International Journal of Recent Advances in Medical & Pharma Research
Vol. 3, Issue 1 – 2020

Design, Development and Characterization of Nano-structured lipid carrier formulation Containing Olmesartan Medoxomil

Ashish Dixit[1], Mansi Singh[2], Sanjesh Kumar[2], Mukesh Kumar Bhardwaj[2]

[1]Professor, Lotus Institute of Pharmacy, Bareilly, Uttar Pradesh-243123.

[2]Assistant Professor, Lotus Institute of Pharmacy, Bareilly, Uttar Pradesh-243123.

Correspondence E-mail Id: *editor@eurekajournals.com*

Abstract

The current study was designed to explore the potential of nanostructured lipid carriers (NLCs) for oral bioavailability enhancement of Olmesartan medoxomil (OLM) by systemic design approach. OLM loaded NLCs were prepared using ultra sonic melt emulsification method. OLM-NLC was characterized by different techniques viz. differential scanning calorimetry (DSC), powder X ray diffraction (PXRD).The prepared NLCs were evaluated for their particle size and shape, polydispersity index, zeta potential and drug entrapment efficiency. The in vitro drug release profiles were evaluated using a dialysis bag with cut off 12KD. The prepared NLCs showed average sizes between 99.4 and 174 nm and polydispersity index in the range of 0.233 to 0.583. The entrapment efficiency was not very high between 51.4% to 83.4%. The scanning electron images showed almost spherical shapes with sizes lower than those obtained by light scattering. The in vitro release followed a bi-phasic pattern with an initial rapid OLM release followed by a slow release varying according to the composition. Formulation F1 showed complete drug release within the first two hours.

Keywords: Olmesartan medoxomil, Ultra sonication, Box-Behnken design, Entrapment efficiency, in-vitro release studies

Introduction

The oral route is the route of preference for the administration of drugs, but the efficient oral delivery of lipophilic drugs with unsteady metabolism is a challenging chore[1,2].

Nanostructure Lipid Carrier (NLC) is the second generation of lipid nanoparticle having a structure like nanoemulsion. The first generation was solid lipid nanoparticle (SLN). The difference between both of them is in its core. Both of them are a colloidal carrier in submicron size in the range of 40-1000 nm. SLN has a core which consists of one of solid lipid or a mixture of solid lipids while the core of NLC consists of a mixture of solid lipid and liquid lipid. Usually, SLN formulation can stable up to 3 year, has a good reproducibility, and can be delivered for various routes of administration such as intravenous, dermal, peroral or topical[1]. SLN also has

the capability to protect degradation of active ingredient, to modulate of drug release, enhance the stability of sensitive active ingredients such as Co-enzyme Q10, Vitamin E, and Vitamin A, and has an ability to control the release of active ingredients[3]. Besides all of the advantages above, SLN still has any disadvantages such as a limitation of drug loading that depend on the solubility of active ingredient in the solid lipid and expulsion of drugs during storage caused by lipid crystallization[4].

NLC is developed to improve the disadvantages of SLN. The unique advantages of NLC such as enhanced of drug loading capacity and prevention of drug expulsion during storages make NLC is more favorable than SLN[3]. Recrystallization in NLC was less then SLN due to crystalline order in NLC was disturbed by oil particle to maintain the system in the form of liquid phase[1]. There were several methods to construct NLC such as: High-Pressure Homogenization[4,5], O/W Microemulsion[6], Emulsification-solvent evaporation[7], multiple emulsion water/oil/water[8]. The high shear homogenization and/or ultrasonication were a dispersion technique which was not use an organic solvent, a large amount of surfactant or additive compound. The melting lipid was added and dispersed in a solution of surfactant using ultrasonication method[8]. The kind of ultrasonic equipment which usually use was probe sonicator[9]. The drug Olmesartan is widely used as main Ingredient in the treat of high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. Olmesartan drug belongs to a class of drugs called angiotensin receptor blockers (ARBs). It works by relaxing blood vessels so that blood can flow more easily[10,11]. The objective of the present study is to design and assess the nanostructured lipid carriers (NLCs) as a potential oral formulation of OLM for enhancement of its oral bioavailability. In this study, OLM loaded NLCs (OLM-NLC) were prepared by hot high shear homogenization, optimized with Box-Behnken design and characterized by different techniques. Further, the formulation was evaluated for in vitro drug release and in vivo pharmacokinetic study. In addition, in vitro cell uptake of nano formulation was discussed through dialysis bag diffusion technique.

Material & Methods

Material

Olmesartan Medoxomil used to be a gift sample from Umedica Pharmaceuticals (P) Ltd. Precirol ato5 was procured from Gattefosse, Mumbai, India. Transcutol HP was also procured from Gattefosse, Mumbai, India. Tween 80 and Span 80 were obtained from S D Fine Chemicals. All other chemicals used were of lab research grade. Clove Oil, Olive oil was purchased from LIPI.

Methods

➢ Preparation of OLM loaded NLCs

NLC formula optimization was done using various combinations of lipid, oil and surfactant concentration. The formulations of Olmesartan medoxomil-NLCs, listed in Table 1, were prepared by melt-emulsification method combined with ultra-sonication technique. Briefly,

appropriate amounts of Olmesartan medoxomil, Precirol ato5, and Transcutol HP were blended and melted at 70˚C to form a uniform and clear oil phase. Meanwhile, the aqueous phase consisting of surfactant Tween 80 and span 80 (1:1ratio) was dissolved in MQ-water and was added drop wise to the oil phase at the same temperature by the aid of agitation at around 500 rpm for 5 min. The coarse emulsion was then treated by probe-sonicator (Qsonica, sonicator) for 7-9 min. Subsequently the dispersion was cooled to room temperature to form nanoparticles and then was stored finally[12,13,14,15,16,17,18,19].

Characterization of OLM-NLCs

Characterization of olmesartan non lipid particles was done by particle size analysis, DSC[20,21,22]

In Vitro Drug Release Studies

In vitro release of drug from NLC_s was evaluated by the dialysis bag diffusion technique reported with slight modification[23,24]. The release studies of Olmesartan from NLC_s were performed in MQ-water and methanol in ratio of 85:15 respectively. The aqueous nanoparticle dispersion of 1ml was placed in a dialysis bag (pre-activated) and sealed at both ends. The dialysis bag (MW 12000-14000 Da,) was immersed in the dissolution vessel containing 100ml of dissolution medium, which was stirred at 100 rpm and maintained at 37±2˚C. The receptor compartment was covered to prevent the evaporation of dissolution medium. An aliquot of 1ml samples were withdrawn at predetermined time interval of 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 24.0 and 48.0 hours the same volume was replaced by fresh dissolution medium. The samples were analysed by UPLC, at 254nm[25,26,27].

$$\% \text{ Drug release} = \frac{\text{Conc.}(\mu g/ml) \times \text{Dilution factor} \times \text{Volume of release media (ml)}}{\text{Initial dose } (\mu g)}$$

Result & Discussion

Physiochemical characterisation

Olmesartan medoxomil was observed to possess the organoleptic properties. The nature of drug was found to be in form of white colour highly crystalline powder.

Preparation of olmesartan medoxomil loaded nanostructured lipid carrier

The formulations of olmesartan medoxomil-NLCs, listed in Table 1, were prepared by melt-emulsification method combined with ultra-sonication technique. Briefly, appropriate amounts of Olmesartan medoxomil, Precirol ato5, and Transcutol HP were blended and melted at 70˚C to form a uniform and clear oil phase. Meanwhile, the aqueous phase consisting of surfactant Tween 80 and span 80 (1:1ratio) was dissolved in MQ-water and was added drop wise to the oil phase at the same temperature by the aid of agitation at around 500 rpm for 5 min. The coarse emulsion was then treated by probe-sonicator (Qsonica, sonicator) for 7-9 min. Subsequently the dispersion was cooled to room temperature to form nanoparticles and then was stored finally.

Selection of optimized formulations

As per the results of Design Expert software seven (7) formulations (F_1, F_2, F_4, F_9, F14, F15 and F16) were selected out of seventeen formulations on the basis of comparative small particle size, small PDI, the primary emphasis was given to the particle size and PDI range between 99-145 nm and 0.2-0.35 respectively, and physical stability (solid lipid settlement) during 1 week. After that entrapment efficiency of those selected seven formulation is calculated. Secondarily those having highest entrapment efficiency was given the preference for final selection of formulation. F1 composition is 3.5% (w/v) lipid mixture, 4% (w/v) surfactant and 8 minutes sonication time. Among all the seven selected formulations, formulation F_1 had highest EE% (83.4) thus, F_1 is selected for the further characterization and evaluation in the present study.

Table 1.Observed responses (EE %) of selected seven formulations

Run	Factor 1 (X_1)	Factor 2 (X_2)	Factor 3 (X_3)	Response (Y_1)	Response (Y_2)	Response (Y_3)
	A: Lipid mixture (7:3%w/v)	B: Surfac-tant (1:1%v/v)	C: Sonica-tion time (min)	P. size (nm)	PDI	EE (%)
F_1	3.5	4	8	140.0	0.253	83.4
F_2	3.5	5	7	116.0	0.513	51.4
F_4	2	3	8	112.0	0.345	78.0
F_9	5	4	9	142.0	0.288	59.7
F_{14}	2	4	9	99.4	0.339	82.2
F_{15}	5	5	8	143.0	0.233	74.4
F_{16}	2	4	7	117.0	0.583	51.8

Characterization of optimized formulation

➢ Particle size and Polydispersity index (PDI)

The particle size and PDI of the optimized formulation F_1 were 140.0 and 0.253 respectively as shown in Figure 1.

			Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm):	138.7	Peak 1:	151.0	97.2	56.03
PdI:	0.283	Peak 2:	5179	2.8	485.6
Intercept:	0.947	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

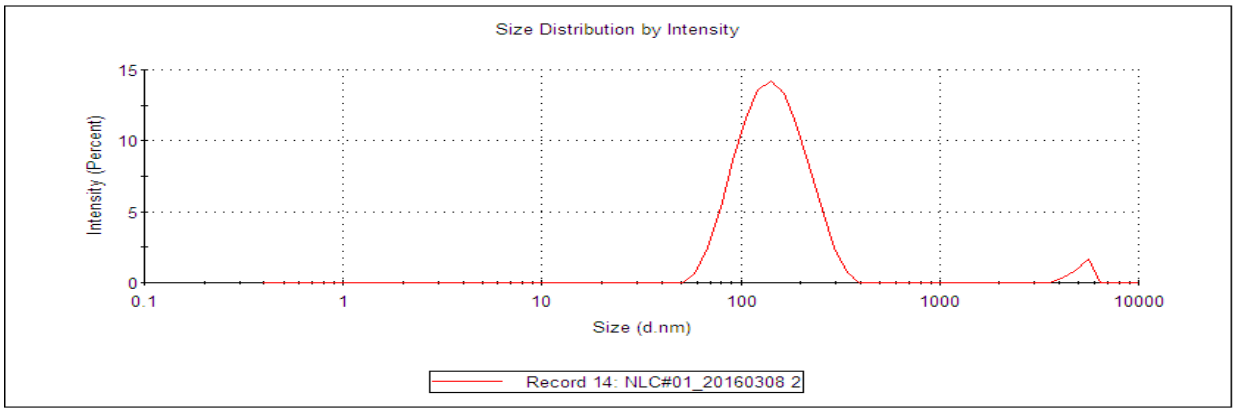

Figure 1.Particle size distribution and PDI of optimized formulation (n=1)

Entrapment efficiency and loading capacity

Among the various formulation prepared, %EE was found to be in the range from 51.4 to 83.4. The % EE of optimized formulation F_1 was found to be 83.4. The drug loading for the optimized formulation F_1 was found to be 1.428%.

Differential scanning calorimetry (DSC)

DSC thermogram of optimized NLC formulation was shown in Figure 2 and 3. Pure Precirol ATO-5 showed the endothermic peak at 57.94^{0}C .

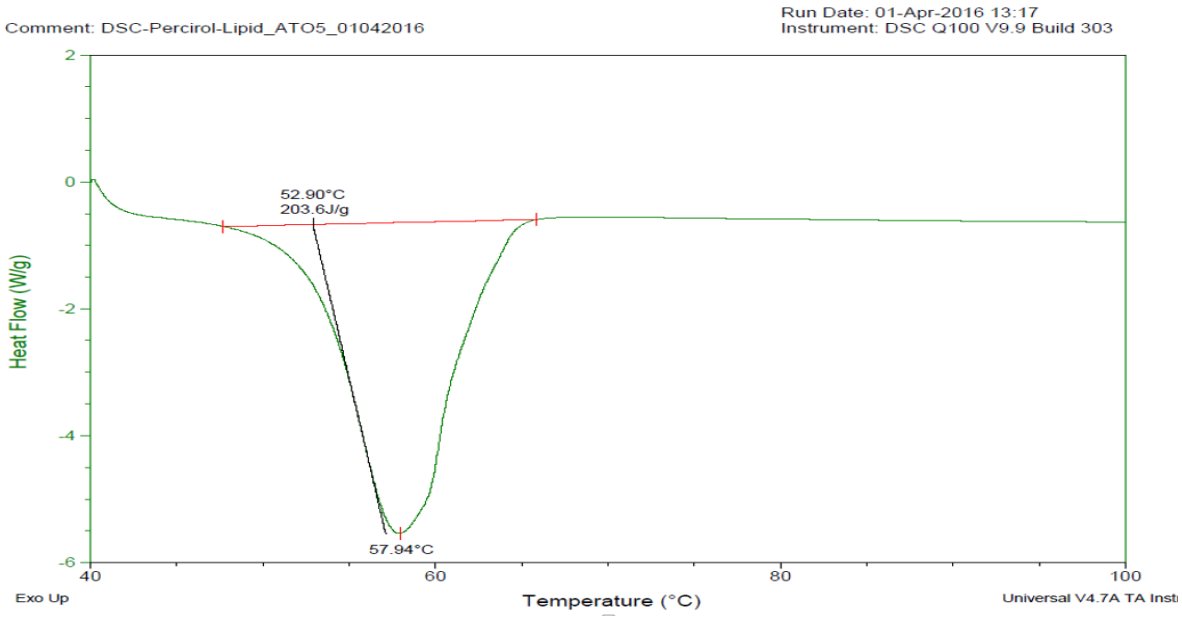

Figure 2.DSC thermogram of solid lipid (Precirol ato5)

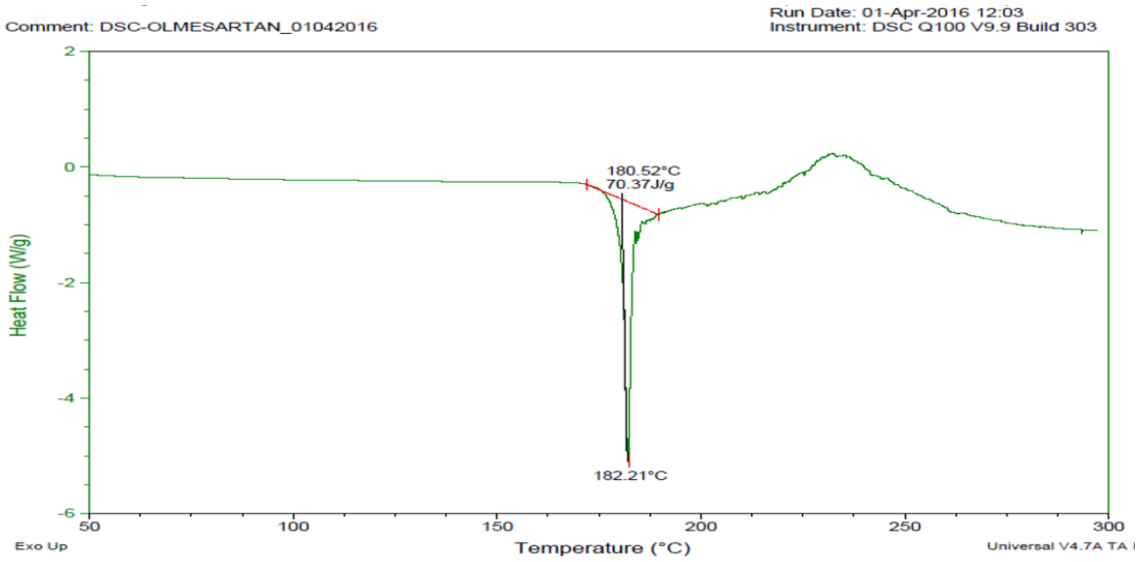

Figure 3.DSC thermogram of pure olmesartan medoxomil (OLM)

In vitro drug release studies

In vitro release data from the NLC_s were carried out for 48 hours and graphically represented as Cumulative Percent drug released v/s time profile. The % CDR after 48 hours for optimized NLC_s formulation was found to be 97.53% and from API suspension it was only 81.90% in MQ-water and methanol in a ratio of 85:15 respectively, as shown in Table 10 and figure 19. The release of drug from the NLC_s can be influenced by the nature of the lipid matrix and its concentration. Since the surfactant concentration was optimized as 3.5% , the drug release profile was affected by other parameters such as lipid nature, solubility of the drug in the lipid, partition coefficient and particle size.

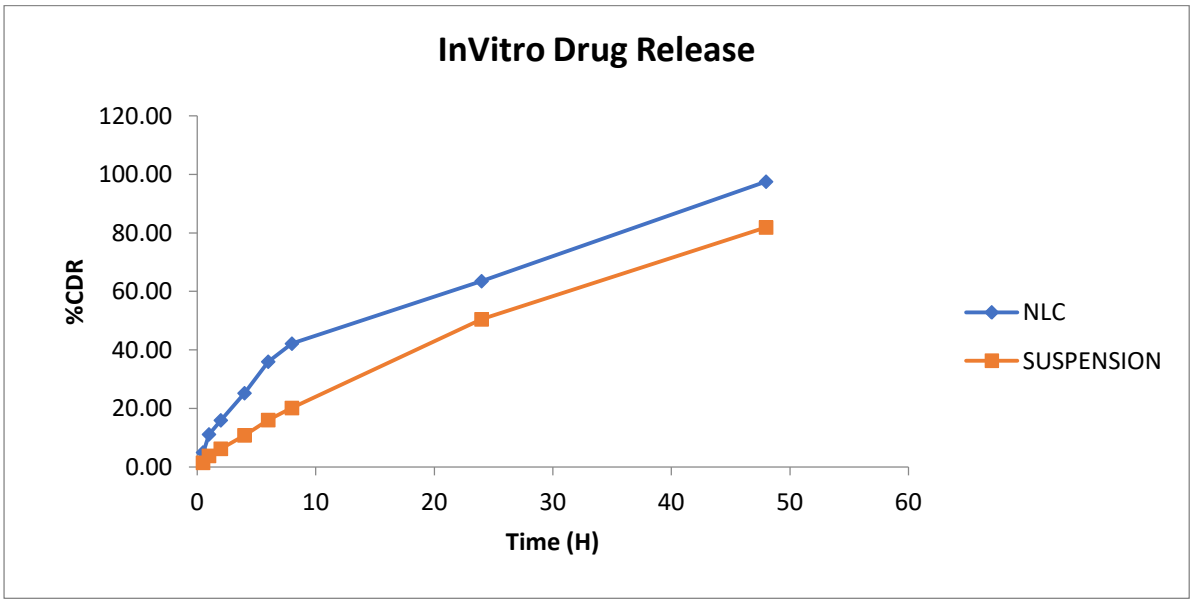

Figure 4.Comparative in vitro release profile of optimized formulation (NLC) and Olmesartan medoxomil suspension in MQ-water and methanol (85:15)

Table 2.In vitro release profile of optimized formulation (NLC) and API suspension

Time (h)	NLC % CDR	Suspension % CDR
0.5	4.98	1.46
1	11.15	3.81
2	15.97	6.24
4	25.24	10.84
6	36.00	16.06
8	42.17	20.18
24	63.52	50.48
48	97.53	81.90

The graphical representation (Figure 4) shows that drug release from NLC was biphasic showing initial burst release followed by sustained release. Initial burst release was due to presence of drug enriched liquid lipid content in the outer shell of NLC. Sustained release pattern was due to drug solubilized lipid core of NLC_s. and optimised NLC formulation was showing more %CDR than corresponding suspension formulation.

Conclusion

Olmesartan is a BCS-class 2 drug. It is highly lipophilic drug (logP=5.55) and is a poorly water soluble drug (aqueous solubility≈0.0105 mg/mL) with a half-life time of about 13 hr, and is cleared by extensive metabolism in the intestinal gut. Due to the slow dissolution rate in the intestinal tract, bioavailability of olmesartan is only approx.26 % (oral tablet) and 20% by oral suspension.

Thus in order to enhance oral bioavailability lipid based Nano carriers like SLN, NLC, and LDC proves to be one of the highly accepted drug delivery system. Among these NLC is the most promising drug delivery system for improving the dissolution rate and thus the bioavailability of poorly water soluble drug. Besides enhancing absorption, NLC also provide additional advantage like greater loading, improved release properties and higher stability of incorporated drug during storage as compared to other lipid based formulation.

Preformulation studies on the drug (Olmesartan) revealed that it had highly crystalline nature, highly soluble in organic solvent like acetonitrile and methanol, partition coefficient of 5.55, melting point of about 182.21^{o}C. The DSC and XRD confirmed the identity as well as nature of olmesartan. Selection of the lipid excipients were performed based on the solubility analysis among various lipid and compatibility studies were performed for any physical changes. Among various lipids Transcutol HP (oil) and Precirol ato5 (solid lipid) had been selected for the production of NLC. Tween 80 and span 80 (1:1) was taken for the production of thermodynamically stable formulations.

For development of formulation (NLC), melt emulsification combined with Ultrasonication was employed and Box Behnken factorial design (n=3) approach was adopted. Lipid concentration,

surfactant concentration and sonication time were successfully optimized. After getting the mathematical relationship between independent and dependent variables same software was used to generate the formula of desired attributes i.e. particle size, PDI, entrapment efficiency. The optimized independent variables were lipid concentration (3.5%), surfactant concentration (4%) and sonication time (8 min). The characteristics of the prepared formulation were found particle size (140 nm), PDI (0.253), % EE (83.40).The results of DSC, XRD, revealed that the drug is almost fully entrapped inside the lipid as no/partial peak of drug was obtained in lyophilized formulation of OLM-NLC.

In vitro drug release study showed the biphasic behaviour with initial burst release and steady release profile. The % CDR after 48 hours for optimized NLC_s formulation was found to be (97.53%) in MQ-water and methanol in ratio of 85:15. and from API suspension it was only 81.90%.

Acknowledgement

Authors are thankful to management Lotus Institute of Pharmacy, Bareilly Uttar Pradesh.

Conflict of Interest: None

References

1. Desai P, Date A, Patravale B. Overcoming poor oral bioavailability using nanoparticle formulations-opportunities and limitations. Drug Discovery Today Technology. 2012; 9: 87-95.
2. Raval Chirag, Joshi Neha, Patel Jitendra, Upadhyay U.M. Enhanced oral bioavailability of olmesartan by using novel solid self-emulsifying drug delivery system (SMEDDS). International Journal of Advanced Pharmaceutics. 2012; 2(2):82-92.
3. Uner M. Preparation, characterization and physicochemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): Their benefits as colloidal drug carrier systems. 2011; 86(1):158-165.
4. Muller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharmcy. 2002; 242: 121-8.
5. Muller RH, Runge SH. Solid lipid nanoparticles (SLN) for controlled drug delivery. In submicron emulsions in drug targeting and delivery edited by S. Benita. Netherlands: Harwood Academic Publishers; 1998, p. 219-34.
6. Gasco MR. Solid lipid nanospheres from warm micro-emulsions. Pharm Technol Eur 1997; 9: 52-8.
7. Cortesi R, Esposito E, Luca G, Nastruzzi C. Production of lipospheres as carriers for bioactive compounds. Biomaterials 2002; 23: 2283-94.
8. Krzic M, Sentjurc M, Kristl J. Improved skin oxygenation after benzyl nicotinate application in different carriers as measured by EPR oximetry Pharmazie 2006; 61: 375-86.

9. Beck R, Guterres S, Pohlmann A. Nanocosmetics and Nanomedicines: New Approaches for Skin Care, Springer-Verlag, Berlin Heidelberg in vivo. J Controlled Release 2001; 70: 203-11.
10. Mei Z, Chen H, Weng T, Yang Y, Yang X. Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. Eur J Pharm Biopharm. 2003; 56: 189-96.
11. Lai J, Che J, Lu Y, Sun J, Hu F, Yin Z, Wu W. Glyceryl monooleate/ poloxamer 407 cubic nanoparticles as oral drug delivery systems. In vitro evaluation and enhanced oral bioavailability of the poorly water-soluble drug olmesartan. AAPS Pharm SciTech. 2009; 10: 960-966.
12. Gurav AS, Sayyad FJ, Gavhane YN, Khakal NN. Development of olmesartan medoxomil-loaded chitosan microparticles: A potential strategy to improve physicochemical and micromeritic properties; International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 7(8): 324-330.
13. Beg S, Sharma G, Thanki K, Jain S, katare O, Singh B. Positively charged self nano emulsifying oily formulations of olmesartan medoxomil: systematic development, in vitro, ex vivo and in vivo evaluation. Int. J. Phar. 2015; 493: 466-482.
14. Liu K, Sun J, Wang Y, He Y, Gao K Z. Preparation and characterization of 10-hydroxycamptothecin loaded nanostructured lipid carriers, Drug Dev. Ind. Pharm. 2008; 34: 465-471.
15. Kasongo KW, Pardeike J, Mullar RH, Walker RB. Selection and characterization of suitable liquid excipients for use in the manufacturing of didanosine-loaded solid lipid nanoparticles and non-structured lipid carriers, J. Pharm. Sci. 2011; 100: 5185-5196.
16. Patel Maulik, Patel Madhabhai, Patel Natvarbhai, Bhandari Anil. Formulation and evaluation of lipid based formulation of valsartan; International Journal of Current Pharmaceutical Research. 2011; 3(4): 76-80.
17. Singh B, Kumar R, Ahuja N. Optimising drug delivery systems using systematic "design of experiments." Part I: fundamental aspects, Crit Rev. Ther. Dug carrier system. 2005; 22: 27-105.
18. Maurya D, Sultana Y, Aquil M. Kumar D, Chuttani K, Ali A, Mishra A. Formulation and optimization of alkaline extracted ispaghula husk microparticles of isoniazid - in vitro and in-vivo assessment, J. Microencapsulation.2011; 28: 472-482.
19. Jain AK, Swarnkar NK, Godugu C, Singh RP, Jain S. the effect of the oral administration of polymeric nanoparticles in the efficacy and toxicity of tamoxifen, Biomaterials. 2011; 32: 503-515.
20. Jain S, Rathi VV, Jain AK, M. Das, Godugu C, Folate-decorated PLGA nanoparticles as a rationally designed vehicle for oral delivery of insulin. Nanomedicine.2012; 7: 1311-1337.
21. Zhuang CY, Li N, Wang M, Zhang XN, Pan WS, Peng JJ, Pan YS, Tang X. Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. Int. J. Pharm. 2010; 394, 179-185.
22. Tiecheng Z, Jianian Chen, Zhang Yi, Shen Qi, Pan Weisan. Characterization and evaluation of nanostructured lipid carrier as a vehicle for oral delivery of etoposide; European Journal of Pharmaceutical Sciences.2011; 43:174-179.

23. Jores K .Mehnert W, Drechsler M, Bunjes H, Johann C, Mader M Investigation on the structure of SLN and oil loaded SLN by photon correlation spectroscopy ,field flow fractionation and transmission electron microscopy. Journal of control release. 2004; 95(2): 217-227.
24. Yuan H, Muller RH, junyaprasert VB .Encapsulation of ascorbyl palmitate in NLC: effect of formulation parameter on physicochemical stability. Int J Pharm 2007; 20:198-206.
25. Ali Hazem, Shirode A B., Sylvester Paul W, Nazza Sami. Preparation and invitro antiproliferative effect of tocotrienol loaded lipid Nanoparticles; Colloids and Surfaces A: Physicochem. Eng. Aspects.2010; 353: 43-51.
26. Vishwanathan A. A thesis on in vitro characterization of olmesartan loaded microspheres in the PolyRing device.2008; 1-79.
27. Khalid M. El-Say, Tarek A. Ahmed, Shaimaa M. Badr-Eldin, Usama Fahmy, Hibah Aldawsari, and Osama A.A. Ahmed. Enhanced permeation parameters of optimized nanostructured OLMESARTAN transdermal films: ex vivo and in vivo evaluation; pharmaceutical development and technology; 2014: 1-8.

