INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

INVESTIGATION OF VARIABLES RELATED TO THE FORMULATION OF APIXABAN NANOSTRUCTURED LIPID CARRIERS

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Article Received on: 23/08/18 Approved for publication: 20/09/18

DOI: 10.7897/2230-8407.099184

ABSTRACT

The objective of this research was to investigate and optimize the potential of nanostructured lipid carriers (NLCs) as a carrier system for Apixaban, which is an inhibitor of coagulation factor Xa have poor solubility and low bioavailability (F=50). Nanostructured lipid carriers (NLC) of apixaban were prepared by the ultra-sonication method with the aim of improving the pharmacokinetic behavior of apixaban and to increase patient compliance. Ten formulas of NLCs were prepared using glyceryl monostearate as solid lipid, and oleic acid as liquid lipid at different ratios in addition to different surfactants include Tween 80, Tween 20, or Poloxamer188 at different ratios. The prepared formulas were characterized regarding drug content, particle size analysis, polydispersity, entrapment efficiency, Zeta-potential, FT-IR, DSC, and *in vitro* dissolution study. All NLC had shown entrapment efficiency within a range of 64.53 to 89.02%. All prepared NLC has a particle size in nanometer but only four formulas particle size lower than 100nm. Both entrapment efficiency and release rate were affected by lipid concentration. Formula F6 which composed of Glyceryl mono-stearate 56.83%, Oleic acid 16.5%, Tween (80) 8.88%, and water up to 100%w/w was considered as a selected formula depending on its smallest particle size (42.1nm) and good physical properties in addition to promising release profile. The optimized formulation did not show remarkable physicochemical changes during preparation according to FT-IR and DSC results. It was concluded that the formulated NLC has a potential approach for controlled release of drug which may reduce the dose frequency and improves patient compliance.

Keywords: Apixaban, Nanostructured lipid carriers, Glyceryl mono-stearate, ultra-sonication

INTRODUCTION

Lipid-based drug delivery systems are expected as a promising oral carrier because of their potential to increase the solubility and improve oral bioavailability of poorly water-soluble or lipophilic drugs [1]. Conventional lipid-based carriers include a wide range of emulsions, liposomes, lipid micro-particles, and nanoparticles. Among the above formulations, the nanostructure lipid carriers (NLCs) are regarded as the second-generation of lipid nanoparticles [2]and are attracting attention as alternative colloidal drug carriers.

NLC developed from solid lipid nanoparticles (SLN) are composed of biocompatible solid lipid matrices and liquid lipid which is considerably different in chemical structure from solid lipid nanoparticles. NLC system possesses many advantages of SLN, such as excellent biocompatibility, controlled drug release, and the possibility of production on the large industrial scale. The lipid formulations loaded with poorly water-soluble drugs for oral route have been investigated and reported to improve the oral bioavailability by many research teams [3,4, 5].

Nanostructured lipid carriers (NLCs) composed of a solid lipid matrix with a liquid lipid content are a new generation of lipid nanoparticles. NLCs are considered smarter nanoparticles which possess improved properties for drug loading, modulation of the delivery profile, and stable drug incorporation during storage[6]. Due to the lipophilic nature of the matrix, NLCs are considered useful means for the administration of lipophilic drugs. NLCs can be prepared by using different methods such as high-pressure homogenization, micro-emulsion template, solvent emulsification, solvent diffusion, reverse double emulsion, homogenization followed by ultra-sonication, and solvent injection method [7].

Apixaban is chemically 1-(4-methoxyphenyl) -7- oxo-6- [4- (2-oxopiperidin-1 yl) phenyl]-1H, 4H, 5H, 6H, 7H-pyrazolo [3,4-c] pyridine-3-carboxamide (fig. 1). Apixaban is an inhibitor of coagulation factor Xa. The drug is indicated for the prevention of deep vein thrombosis [8,9,10]. FDA approved apixaban (Eliquis, Bristol-Myers Squibb/Pfizer) on December 28, 2012, for the prevention of stroke and systemic embolism in patients with atrial fibrillation (AF) [11].

Apixaban has poor solubility in water and relative low oral bioavailability (about 50% for a single 10 mg dose) [12]. The T_{max} of the apixaban tablet formulation is ~3 h after oral administration in healthy subjects, and the $t_{1/2}$ is ~12 h [13].

The aim of this study is to develop a simple approach for the apixaban NLC that may improve the pharmacokinetic behavior of apixaban and to increase patient compliance. Ten formulations were prepared using various lipid concentrations, to study maximum entrapment efficiency (EE). In addition to this in-vitro drug release, particle size, zeta potential, FT-IR, and DSC were also determined.

MATERIALS AND METHODS

Apixaban was obtained from HEC pharmaceutical Co., Ltd China. Glyceryl monostearate was bought from Hyperchemical, China. Oleic Acid was purchased from Central Drug House

(CDH), India. All other materials used in this research were of analytical grade.

Calibration curve of apixaban in methanol

Accurately weighed 30 mg quantity of apixaban was transferred into 250 ml volumetric flask, to this 100 ml of methanol was added to get 300 µg/ml (stock solution). This solution also diluted with methanol to obtained desired concentrations for working standard solutions in the range of 1.25- 12.5 µg /ml.

Calibration curve of apixaban in buffer with SLS

Accurately weighed 30 mg quantity of Apixaban was transferred into 250 ml volumetric flask, to this 100 ml of buffer pH 6.8 and 0.05% SLS was added to get (stock solution), this solution also diluted with methanol to obtained desired concentrations for working standard solutions in the range of 2.5 -15 µg /ml.

Preparation of NLC

Different concentrations of apixaban NLC dispersions were prepared using an ultra-sonication technique (table 1). In this technique, an accurately weighed solid lipid (glyceryl monostearate), and liquid lipid (oleic acid) were heated at 5-10°C above the melting point of lipid mixture, to this lipid mixture, the drug was added to obtain a clear melting solution.

An aqueous phase was prepared by dissolving surfactant in deionized water and heated to the same temperature as that of the oil phase. Then, this hot aqueous phase was added drop-wise to the lipid phase at a constant rate (2ml/min) under magnetic stirring.

After that this pre-emulsion sonicated for 20 minutes using probe sonicator. The resulting hot nano-emulsion was cooled to room temperature to induce crystallization[14]. Ten formulas were prepared by this method (table 1).

Characterization of prepared NLC's

Assay; (drug content)

For determination of the drug content, an accurate quantity of the formulated NLC from each formula was dissolved in methanol and filtrate them, then aliquot of the filtrate was diluted suitably and analyzed spectrophotometrically at λ_{max} of 280 nm, and calculates drug content using calibration curve of apixaban in methanol.

FT-IR Spectroscopy

The FT-IR was used to detecting the presence of any interactions between the drug and carrier. RT-IR spectrums of pure apixaban and selected formula were taken. Spectra's are scanned within range of 400 and 4000 cm^{-1}.

DSC analysis

Differential Scanning Calorimetry (DSC) was performed using Shimadzu DSC-60, Japan. It was done by taking 5mg of sample into an aluminum pan and sealed. The scan was recorded within a temperature range of 30 to 300 °C. Pure apixaban powder, and selected formula thermograms were recorded [15].

Particle size analysis

Distribution of size in the mean diameter of the nanoparticle was measured using particle size and polydispersity index measurement. The particle size analysis of formulas was performed using ABT-9000 Nano Laser Particle Size Analyzer. Before measurements, NLCs dispersion was diluted suitably using de-ionized water.

Data were analyzed by software and values of mean particle size, polydispersity index (PDI) and particle size distribution curve were recorded [16].

Polydispersity index (PDI)

The polydispersity index (PDI) also be measured using ABT-9000 Nano Laser Particle Size Analyzer. PDI can be termed as an index or difference within the particle size distribution. It can be calculated by using the following equation [17].

Polydispersity = [D90-D10] / D50
Where; D50, D90, and D10 are the percentiles of the undesired particles.

Entrapment efficiency (EE)

Entrapment efficiency can be measured by using the following equation;

Entrapment efficiency = (Estimated % drug content/ Theoretical % drug content) X 100

Estimated drug content can be obtained according to the mentioned method where theoretical drug content can be achieved from the fraction employed to prepare the NLC.

Zeta-potential

Zeta potential is employed to measure the charge on the surface of the particles. It permits an estimate about the storage stability of colloidal dispersion due to repulsion among particles[18].

In-vitro dissolution studies

Dissolution studies were performed for all the prepared formulas in triplicate, using USP- II dissolution apparatus (i.e., paddle) in 900 ml of 6.8 phosphate buffer (0.05 % SLS), the stirrer was modified to rotate at 75 rpm, and the temperature of the medium was adjusted at 37±0.5 ºC during the procedure.

An aliquot of 5ml was taken out periodically from the medium and replaced with fresh medium. The samples were analyzed spectrophotometrically at 280 nm using UV-spectrophotometer and calibration curve of apixaban in buffer [19].

RESULTS AND DISCUSSION

A. FT-IR Studies
The spectra of the selected formula with the pure drug had shown all the characteristic peaks of the drug, from which we can suggest the stable nature of the drug during the process.

B. DSC analysis
Pure apixaban showed a sharp endothermic point at 239.61ºC, which resembles with the melting point of pure apixaban. The DSC curve of the selected formula had shown a sharp endothermic peak at 70.21ºC which resembles glyceryl

monostearate peak and disappearance of the drug peak indicates that the drug was dispersed in the lipid.

C. Particle size analysis

Data consisting about the particle size of the formulated NLC for apixaban was given in table 2. According to the obtained data, it is obvious that the particle size for formulations F1 to F8 was within a range of 42.10 to 421nm.

D. Polydispersity Index (PDI)

Polydispersity was employed to determine the width of particle size distribution. Polydispersity of the entire drug loaded NLCs were given in table 2. From the table, it is shown that the polydispersity index of all the formulations was within a range of 0.018 to 0.308, which indicates all the formulations were within the acceptable size distribution.

E. Entrapment efficiency (EE)

From the obtained data, it can be inferred that the concentration of liquid lipid and surfactant affect apixaban entrapment efficiency. The EE of apixaban NLCs were set in table 2. From the obtained data, it is clear that EE were found in a range of 64.53 to 89.02%. The results show that the increasing the amount of liquid lipid and surfactant leading to increasing the EE, this is attributed to the using of a higher amount of lipid which leads to increase in the particle size that will affect the adsorption of the drug found on the surface of NLCs.

F. Zeta potential

Zeta potential is an important parameter to predict the physical stability of the prepared NLC. Higher the electrostatic repulsions between the particles cause higher the stability. NLC with a zeta potential more than +20 mV or less than -20 mV can be termed as physically stable dispersion. The zeta potential of the optimized formulation F6 was found to be -18.59 mV, from which it can be concluded that the dispersion has reliable physical stability during storage time.

G. In-vitro drug release

The dissolution study was employed for formulas have a particle size lower than 100 nm. The in-vitro dissolution profiles are biphasic with an initial burst effect which is connected with the drug present on the surface of the particles. Drug release profiles of formulated NLCs were given in figure 7. From the figure, it was evident that particle size has a greater effect on the drug release. F6 with small particle size had shown a burst release of 33.59 % after 2hrs and 97.33% after 12 h, and formulas with higher particle size show slower drug release. From these findings, it can be inferred that NLCs with a small particle size has a higher surface area which gives an initial burst effect and sustained drug release.

Table 1: Composition of prepared apixaban nanostructured lipid carriers

Formulas	Drug (mg)	Solid lipid	Liquid lipid	Total lipid weight (mg)	Glyceryl mono-stearate: oleic acid ratio	Type of surfactant (mg)			Water Q.S (ml)
		Glyceryl mono-stearate (mg)	Oleic acid (mg)			Tween 80 (mg)	Tween 20 (mg)	Poloxamer 188 (mg)	
F1	5	305.25	24.75	330	12.33: 1	80			450
F2	5	255.75	74.25	330	03.44: 1	80			450
F3	5	255.75	74.25	330	03.44: 1		40		450
F4	5	305.25	24.75	330	12.33: 1	40			450
F5	5	305.25	24.75	330	12.33: 1		40		450
F6	5	255.75	74.25	330	03.44: 1	40			450
F7	5	255.75	74.25	330	03.44: 1		80		450
F8	5	305.25	24.75	330	12.33: 1		80		450
F9	5	255.75	74.25	330	03.44: 1			40	450
F10	5	255.75	74.25	330	03.44: 1			80	450

Table 2: Particle size distribution and evaluation data of different formulations of NLC

Formula No.	Particle size (nm)	PDI	SSA(m^2/g)	Zeta potential mV	Entrapment efficiency (%)
F1	53.05	0.125	44.81	-11.96	72.59
F2	132.5	0.087	18.18	-19.31	81.15
F3	105.3	0.236	20.43	-19.87	88.40
F4	84.05	0.134	27.33	-18.70	81.31
F5	118.0	0.035	21.45	-14.75	77.51
F6	42.10	0.118	56.42	-18.59	89.02
F7	94.30	0.308	23.75	-27.29	78.37
F8	167.5	0.106	13.85	-14.20	77.47
F9	421.0	0.035	7.340	-28.77	64.53
F10	334.0	0.018	7.510	-29.45	75.32

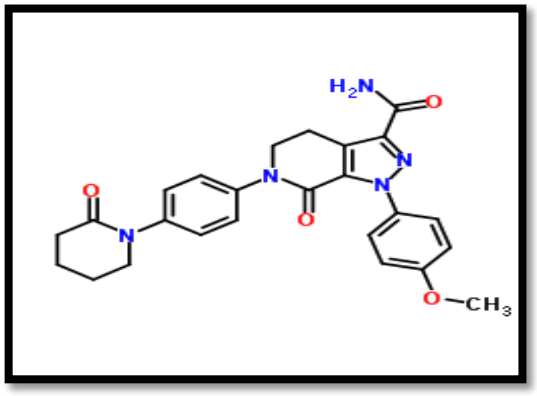


Figure 1: Chemical structure of apixaban

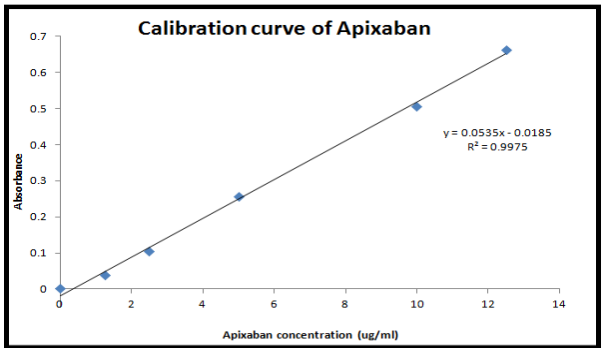


Figure 2: Calibration curve of Apixaban in methanol

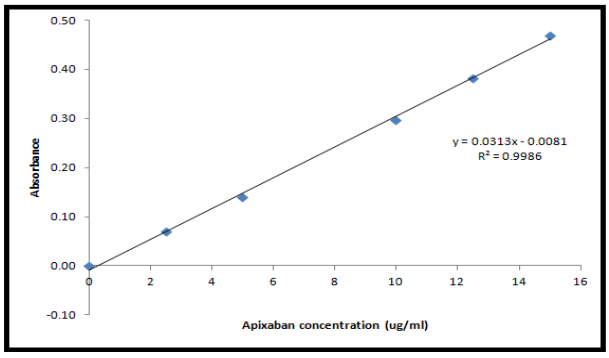


Figure 3: Calibration curve of Apixaban in buffer pH 6.8 with 0.05% SLS

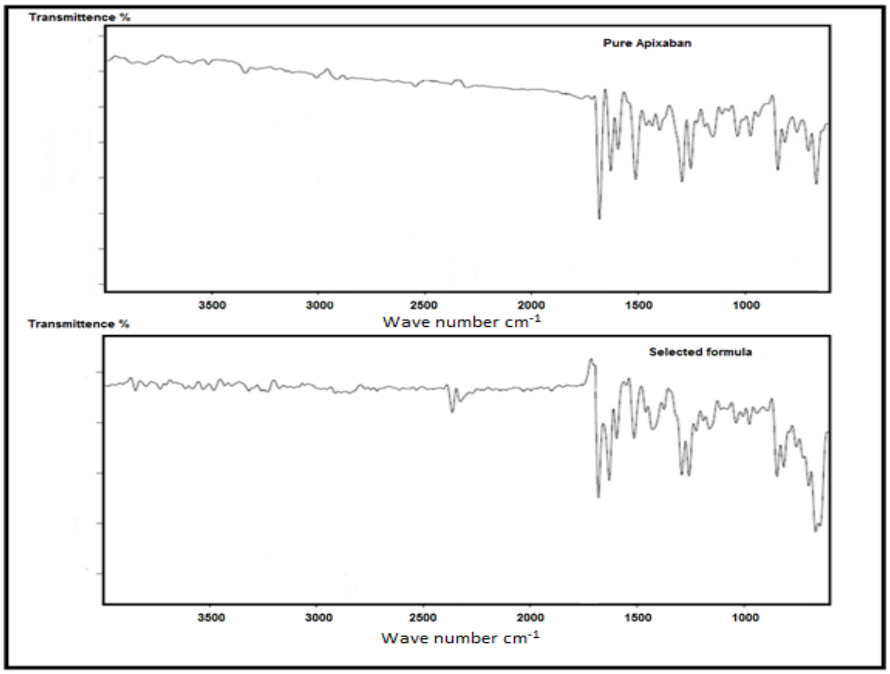


Figure 4: FT-IR spectra of pure Apixaban and selected formula of prepared NLC

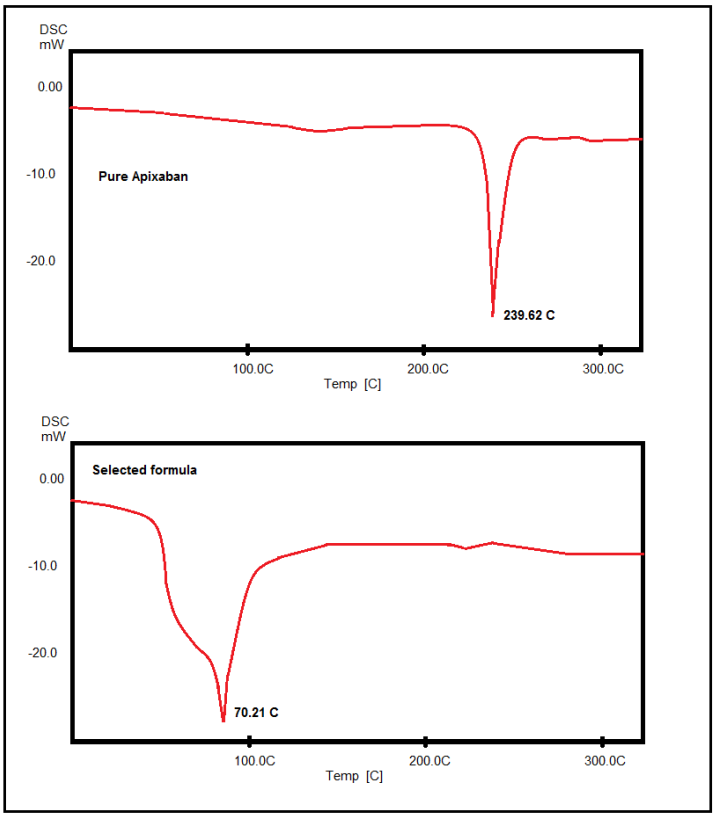
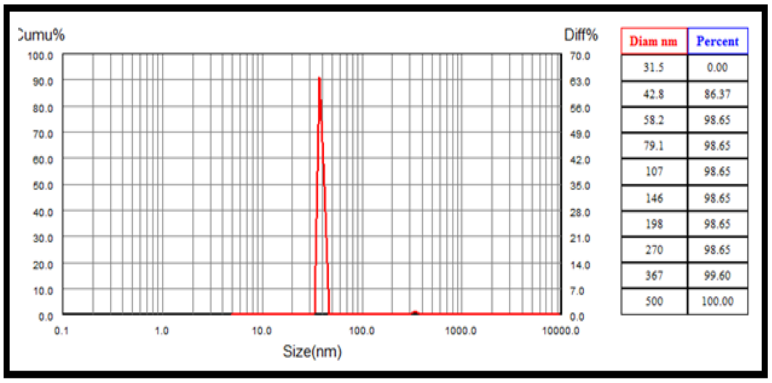


Figure 5: DSC thermogram of pure Apixaban and selected formula of prepared NLC



Diam nm	Percent
31.5	0.00
42.8	86.37
58.2	98.65
79.1	98.65
107	98.65
146	98.65
198	98.65
270	98.65
367	99.60
500	100.00

Figure 6: Particle size distribution of selected formula of prepared NLC of apixaban

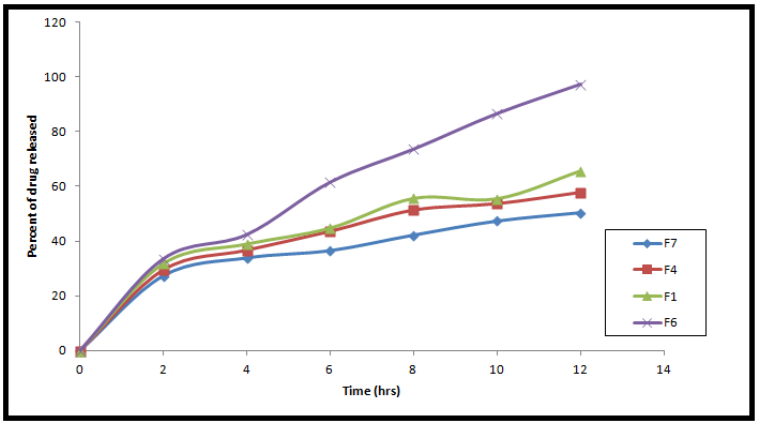


Figure 7: In vitro dissolution of selected formulas of prepared NLC of apixaban

CONCLUSION

Nanostructured lipid carriers were formulated using glyceryl monostearate, oleic acid and tween 80. FT-IR and DSC studies revealed that there were no significant interactions between the drug and excipients. Both particle size and entrapment efficiency were increased with the increase in liquid lipid amount and surfactant. Apixaban release profile was dependent on the particle size; the larger particles had shown a slow release whereas smaller particles had shown a faster dissolution. Formulation F6 was considered as an optimized formulation based on its particle size and % drug release when compared with other formulations. Zeta potential value had suggested good particle stability. The optimized formulation did not show remarkable physicochemical changes during preparation. Controlled release achieved by these formulations may reduce the dose frequency and improves patient compliance in addition to expected bioavailability enhancement.

REFERENCES

1. O'driscoll CM, Griffin BT. Biopharmaceutical challenges associated with drugs with low aqueous solubility□the potential impact of lipid-based formulations. Adv Drug Deliv Rev. 2008;60(6):617–24. https://doi.org/10.1016/j.addr.2007.10.012
2. Müller RH, Radtke M, Wissing SA. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Adv Drug Deliv Rev. 2002;54:S131--S155. https://doi.org/10.1016/S0169-409X(02)00118-7
3. Shah NH, Carvajal MT, Patel CI, Infeld MH, Malick AW. Self-emulsifying drug delivery systems (SEDDS) with polyglycolyzed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. Int J Pharm. 1994;106(1):15–23. https://doi.org/10.1016/0378-5173(94)90271-2
4. Al-Meshal MA, Khidr SH, Bayomi MA, Al-Angary AA. Oral administration of liposomes containing cyclosporine: a pharmacokinetic study. Int J Pharm. 1998;168(2):163–8. https://doi.org/10.1016/S0378-5173(98)00066-0
5. Paliwal R, Rai S, Vaidya B, Khatri K, Goyal AK, Mishra N, et al. Effect of lipid core material on characteristics of solid lipid nanoparticles designed for oral lymphatic delivery. Nanomedicine Nanotechnology, Biol Med. 2009;5(2):184–91. https://doi.org/10.1016/j.nano.2008.08.003
6. Müller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Adv Drug Deliv Rev. 2007;59(6):522–30. https://doi.org/10.1016/j.addr.2007.04.012
7. Thatipamula RP, Palem CR, Gannu R, Mudragada S, Yamsani MR. Formulation and in vitro characterization of domperidone loaded solid lipid nanoparticles and nanostructured lipid carriers. Daru J Fac Pharmacy, Tehran Univ Med Sci. 2011;19(1):23.
8. Becattini C, Vedovati MC, Agnelli G. Old and new oral anticoagulants for venous thromboembolism and atrial fibrillation: a review of the literature. Thromb Res. 2012;129(3):392–400. https://doi.org/10.1016/j.thromres.2011.12.014
9. Dentali F, Riva N, Crowther M, Turpie AGG, Lip GYH, Ageno W. Efficacy and safety of the novel oral anticoagulants in atrial fibrillation: a systematic review and meta-analysis of the literature. Circulation. 2012;CIRCULATIONAHA--112.
10. Pinto DJP, Orwat MJ, Koch S, Rossi KA, Alexander RS, Smallwood A, et al. Discovery of 1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl) phenyl)-4, 5, 6, 7-tetrahydro-1 H-pyrazolo [3, 4-c] pyridine-3-carboxamide (Apixaban, BMS-562247), a highly potent, selective, efficacious, and orally bioavailable inhibitor of blood coagulation factor Xa. J Med Chem. 2007;50(22):5339–56. https://doi.org/10.1021/jm070245n
11. Rosenthal L. Contributor Information and Disclosures.
12. Eriksson BI, Quinlan DJ, Weitz JI. Comparative pharmacodynamics and pharmacokinetics of oral direct thrombin and factor Xa inhibitors in development. Clin Pharmacokinet. 2009;48(1):1–22. https://doi.org/10.2165/0003088-200948010-00001
13. Frost C, Nepal S, Wang J, Schuster A, Byon W, Boyd RA, et al. Safety, pharmacokinetics and pharmacodynamics of multiple oral doses of apixaban, a factor Xa inhibitor, in healthy subjects. Br J Clin Pharmacol. 2013;76(5):776–86. https://doi.org/10.1111/bcp.12106
14. Ricci M, Puglia C, Bonina F, Di Giovanni C, Giovagnoli S, Rossi C. Evaluation of indomethacin percutaneous absorption from nanostructured lipid carriers (NLC): in vitro and in vivo studies. J Pharm Sci. 2005;94(5):1149–59. https://doi.org/10.1002/jps.20335
15. Riekes MK, Pereira RN, Rauber GS, Cuffini SL, de Campos CEM, Silva MAS, et al. Polymorphism in nimodipine raw materials: development and validation of a quantitative method through differential scanning calorimetry. J Pharm Biomed Anal. 2012;70:188–93. https://doi.org/10.1016/j.jpba.2012.06.029
16. Chidi E, Zainab A, John DF, others. Development and evaluation of nanoemulsion formulations for improved oral delivery of carvedilol. Univers J Pharm Res. 2017;
17. Kapil A, Aggarwal G, Harikumar SL. Nanotechnology in novel drug delivery system. J Drug Deliv Ther. 2014;4(5):21–8.
18. Lin P-C, Lin S, Wang PC, Sridhar R. Techniques for physicochemical characterization of nanomaterials. Biotechnol Adv. 2014;32(4):711–26. https://doi.org/10.1016/j.biotechadv.2013.11.006
19. Fotaki N, Brown W, Kochling J, Chokshi H, Miao H, Tang K, et al. Rationale for selection of dissolution media: three case studies. Dissolution Technol. 2013;20(3):6–13. https://doi.org/10.14227/DT200313p6

Cite this article as:

Mowafaq M. Ghareeb. Investigation of variables related to the formulation of apixaban nanostructured lipid carriers. Int. Res. J. Pharm. 2018;9(9):35-40 http://dx.doi.org/10.7897/2230-8407.099184

Source of support: Nil, Conflict of interest: None Declared

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