 10% FBS and 1% of antibiotics. Afterward, cells were incubated at 37 °C till confluence reached 70%. Cells were then treated with SLNs (before and after incubation) and further incubated for 12 h, 24 h and 48 h. After desired hours of exposure, MTT (20 µl) was added to each well and cells were further incubated for 3 h at 37 °C. Metabolically active cells were able to reduce MTT with the help of enzyme mitochondrial succinate dehydrogenase and from purple colored insoluble formazan. To dissolve these formazan crystals, DMSO (200 µl) was added to each well. To test the cell viability, absorbance was taken at 570 nm using a microplate reader (Bio-Rad, Model 680). The absorbance of untreated cells was taken as 100% to determine cytotoxicity. All the experiments were performed in triplicates.

Visual determination of SLNs effect on cell morphology by acridine orange (AO)/ethidium bromide (EtBr) dual staining

Further, SLNs and D-SLNs were analyzed for their cell morphological changes in MDCK cell line by dual AO and EtBr staining. Briefly, the MDCK cells were seeded at a density of 2×10^5cells/well in 6 well plates in 1 ml of growth medium. Till the confluence reached 70% cells were incubated at 37 °C in CO_2 incubator. Cells were then treated with SLNs and D-SLNs and further incubated for 12 h, 24 h and 48 h. After desired time period of exposure, cells were harvested, washed with ice-cold PBS and fixed with 4% of paraformaldehyde for 30 min. Afterward, the supernatant was discarded, and cells were treated with 1% triton X-100 (non-ionic surfactant) for 15 min followed by washing with PBS. Cells were stained with dye mixture; comprising 5 µl of acridine orange (1 mg/ml) and 5 µl of EtBr (1 mg/ml) for 5 min and then washed thrice with PBS. The morphological changes in the stained cell were observed by using a fluorescence microscope at 200 X (Nikon Eclipse-80i, Japan).

Statistical analysis

Data in the present study was expressed as mean±standard deviation of triplicate recordings using GraphPad Prism-5 one way ANOVA.

RESULTS

Morphological investigation

SEM and TEM are complimentary to each other and enable us to recognize the morphological changes in formulations by quantitatively observing the particle size and shape. NPs size is a key property for a delivery system that influence biodistribution of the compound, likewise spherically small magnitude of particle possess less protein surface absorption properties, leading to a reduction in hepatic uptake and longer blood circulation half-life [18]. From the SEM micrograph investigation, we observed that the formulation had deformed surface after incubation of SLNs (fig. 1c) and particles also get aggregated to each even after sonication (fig. 1b) as in comparison to SLNs before incubation, representing smooth spherical morphology (fig. 1a).

TEM micrograph analysis revealed that spherical morphology of the prepared nanoparticles (fig. 2a) was deformed after incubation (fig. 2b). Hence both, SEM and TEM indicate that after incubation of SLNs at 4 °C, they lose their surface integrity.

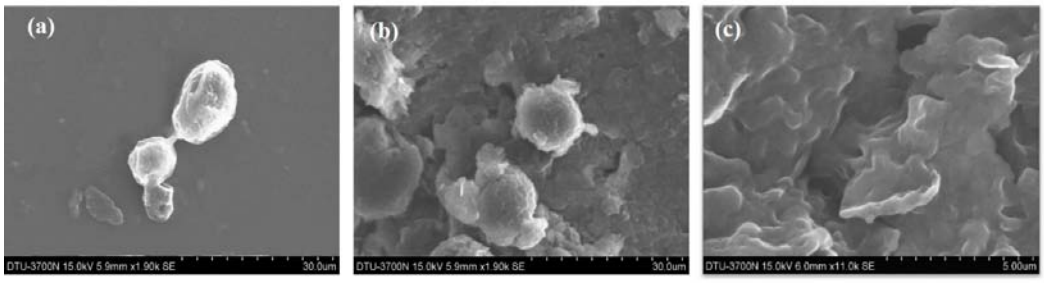

Fig. 1: Scanning electron micrograph (SEM) of formulated SLNs (a), D-SLNs after incubation period (b) and morphological examination of D-SLNs at higher magnification

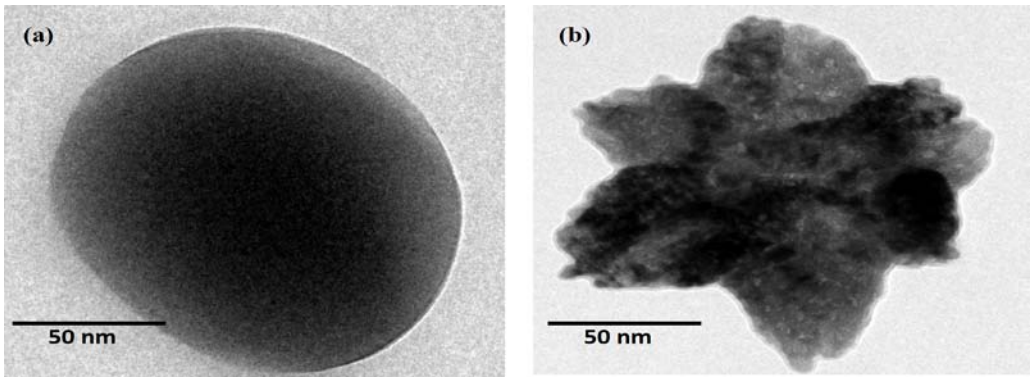

Fig. 2: Transmission electron micrograph (TEM) of formulated SLNs showing spherical morphology (a) and D-SLNs representing deformed morphology (b)

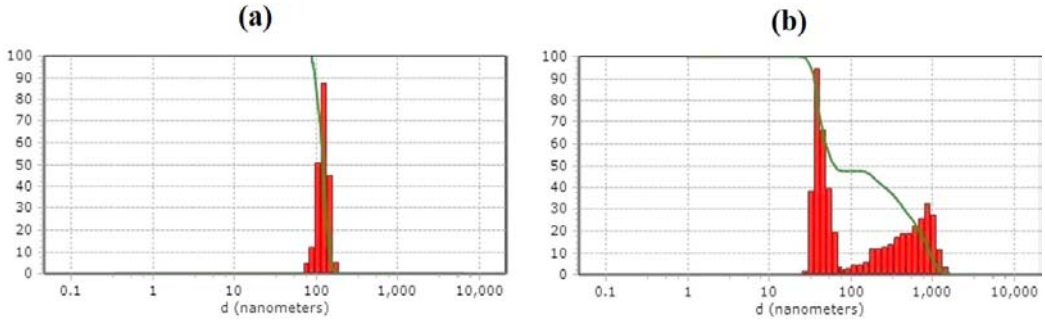

Fig. 3: Particle size distribution of formulated SLNs (a) and D-SLNs (b)

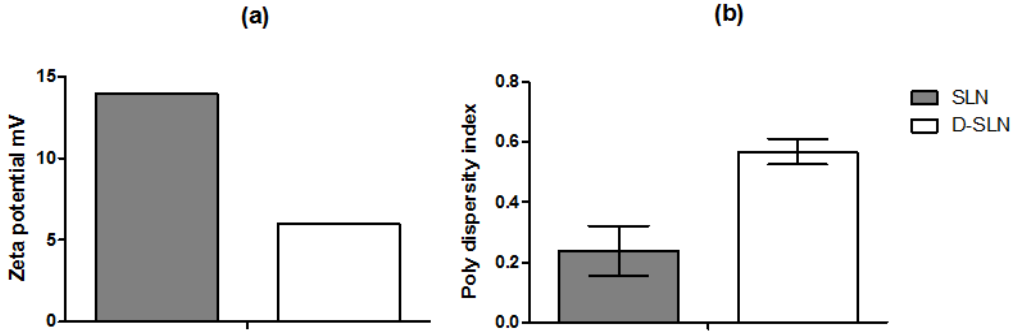

Fig. 4: Physicochemical investigation of SLNs and D-SLNs; (a) zeta potential and (b) polydispersity index

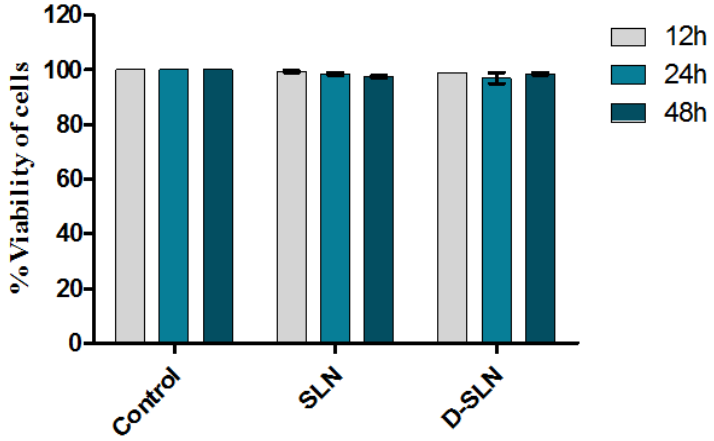

Fig. 5: MTT cell-cytotoxicity study on MDCK cell line incubated with SLNs for different time intervals. (n=3)

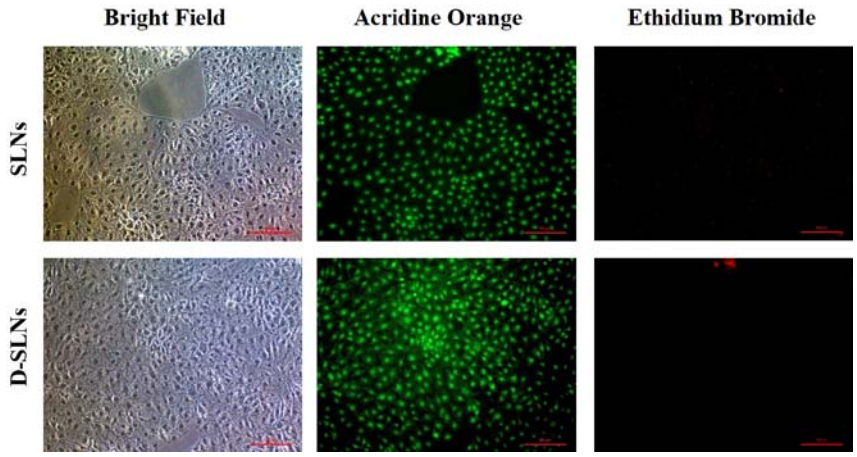

Fig. 6: Effect of SLNs and D-SLNs on cell and nuclear morphology in MDCK cell line after 48 h of incubation time

Particle size distribution, zeta potential, and polydispersity index

Particle size distribution for SLNs prior to incubation was found with the mean value of 188 nm and after incubation of SLNs at 4 °C the particle size increased to 327 nm. Mean particle size distribution graph for SLNs before incubation and after incubation has been represented in fig. 3a and 3b.

Zeta potential is very sensitive and sophisticated technique to measure surface ionic environment, or charge of the NPs and the surface potential of particles varies depending on functional end group on the surface [19]. Zeta potential of SLNs before and after incubation was found to be±14mV and±6mV (fig. 4a) which is the most accountable physicochemical parameter for the stability of nanoformulation. The increased mean particle size of SLNs may be the cause of decreased zeta potential, which further signifies that with a decrease in surface charge, particles get closure to each other, and there may be the aggregation of particles as we have seen in SEM and TEM micrographs.

The polydispersity index (PDI) is a measure of the distribution of the molecular mass in given polymeric sample. PDI was found to be 0.297±0.18 for SLNs without incubation and 0.538±0.07for SLNs after incubation (fig. 4b) as it's well-established fact that, greater the homogeneous population of nanoparticle in the system lesser will be the PDI [20]. Hence the particles after incubation are more unstable and deformed.

Cell cytotoxicity

SLNs were investigated for their cytotoxicity though MTT exclusion method by using normal epithelial MDCK cells in a time-dependent manner (12 h, 24 h and 48 h). The percentage cell viabilities for SLNs after and before incubation was measured with definitely non-significant change (fig. 5) and it was noted that the lowest % cell viability of the formulated SLNs was found approximately up to 98 % for the 48 h of incubation in comparison to control. This might be due to the prolonged cell incubation i.e. for 48 h and depicts the natural cell death. Results revealed that the fabricated SLNs have no effect on normal epithelial cell line and hence considered as safe for further biological evaluation.

Visual determination of SLNs effect on cell morphology by acridine orange (AO)/ethidium bromide (EtBr) dual staining

For the appropriate understanding of the outcomes from cell cytotoxicity, SLNs were further visualized by AO/EtBr staining on MDCK cells, viewed under a fluorescence microscope (Nikon Eclipse-80i, Japan) at 200X as previously mentioned in materials and methods. The viable cell will possess a uniform bright green nucleus; early apoptotic cells will have bright orange areas of condensed or fragmented chromatin in the nucleus, and late apoptotic cells will have a uniform bright red nucleus. No cellular morphological damage was observed at 48 h of incubation with MDCK cells as represented in fig. 6. This experimental result shows that SLNs have no effect on the normal cell proliferation rate and found to be safe their further biological applications.

CONCLUSION

In the presented study, SLNs were fabricated by nanoprecipitation and auxiliary investigation were carried out to evaluate various physicochemical parameters during prolonged storage of lipid nanoformulation. SEM and TEM investigation revealed morphological variation, as deformity was observed after and before the incubation of SLNs at 4 °C. Dissimilarity in the mean particle size, zeta potential and polydispersity index indicates the increase in size and aggregation of nanoparticles as the same was observed from the surface morphological analysis. On no account, the cytotoxic signs were observed in normal cells, suggesting storage of nanoformulation in the aqueous state has no effect in context to cytotoxicity, therefore safe for the further usage. Hence we demonstrated that prolonged storage of formulation at cold temperature also causes the deterioration of polymeric formulation. Further for better thoughtful in this direction drug release pattern analysis is suggested along with the atomic force micro-graphical investigation for surface analysis.

ACKNOWLEDGEMENT

Authors would like to acknowledge the Jaypee University of Information Technology for providing facilities to do this work.

CONFLICT OF INTERESTS

Authors declare no conflict of interest amongst themselves

REFERENCES

1. Jain PK, Huang X, Sayed EL. Review of some interesting surface plasmon resonance-enhanced properties of noble metal nanoparticles and their applications to biosystems. Plasmonics 2007;2:107-18.
2. Chen Y, Chen H, Shi J. *In vivo* biosafety evaluations and Diagnostic/Therapeutic applications of chemically designed mesoporous silica nanoparticles. Adv Mater 2013;25:3144-76.
3. Benita S. Microencapsulation: methods and industrial applications: CRC Press; 2005. p. 784.
4. Sharma A, Sood A, Metha V, Malairaman U. Formulation and physicochemical evaluation of nanostructured lipid carrier for codelivery of clotrimazole and ciprofloxacin. Asian J Pharm Clin Res 2016;9:1-5.
5. Sareen R, Bhardwaj V, Mehta V, Sharma A. Topical gel incorporated with a non-ionic surfactant based solid lipid microspheres of ketoprofen: physicochemical analysis and anti-inflammatory evaluation. Int J Pharm Pharm Sci 2015;7:199-206.

6. Muller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Delivery Rev 2002;54:131-55.
7. Pardeike J, Hommoss A, Muller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int J Pharm 2009;366:170-84.
8. Buse J, El-Aneed A. Properties, engineering, and applications of lipid-based nanoparticle drug-delivery systems: current research and advances. Nanomedicine 2010;5:1237-60.
9. Attama AA, Momoh MA, Builders PF. Lipid nanoparticulate drug delivery systems: a revolution in dosage form design and development: INTECH Open Access Publisher; 2012.
10. Dong Y, Ng WK, Shen S, Kim S, Tan RB. Solid lipid nanoparticles: continuous and potential large-scale nanoprecipitation production in static mixers. Colloids Surf B 2012;94:68-72.
11. Olbrich C, Kayser O, Muller RH. Lipase degradation of dynasan 114 and 116 solid lipid nanoparticles (SLN) effect of surfactants, storage time and crystallinity. Int J Pharm 2002;237:119-28.
12. Westesen K, Bunjes H, Koch M. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J Controlled Release 1997;48:223-36.
13. Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: formulation, process, and storage considerations. Adva Drug Delivery Rev 2006;58:1688-713.
14. Baek JS, Cho CW. 2-Hydroxypropyl-β-cyclodextrin-modified SLN of paclitaxel for overcoming p-glycoprotein function in multidrug-resistant breast cancer cells. J Pharm Pharmacol 2013;65:72-8.
15. Win KY, Feng SS. Effects of particle size and surface coating on cellular uptake of polymeric nanoparticles for oral delivery of anticancer drugs. Biomaterials 2005;26:2713-22.
16. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Controlled Release 2001;70:1-20.
17. Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends Pharmacol Sci 2009;30:592-9.
18. Panyam J, Williams D, Dash A, Leslie-Pelecky D, Labhasetwar V. Solid-state solubility influences encapsulation and release of hydrophobic drugs from PLGA/PLA nanoparticles. J Pharm Sci 2004;93:1804-14.
19. Fan X, Xia C, Advincula RC. On the formation of narrowly polydispersed PMMA by surface initiated polymerization (SIP) from AIBN-coated/intercalated clay nanoparticle platelets. Langmuir 2005;21:2537-44.

How to cite this article

- Arun Sharma, Vineet Mehta, Arun Parashar, Riddhi Patrwal, Udayabanu Malairaman. Solid lipid nanoparticle: fabricated through nanoprecipitation and their physicochemical characterization. Int J Pharm Pharm Sci 2016;8(10):144-148.