RESEARCH ARTICLE

Improvement of Entrapment and Ocular Permeability of Ganciclovir Nanostructured Lipid Carriers Using Various Conditions of Preparations

Zainab T. Salih*, Fatima Al-Gawhari

Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Received: 05th January, 2023; Revised: 10th February, 2023; Accepted: 12th March, 2023; Available Online: 25th March, 2023

ABSTRACT

Ganciclovir (GCV) is a drug included in BCS-Class III, having high solubility and low permeability. It is a synthetic acyclic nucleoside analog of 2′-deoxyguanosine, considered a potent inhibitor of herpes viruses and cytomegalovirus (CMV) infection. Herpes simplex virus (HSV) infections are very common and are also considered a major cause of corneal blindness.

This study intended to advance a pioneering nanostructured lipid carriers (NLCs) system for improving the ocular permeability of GCV. Several procedures were used for the preparation. Cold homogenization, solvent injection, and emulsification-ultrasonication methods. A mixture of palmitic acid (PA) and oleic acid (OA) as a lipid matrix, cremophore EL, and transcutol HP were used as emulsifiers. To evaluate the optimum method, the particle size (PS), polydispersity index (PDI), zeta potential (ZP), entrapment efficiency (EE), and drug loading (DL%) were determined for the prepared NLCs. Due to the decreased particle size value, the polydispersity index, and the high value of EE%, emulsification/ultrasonication outcomes were more practical than cold homogenization and solvent injection procedures.

The findings demonstrated that the preparation procedure had a substantial impact on the EE%.The emulsification method can prepare the NLCs of GCV successfully.

Keywords: Cold homogenization, Emulsification, Ganciclovir, Nanostructured lipid carriers, Solvent injection.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.1.55

How to cite this article: Salih ZT, Al-Gawhari F. Improvement of Entrapment and Ocular Permeability of Ganciclovir Nanostructured Lipid Carriers Using Various Conditions of Preparations. International Journal of Drug Delivery Technology. 2023;13(1):341-346.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Ophthalmic medicines that are used topically and without pain are the most advantageous way of drug administration and constitute more than 90% of treatment to the anterior segment of the eye A number of elimination processes, including tear turnover, drug drainage through the nasolacrimal duct, systemic absorption, enzymatic degradation, protein binding, and complex barriers that hinder permeation cause drug loss from the site of installation, which lowers the bioavailability of therapeutic agents. Therefore, repeated instillation of conventional eye drops is needed to achieve therapeutic effects, which may result in toxic side effects.[1]

In ocular medication delivery, significant attempts have been undertaken to extend drug retention time, improve corneal permeability, augment drug effectiveness, and minimize side effects.[2] Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) have become the first and second generation of It is considered a second-generation lipid nanoparticle. It has been identified as a promising approach to drug delivery. The use of NLC has many advantages in ophthalmic treatment. NLCs can offer small particle sizes (50–400 nm) while containing low levels of emulsifiers and thus may be an alternative delivery system to microemulsions or liposomes for ophthalmic administration.[3]

Due to the reduced lipid crystallinity, NLCs have higher drug entrapment efficiencies compared to liposomes.[4] They can enhance corneal penetration and bioavailability in a safe, non-invasive, and patient-friendly manner. In addition, the potential mucoadhesive properties of NLCs enhance their interaction with the corneal membrane. Which may result in longer residence times, improved bioavailability, and reduced local side effects. An advantage of these systems is the potential for scale-up to industrial production.[5]

NLCs can be created using various approaches divided into high- and low-energy groups based on the amount of energy used. They can be applied in various ways and through various routes, including oral, cutaneous, ophthalmic, and pulmonary.

In the study, the method used in preparation was optimized for cold homogenization, sonication, and solvent injection. The effect of various methods of preparation on the drug entrapment efficiency of these nanoparticles was investigated.

**Author for Correspondence: zainab.saleh@copharm.uobaghdad.edu.iq*

MATERIALS AND METHODS

Materials

Ganciclovir (GCV) was supplied from HperChem LTD CO, China. Oleic acid (OA), CDH, and palmitic acid (PA) were supplied from Himedia Laboratories, India). Cremophor EL (CR-EL) was provided from China. Transcutol HP (TR-HP) from Gattefosse Co. (St.Priest, France). Ethanol, methanol and deionized water were bought from France. Disodium hydrogen phosphate and potassium dihydrogen phosphate were bought from BDH Chemicals Ltd., India, Amicone® Ultracentrifuge tube 10k MWCO was supplied from PALL Corporation, USA, and all other chemicals and reagents obtained are of analytical grade.

Methods

Preparation of Drug-Loaded NLC

The formulas were created using PA to OA ratio set at 60:40 with the total amount of lipid phase remaining constant (6% w/w). All formulations use an aqueous surfactant solution with co-emulsifier TR-HP at 1% by weight and CR-EL concentration (CR-EL, 2% w/w). Table 1 lists the ingredients in the formulations that were created and assessed during the study.

Formula 1: Cold homogenization was used to create GCV- NLCs, and the following technique was used with slight changes.[6]

The solid lipid PA and OA as a liquid lipid were melted, then 1% transcutol was added as a solubilizer and mixed into the melted lipid. Finally, 75 mg of GCV was added, and the mixture was magnetically stirred for two minutes. It was quickly cooled by putting this fused lipid phase in a tiny glass tube and submerging it in an ice bath. The solid lipid containing the medication was then milled in a mortar to create microparticles in an aqueous solution containing 2% cremophore at 4°C. Microparticles were dispersed in the final volume and the suspension was then run through a homogenizer three times for 20 seconds each.

Formula 2: GCV-loaded NLCs were created using the solvent injection technique with slight adjustment,[7] the prepared organic phase containing PA and OA, and the drug (GCV) dissolved in ethanol at 70 ± 2°C. The organic phase was rapidly injected into the aqueous phase using a special needle under continuous magnetic stirring for 30 minutes at 600 rpm. After the emulsifier mixture was added to double distilled water that had been pre-heated to a specific temperature at 4 ± 2°C so that NLCs must solidify before being subjected to sonication for five minutes.

Formula 3: GCV-NLCs were prepared with slight modification by the hot emulsification-ultrasonication method.[5]

A binary lipid mixture of both solid and liquid lipid was blended and heated using a hot water bath to about 10 ± 0.5°C above the melting point of the solid lipid, along with 75 mg of GCV to form a uniform oil phase. The CR-EL and TR-HP blends were dissolved in double-distilled water previously heated to the same temperature as that of the lipid phase. By adding the hot aqueous surfactant solution dropwise to the melted lipid phase with constant magnetic stirring at 900 rpm, an oil in water (o/w) pre-emulsion was produced. The hot pre-emulsion was then sonicated using a probe sonicator for 10 minutes at 75% amplitude with 30 seconds on, 5 seconds off periods to create hot nanoemulsion, which was then cooled in an ice bath where the lipid nanodroplets were formed.

Formula 4: GCV-NLCs were made utilizing the same procedure as formula 3 with a minor modification that involved employing GCV-saturated aqueous surfactant solution while keeping the rest of the formula's conditions and concentrations constant.

GCV-NLCS Characterization and Optimization

Particle Size (PS) and Poly Dispersity Index (PDI) Determination

Zetasizer (Malvern Instruments Ltd., United Kingdom) was used to measure the PS and PDI of the designed GCV-NLC formulations. At 25 ± 0.5°C, analyses were conducted with a 90° scattering angle. After the materials were diluted with double-distilled water, the particle means diameter and PDI measurements were made.[6]

Determination of Zeta Potential (ZP)

The ZP of GCV-NLC formulas was evaluated in double distilled water, and all determination was made in triplicate.[7]

Determination of Entrapment Efficiency (EE%) and Drug Loading (DL%)

The ultrafiltration method was used to determine EE%. Using an ultrafilter (Amicon ultra, MWCO 10kDa; Millipore Company), 4 mL of the GCV-NLC formulation was placed in the upper chamber of a centrifuge tube and centrifuged for 30 minutes at 5000 rpm. UV spectrophotometer at 254 nm was used to identify the free drug present in the filtrate. The following equations below were used to compute the EE% and DL%.

Table 1: The composition of GCV-NLCs

Method of preparation	*Form. cod*	*GCV (mg)*	*PA (g)*	*OA (g)*	*CR. EL (% w/w)*	*TR. HP (% w/w)*	*ethanol (mL)*	*DDH2O (g)*
Cold homogenization	F1	75	1.8	1.2	2	1		50
Solvent injection	F2	75	1.8	1.2	2	1	5	50
Emulsification/ ultrasonication	F3	75	1.8	1.2	2	1		50
Emulsification/ ultrasonication with saturation	F4	400	1.8	1.2	2	1		50

$$EE\% = \frac{(W initial\ drug - W\ free\ drug)}{W\ initial\ drug} \times 100 \quad \text{----------eq (1)}$$

$$DL\% = \frac{(W initial\ drug - W\ free\ drug)}{W\ lipid} \times 100 \quad \text{---------- eq (2)}$$

where W_{total}, W_{free}, and W_{lipid} are the total weight of the drug in the GCV-NLC formulation, the free drug in the ultrafiltrate, and the lipid in the GCV-NLC formulation, respectively. The experiments were performed in triplicate.[8,9]

In-vitro Release Study

The optimum GCV-NLC formulations were looked for *in-vitro* drug release based on the results from PS, PDI, ZP, EE%, and DL%. The dialysis membrane, whose molecular weight ranges from 12,000 to 14,000 Da, was used to create the *in-vitro* release profiles; it had previously immersed overnight in phosphate buffer pH 7.4 has been placed on diffusion cells; the commercial Virgan® ophthalmic gel served as control.

The *in-vitro* release studies were carried out using Franz-type diffusion chambers and stirring at 100 rpm. A certain quantity of GCV-NLCs dispersion was placed in the donor chamber. The receiver compartment was filled with freshly prepared worm PBS pH 7.4 at 34 ± 0.5°C, Samples of a specific volume were taken between 0.25 and 24 hours and were replaced with a fresh buffer sample of the same volume. Then using UV spectrophotometer and the calibration curve for GCV in phosphate buffer 7.4, the amount of GCV in the receptor compartment was calculated. The sink conditions were conserved in the receptor's compartment throughout the process. the cumulative drug release% was plotted versus the time to produce the release profiles, and all tests were performed in triplicate.[10,11]

Ex-vivo Corneal Permeation Study

Permeation of the optimized GCV-NLCs and commercial Virgan® ophthalmic gel as the control were studied on the carefully excised cornea from sheep's eyes after washing the isolated cornea with the PBS solution, pH 7.4. The cornea was placed in between the two half-Franz cells with the epithelial layer towards the donor cell at which the preparations were placed. While the receiver champed contained, PBS solution with pH 7.4 which was kept continuously magnetically stirred and maintained at 34 ± 0.5°C for all permeation studies. Samples were taken out of the receiver chamber at various time intervals ranging from 0 to 12 hours and refilled with an equal volume of fresh, warm PBS. Analysis for all the samples was carried out in triplicate using UV spectroscopy at 254 nm. The cumulative amount of GCV permeated (M), steady-state flux (J), and trans-corneal permeability (P) across the sheet cornea were estimated from the following equation:[12,13]

$$J = \left(\frac{dM}{dt}\right) A \quad \text{---------eq (3)}$$

$$p = \frac{J}{Cd} \quad \text{----------eq (4)}$$

Where: dM/dt is the cumulative amount (M) permeated per unit of time (t);(A) is the area of the cornea where drug penetration occurs; Cd is the initial drug concentration in the donor chamber.

Statistical Analysis

The Graph Pad Prism software version 8 was used to conduct the one-way analysis of variance (ANOVA) test. The level of significance of individual factors and the interaction between them was set at α = 0.05, and all the results were expressed as the mean values ± standard deviation.

RESULTS AND DISCUSSION

Table 2 displays the findings of the PS, PDI, ZP, EE%, and DL% evaluations.

Impact of Preparation Technique on PS

Particle size and size distribution have an impact on the bioavailability and drug release rates of nanoparticles, which in turn affects their pharmacodynamics. The average PS and PDI were measured for the prepared GCV-NLC formulations, and their values were represented in Table 2.

The results demonstrated that the GCV-NLCs mean PS and PDI were (62 ± 3.33 to 385 ± 5.1 nm), (0.281 ± 0.04 to 0.368 ± 0.012), respectively and all the formulated GCV-NLCs had particle sizes in the nanometer range (< 1 μm), and they were monodispersed systems (their PDI was less than 1).

The PS were significantly lower ($p < 0.05$) in formula F9 than two other methods, as shown in Figure 1 and this variation in PS may be due to the type of instruments utilized (*i.e.,* ultrasonication that produce a cavitation force which act to reduce the PS to a very limited size) and their working conditions like the time and temperature alongside sample properties and compositions.[14] Meanwhile, the large PS produced by cold homogenization and solvent injection techniques may be due to lower energy used, and the lipids in the semisolid or melting state could easily coalesce together to create larger droplets in these techniques compared to first one.[15]

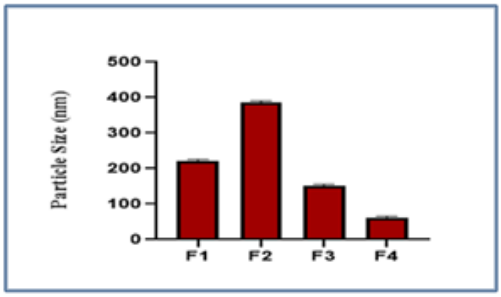

Figure 1: The impact of the preparation procedure on GCV-NLC PS.

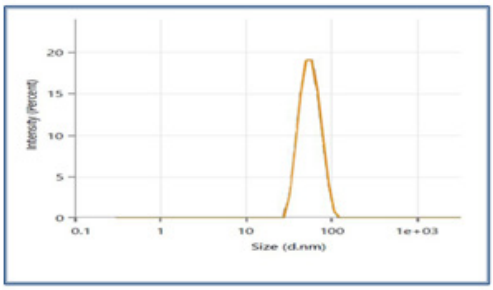

Figure 2: The particle size of formula (4).

The results revealed that increasing GCV concentration significantly reduced PS ($p < 0.05$). This finding is comparable to one from an earlier study by Jansook *et al*., who conclude that the addition of the drug (amphotericin) in the NLCs lead to the reduction of the nanoparticle diameter and this outcome may be related to the physical properties of the intrinsic drug (emulsifying property),[16] as shown in Figures 1 and 2.

Determination of DL% Capacity and EE%

Hydrophilic medicines have a higher tendency to partition in water during the fabrication process, making it difficult to entrap them within the lipophilic matrix of NLCs.

The results show that the treatment method has a large effect on EE%. In addition, changes in the manufacturing process slightly increased the drug load. Since the GCV is hydrophilic and the nanoparticles are lipophilic, the low EE% of the NLCs produced by the solvent injection technique at 0.15% drug concentration is due to the increased solubility of the drug. The aqueous phase stated that due to the high water solubility of GCV, the highly hydrophilic drug was distributed on the nanoparticle surface during the diffusion process, and there was also leakage of GCV into the aqueous phase, resulting in lower EE% and DL%.[17]

The EE% and DL% of NLC significantly increased with aqueous phase saturation, reaching 75 and 9.6%, respectively. The limited diffusion of drugs may explain these results from the lipid phase into the aqueous phase, which caused more drug molecules to be entrapped into the lipid matrix when the initial drug concentration and aqueous phase saturation were increased. Additionally, solid lipids in the NLC develop tortuosity by enclosing liquid lipid portions in a highly structured crystal matrix, resulting in further empty spaces where a considerable amount of drug might be trapped.[18]

ZP

The physical stability of aqueous nano-dispersions is frequently quantified using ZP. It describes the strength of the repulsion between nearby similarly charged particles in the dispersion, which is crucial in preventing particle aggregation. The stability increases as the electrostatic repulsion between the particles increases.[18]

ZP showed a negative charge with values ranging from −46.5 mV ± 2.5 to -38.79 ± 1.2 mV. Because of the repulsive forces, this value was high enough to provide a stable colloidal suspension to manufacture NLC. The anionic structure of the lipid was thought to be responsible for the negative charge. The small drop in ZP values shows that formulas still possess strong physical stability. These better physical features can facilitate the creation of effective NLCs for the delivery of GCV.[17,19]

This suggests that the operating conditions utilized had little effect on the ZP, so changing the production process had little effect on the stability of the NLCs produced.[20,21]

In-vitro Release Study

The *in-vitro* release profile of the optimized GCV-NLC and commercial GCV ophthalmic gel Virgan®. The Virgan released GCV more quickly than the NLCs and this rapid drug release from the ophthalmic gel may be explained by GCV's water-soluble properties and availability in direct contact with the diffusion membrane with the constant removal of the release medium from the receiver chamber ensuring sink conditions.[23] The cumulative percentage of GCV released from GCV gel was 100% after 8 hours, whereas GCV-NLC showed values of 72% during the same period.[22]

The release of GCV-NLCs should be dissimilar from the release of the drug in the gel due to the solid matrix of the NLC and subsequent drug immobilization. A biphasic release pattern was seen, with the medication released initially in a burst pattern and then continuously after that. Approximately 68% of medication was released from F4 in the first five hours, with a slower release continuing for the next 24 hours as shown in Figure 3.

The initial burst release was caused by the GCV being on the surfaces of the NLCs and the external phase during the supersaturation period. Another explanation could be that GCV was introduced into the NLC system with a drug-enriched shell model, resulting in a short drug diffusion path to the release media. GCV was released from the deeper lipid matrix/core in a sustained manner in the second stage, using diffusion and erosion mechanisms to release the drug.[23] Furthermore, the uniform distribution of oleic acid in nanoparticles may have contributed to the consistent release of NLC formulations.[24]

Ex-vivo Study (Corneal Permeation)

Plot 4 shows the GCV amount permeated from the market formula Virgan® ophthalmic gel as the control and the optimized formula F4 through the cornea of sheep,

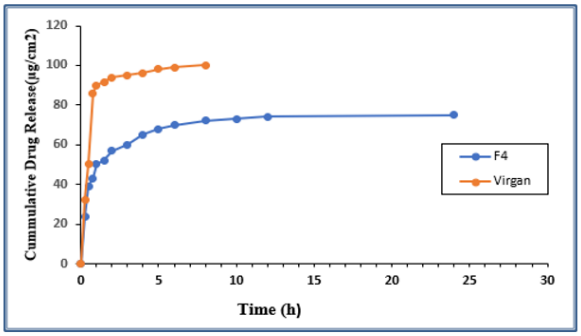

Figure 3: The cumulative amount released of formula F4 (NLCs) and Virgan ®

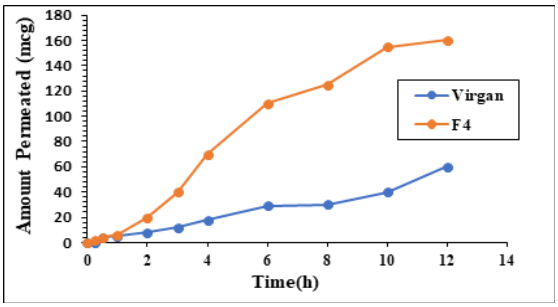

Figure 4: Permeation of GCV from optimized formula F4 and Virgin® ophthalmic gel through the corneal membrane.

Table 2: Evaluation Parameters of Different GCV-NLCs

Formulation methods	*EE%*	*DL%*	*APS (nm)*	*PDI*	*ZP (mv)*
F1	35.4 ± 3.52	0.37 ± 0.22	222 ± 3.2	0.351 ± 0.005	-45.28 ± 2.4
F2	37.63 ± 2.59	0.48 ± 0.14	385 ± 5.1	0.368 ± 0.012	-46.5 ± 2.5
F3	40.2 ± 1.02	1.01 ± 0.23	151 ± 4.0	0.293 ± 0.011	-39.97 ± 1.1
F4	75.3 ± 2.41	9.6 ± 0.2	62 ± 3.33	0.281 ± 0.04	-38.79 ± 1.2

represented in Figure 4. The findings indicated that the hydrophilic nature of GCV and the lack of any carrier molecules in the gel hindered the drug's potential to penetrate the cornea, while GCV from F4 significantly increased corneal permeability when compared to the control. The selected nanoformulations produced significantly higher ($p < 0.05$) flux and permeability coefficient through the excised cornea after 12 hours. These results were similar to the results obtained with ribavirin.[25]

The GCV flux from Virgan® gel was 4.668 ± 0.006 (μg/cm^2 h), while drug flux from formula F4 was found to be 16.13 ± 0.13 (μg/cm^2.h) correspondingly, suggesting a 3.5 fold. In comparison, doubling of drug penetration for F4 with Virgan®, these result was consistent with Liu *et al.* finding.[8]

The presence of lipid carriers, surfactants, and co-surfactant in the NLCs composition that acts as permeability enhancers and the small particle size of the product all contribute to improved passive drug transport through the cornea. In addition, the corneal flux was improved due to the preservative benzalkonium chloride. Because lipid-based drug delivery systems (LBDDS) forming a film capable of sustained drug release can be formed on the corneal surface, it will be possible to further enhance the promotion of corneal permeation and accumulation of GCV. The presence of liquid lipids acting as permeation enhancers could have affected the surface properties of the cornea, leading to the uptake and transport of intact NLCs to the opposite side of the membrane.[9,25]

CONCLUSION

The findings of this investigation demonstrated that the preparation process changed the obtained NLCs' EE% and physicochemical characteristics, and the ultrasonication with saturation was found to be the optimum method of preparation as this procedure showed the lowest PS of 62 nm and PDI of 0.281.Compared to the commercial ophthalmic gel of GCV, the NLCs loaded with GCV produced by emulsification/ultrasonication with saturation demonstrated extended ocular release and greater permeate. Therefore, LBDDS may be considered a promising ophthalmic drug administration method of GCV since the emulsification/ultrasonication approach is suitable for producing NLCs for the applied topically of ocular keratitis.

REFERENCES

1. Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J, Lu J, Li J, Du S, Liu Z. Research progress of in-situ gelling ophthalmic drug delivery system. Asian journal of pharmaceutical sciences. 2019 Jan 1;14(1):1-5.
2. Gan L, Wang J, Jiang M, Bartlett H, Ouyang D, Eperjesi F, Liu J, Gan Y. Recent advances in topical ophthalmic drug delivery with lipid-based nanocarriers. Drug discovery today. 2013 Mar 1;18(5-6):290-7.
3. Salvi VR, Pawar P. Nanostructured lipid carriers (NLC) system: A novel drug targeting carrier. Journal of Drug Delivery Science and Technology. 2019 Jun 1;51(990):255-67.
4. Battaglia L, Serpe L, Foglietta F, Muntoni E, Gallarate M, Del Pozo Rodriguez A, Solinis MA. Application of lipid nanoparticles to ocular drug delivery. Expert opinion on drug delivery. 2016 Dec 1;13(12):1743-57.
5. Liu R, Liu Z, Zhang C, Zhang B. Nanostructured lipid carriers as novel ophthalmic delivery system for mangiferin: improving in vivo ocular bioavailability. Journal of pharmaceutical sciences. 2012 Oct 1;101(10):3833-44.
6. Abed HN, Hussein AA. Ex-vivo absorption study of a novel dabigatran etexilate loaded nanostructured lipid carrier using non-everted intestinal SAC model. Iraqi J Pharm Sci. 2019;28(2):37-45.
7. Allah AKA, Hussein AA. Preparation and Evaluation of Darifenacin Hydrobromide Loaded Nanostructured Lipid Carriers for Oral Administration. Iraqi Journal of Pharmaceutical Sciences. 2018;27(1):53-68.
8. Karami S, Rostamizadeh K, Shademani N, Parsa M. Synthesis and investigation of the curcumin-loaded magnetic lipid nanoparticles and their cytotoxicity assessment on human breast carcinoma cell line. Jundishapur Journal of Natural Pharmaceutical Products. 2020 May 31;15(2).
9. Baig MS, Owida H, Njoroge W, Yang Y. Development and evaluation of cationic nanostructured lipid carriers for ophthalmic drug delivery of besifloxacin. Journal of Drug Delivery Science and Technology. 2020 Feb 1;55:101496.
10. Yoon G, Park JW, Yoon IS. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs): recent advances in drug delivery. Journal of pharmaceutical investigation. 2013 Oct;43(5):353-62..
11. Acharya A, Goudanavar P, Chitti R, Dinnimath BM. Preparation of gellan gum and chitosan based in-situ gel of timolol maleate for ophthalmic drug delivery and evaluation of physicochemical properties and drug release profile. Acta Sci. Pharm. Sci. 2019;3(2):68-78.
12. Youssef A, Dudhipala N, Majumdar S. Ciprofloxacin loaded nanostructured lipid carriers incorporated into in-situ gels to improve management of bacterial endophthalmitis. Pharmaceutics. 2020 Jun 19;12(6):572.
13. Bahari LA, Hamishehkar H. The impact of variables on particle size of solid lipid nanoparticles and nanostructured lipid carriers; a comparative literature review. Advanced pharmaceutical bulletin. 2016 Jun;6(2):143-151.
14. Gardouh AR, Faheim SH, Noah AT, Ghorab MM. Influence of formulation factors on the size of nanostructured lipid carriers and nanoemulsions prepared by high shear homogenization. International journal of pharmacy and pharmaceutical sciences. 2018 Apr 1;10(4):61-75.
15. Duong VA, Nguyen TT, Maeng HJ, Chi SC. Preparation of ondansetron hydrochloride-loaded nanostructured lipid

carriers using solvent injection method for enhancement of pharmacokinetic properties. Pharmaceutical Research. 2019 Oct;36(10):138.
16. Jansook P, Pichayakorn W, Ritthidej GC. Amphotericin B-loaded solid lipid nanoparticles (SLNs) and nanostructured lipid carrier (NLCs): effect of drug loading and biopharmaceutical characterizations. Drug Development and Industrial Pharmacy. 2018 Oct 3;44(10):1693-700.
17. Schubert MA, Müller-Goymann CC. Solvent injection as a new approach for manufacturing lipid nanoparticles–evaluation of the method and process parameters. European journal of pharmaceutics and biopharmaceutics. 2003 Jan 1;55(1): 125-31.
18. Houacine C, Adams D, Singh KK. Impact of liquid lipid on development and stability of trimyristin nanostructured lipid carriers for oral delivery of resveratrol. Journal of Molecular Liquids. 2020 Oct 10;316:113734.
19. Kiss EL, Berkó S, Gácsi A, Kovács A, Katona G, Soós J, Csányi E, Gróf I, Harazin A, Deli MA, Budai-Szűcs M. Design and optimization of nanostructured lipid carrier containing dexamethasone for ophthalmic use. Pharmaceutics. 2019 Dec 14;11(12):679.
20. Witayaudom P, Klinkesorn U. Effect of surfactant concentration and solidification temperature on the characteristics and stability of nanostructured lipid carrier (NLC) prepared from rambutan (Nephelium lappaceum L.) kernel fat. Journal of Colloid and Interface Science. 2017 Nov 1;505:1082-92.
21. Badie H, Abbas H. Novel small self-assembled resveratrol-bearing cubosomes and hexosomes: Preparation, charachterization, and ex vivo permeation. Drug Development and Industrial Pharmacy. 2018 Dec 2;44(12):2013-25.
22. Ibrahim MM, Maria DN, Wang X, Simpson RN, Hollingsworth TJ, Jablonski MM. Enhanced corneal penetration of a poorly permeable drug using bioadhesive multiple microemulsion technology. Pharmaceutics. 2020 Jul 26;12(8):704.
23. Seyfoddin A, Shaw J, Al-Kassas R. Solid lipid nanoparticles for ocular drug delivery. Drug delivery. 2010 Oct 1;17(7):467-89.
24. Kelidari HR, Saeedi M, Akbari J, Morteza-Semnani K, Valizadeh H, Maniruzzaman M, Farmoudeh A, Nokhodchi A. Development and optimisation of spironolactone nanoparticles for enhanced dissolution rates and stability. AAPS PharmSciTech. 2017 Jul;18:1469-1474.
25. Patel R, Gajra B, Parikh RH, Patel G. Ganciclovir Loaded Chitosan Nanoparticles: Preparation and Characterization. J Nanomed Nanotechnol. 2016;07(06).