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PREPARATION AND EVALUATION OF NANOPARTICLES CONTAINING ANTI-HYPERTENSIVE DRUG BY SONICATION METHOD.

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ABSTRACT

The present study describes preparation and evaluation of nanoparticulate systems containing losartan potassium by using polymers eudragit RS100 and eudragit RSPO by sonication method (Probe sonicator) for the treatment of hypertension to increase the oral bioavailability. The prepared Nanoparticles were evaluated for Surface morphology, Drug entrapment efficiency, Differential scanning colorimetry, particle size, FTIR, *in-vitro* drug release and stability studies. The prepared Nanoparticles are smooth in surface and showing spherical shape and average particle size of the Nanoparticles were found in the range of 300 nm to 500 nm. The drug entrapment efficiency (EE) of prepared nanoparticles were found in the range of 78.14% to 91.64%. The *in-vitro* drug release data of all the formulations were found to be zero order and shown maximum release over a period of 24hrs. The FTIR Spectra of Nanoparticles formulation are compared with the spectra of pure drug of losartan and there is no much deviation in the spectra's and not observed any drug and polymer interactions. The short-term stability of optimized formulation was conducted for drug entrapment efficiency and *In-vitro* drug release studies, where results shown that there is no significant change in the formulation.

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INTRODUCTION

As oral drug delivery is simple, most convenient, safest, noninvasive and most economical, it continues to be the preferential route of administration and researchers are seeking ways to incorporate various technologies in oral formulations. Drug delivery systems (DDS) that can precisely control the release rates or drug targetto specific body site have had an enormous impact on the health care system. Nanotechnology is a promising strategy in the development of drug delivery systems especially for those potent drugs whose clinical development failed due to their poor solubility, low permeability, inadequate bioavailability, and other poor biopharmaceutical properties. Nano-particles can be defined as solid, sub-micron, colloidal particles ranging in size from 10 nm to 1000 nm in diameter, generally but not necessarily made of natural or syntheticpolymers, in which drugs can be adsorbed, entrapped, encapsulated or covalently attached and are produced by mechanical or chemical means. Above mentioned method is selected for the preparation of Nanoparticles is to overcome the poor characteristics of the BCS class II and class III drugs. The objective of this study was to prepare and evaluate the prepared Nanoparticles containing Anti-hypertensive drug (Losartan potassium) by sonication methodology.

Materials And Method

Materials

Losartan potassium was procured from Nirupama K V, Dept. of pharmaceutics, Bharathi college of pharmacy, Mandya as a gift sample, Eudragit RS 100, Eudragit RSPO was supplied from Yarrow chemicals, Polyvinyl alcohol (PVA) was supplied from Fisher scientifics, Iso propyl Alcohol, Dichloromethane, NaOH and Potassium Dihydrogen Orthophosphate was supplied from SD Fine chemicals.

Method

Solution of polymers, Eudragit RS 100 and Eudragit RSPO was dissolved in dichloromethane containing losartan was mixed with an aqueous phase solution of surfactant polyvinyl alcohol, by using controlled flow rate syringe pump 3ml/min rate. During this mixing the aqueous phase was sonicated using a probe sonicator set at 10 Khz of energy output (LABMAN PRO-650) to produce an emulsion, here the sonication is done for 15 to 20 min. Then Place this preparation for magnetic stirring at 1000 rpm until the organic solvent is to evaporate. Then obtained nanoparticles were recovered by centrifugation (Remi PR 24) at 10,000 rpm for 10 to 15 min and washed thrice with distilled water. The washing water is been removed by further centrifugation and obtained nanoparticles were dried.

Table 1: Formulation table of SNEDDS containing Losartan potassium.

Formulation code	Losartan potassium (mg)	Polyvinyl alcohol (PVA)(%)	Eudragit RS100 (w/v)	Eudragit RSPO(w/v)	Isopropyl alcohol (ml)
F1	100	0.5	100	-	20
F2	100	0.5	150	-	20
F3	100	0.5	200	-	20
F4	100	0.5	-	100	20
F5	100	0.5	-	150	20
F6	100	0.5	-	200	20
F7	100	0.5	100	100	20

Characterization of Nanoparticles.

Drug - Excipient Compatibility Study

The compatibility of components and drug was evaluated by FT-IR study.

FTIR Study:

FTIR spectra of pure drugs, physical mixture Eudragit RS 100, RSPO and drug loaded Nanoparticles were recorded on a BRUKER IR spectrophotometer and scanned in the spectral region between 4000 cm-1 and 600 cm-1.

Surface morphology:

The surface morphology is most commonly measured by Scanning Electron microscopy. The surface morphology has been studied by using JEOL JSMT -330A Scanning electron microscopy (SEM).

Particle size:

The particle size and distribution is measured by Malvern Zeta sizer by Wet technique. The average particle sizes of the individual batch of Nanoparticles were reported.

Zeta potential.

The zeta potential of a nanoparticle is commonly usd to characeterize the surface charge property of nanopartilces. Zeta potential is measured by using Malvern zeta anaiyzer.

Drug Entrapment efficiency.

$$\text{Drug entrapment efficiency} = \frac{\text{amount of drug released from the lysed nanoparticle}}{\text{Amount of drug initially taken to prepare nanoparticle}} \times 100$$

Evaluation parameters of prepared Nanoparticles.

Dissolution Rate study.

In-vitro drug release studies were performed in USP Type II dissolution apparatus at rotation speed of 50 rpm. The prepared Nanoparticles were immersed in 900ml of phosphate buffer solution in a vessel, and temperature was maintained at 37±0.20°C. Required quantity 5ml of the medium was withdrawn at specific time periods and the same volume of dissolution medium was replaced in the flask to maintain a constant volume. The withdrawn samples were analyzed using UV spectrophotometer (SHIMADZU 1700).

Stability studies

The prepared Nanoparticles were packed in screw capped HDPE bottles and were stored at 40± 20 C and 75 % RH for 45 days. After storage for 45 days, the products were tested for drug entrapment efficiency and drug release study as per the ICH guidelines.

RESULTS AND DISCUSSION

I. Determination of λ max of Losartan potassium

The λ max of Losartan potassium was found to be 234 nm. The spectrum traced using UV spectrophotometer (UV1700, Shimadzu, Japan).

II. Standard plot of Losartan potassium

The standard plot was established for Losartan potassium in Phosphate buffer pH 7.4. Absorbance measured at 234 nm and a graph of concentration versus absorbance was plotted.

Table 2: Standard Calibration curve of Losartan in Phosphate buffer 7.4.

Sl. No	Losartan potassium (µg/ml)	Absorbance	
		ẍ	RSD (%)
01	0	0	0
02	2	0.152	0.152±0.059
03	4	0.283	0.283±0.012
04	6	0.426	0.426±0.063
05	8	0.568	0.568±0.052
06	10	0.729	0.729±0.075

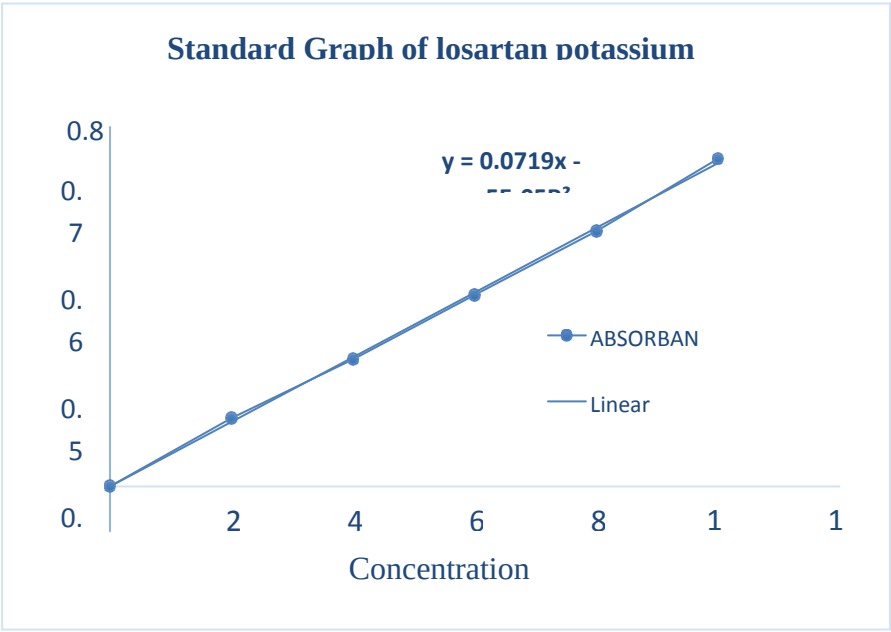

Figure 1: Calibration curve of Losartan in phosphate buffer pH 7.4.

Solubility analysis of drug (Losartan)

Practically completely soluble in Dichloromethane, Isopropyl alcohol, Ethanol, Methanol and Water.

Characterization of nanoparticles

Compatibility studies.

From the FTIR spectra of the pure drug and the combination of drug with the polymers was observed that all the characteristics peaks of Losartan is present in the combined as well thus indicating the compatibility of the drug with the polymer. The spectra individual FTIR spectra of the pure drug Losartan and physical mixture of Eudragit RS100 and Eudragit RSPO as well as combination spectra of the drug and polymers (formulation F7) are shown in Figure **No. 13, 14 and 15.** It was found that the drug was compatible with polymer in physical mixture

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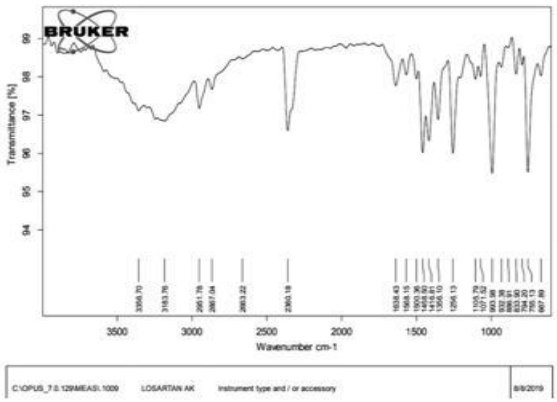

Figure 2: IR spectra of losartan potassium.

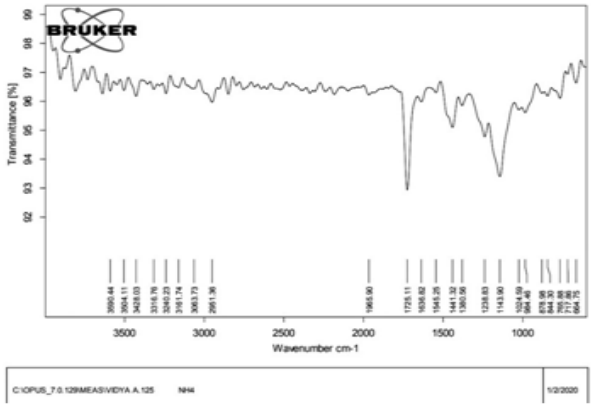

Figure 3: IR spectrs of F7.

Scanning Eectron Microscopy (SEM)

Determination of surface morphology and shape was done by ZEISS EVO. US Scanning Electron Microscope. The F7 formulation shows the spherical shape and smooth in surface area.

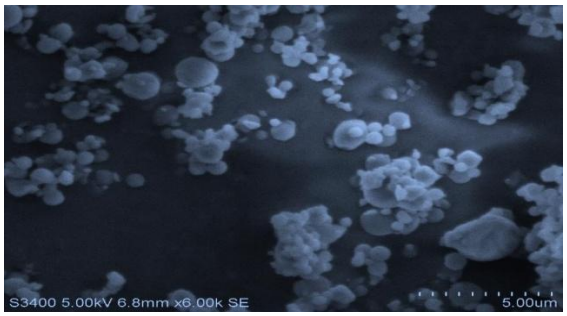

Figure 4: SEM images of LP.

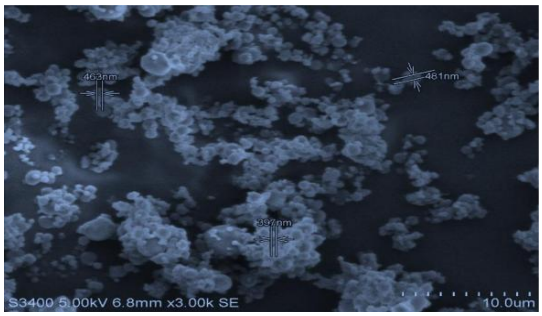

Figure 5: SEM images of F7.

Table 3: Particle size and drug entrapment efficiency of Nanoparticles.

Sl no.	Formulation code	Particle size (nm)	Drug EE(%)
1	F1	364	74.14
2	F2	486	76.56
3	F3	584	78.14
4	F4	345	71.14
5	F5	438	78.23
6	F6	492	81.06
7	F7	407	86.64

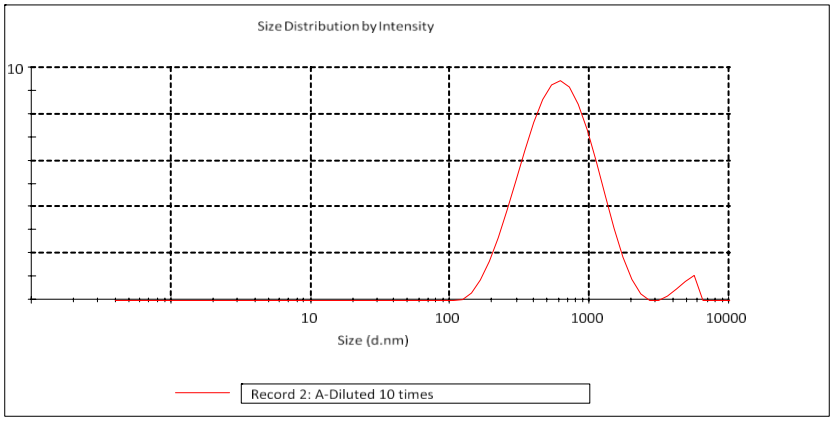

Figure 6: Particle size distribution of losartan Nanoparticles of F7.

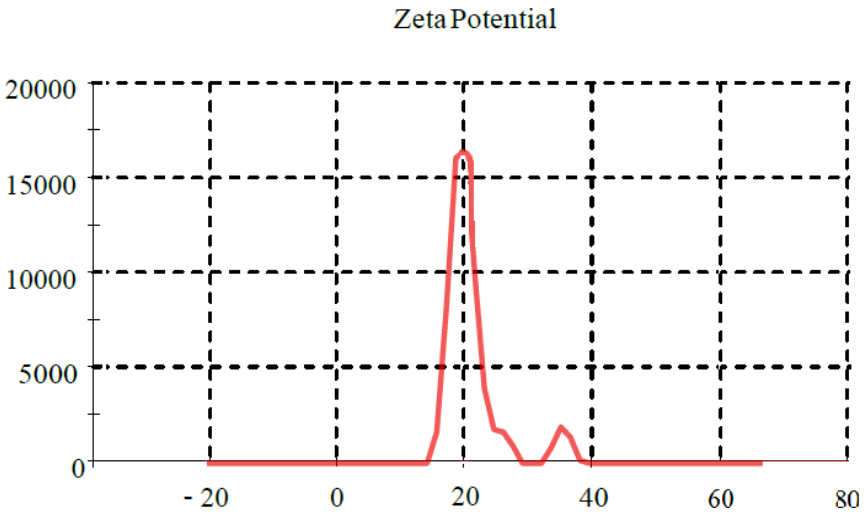

Figure 7: Particle size analysis by Zeta Potential distribution.

***In-vitro* drug release study**

Table 4: *In-vitro* drug release study of Nanoparticles formulations F1 to F7.

Time (hrs)	% of drug Release (X̄SD)						
	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
0.5	14.25 ±0.014	13.23 ±0.015	18.25 ±0.028	12.98 ±0.022	11.09 ±0.018	13.06 ±0.018	15.62 ±0.018
1	22.56 ±0.025	23.58 ±0.022	27.23 ±0.026	19.28 ±0.028	22.09 ±0.016	22.26 ±0.022	38.05 ±0.015
2	34.26 ±0.023	33.44 ±0.016	38.25 ±0.032	27.18 ±0.026	30.98 ±0.016	31.24 ±0.026	49.68 ±0.029
4	48.23 ±0.018	49.89 ±0.031	45.26 ±0.026	35.25 ±0.024	38.91 ±0.018	48.26 ±0.028	63.19 ±0.031
6	59.36 ±0.026	58.56 ±0.032	55.58 ±0.021	43.98 ±0.021	45.98 ±0.014	56.29 ±0.026	65.25 ±0.029
8	68.86 ±0.028	69.48 ±0.024	63.28 ±0.026	56.29 ±0.018	58.56 ±0.020	67.05 ±0.024	74.25 ±0.022
10	79.56 ±0.014	77.26 ±0.031	73.25 ±0.028	63.29 ±0.016	65.37 ±0.026	75.26 ±0.021	79.29 ±0.017
12	81.66 ±0.028	81.25 ±0.021	78.26 ±0.028	70.29 ±0.019	73.46 ±0.022	79.26 ±0.062	83.21 ±0.035
24	82.25 ±0.032	87.01 ±0.027	89.25 ±0.018	78.94 ±0.020	80.52 ±0.024	84.26 ±0.028	92.54 ±0.014

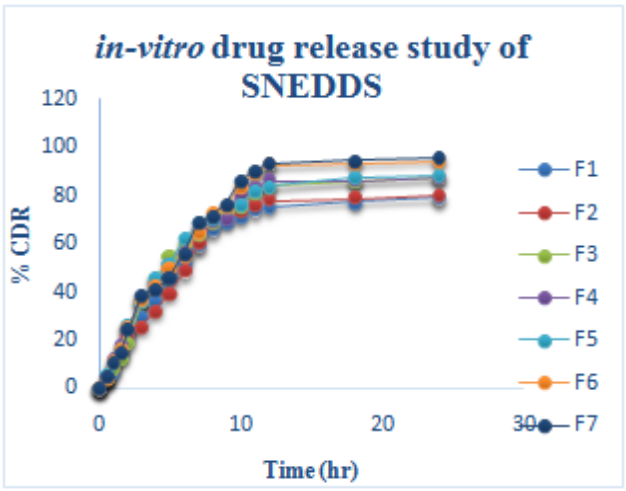

Figure 8: Graph of *in-vitro* drug release kinetics for formulations F1 to F7.

Figure 9: Graph of First order SNEDDS formulation from F1 to F7.

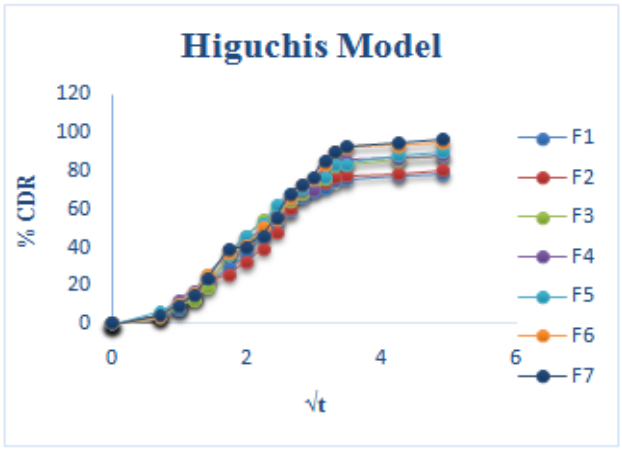

Figure 10: Graph of Higuchi's model for *in-vitro* drug release of formulation F1 to F7.

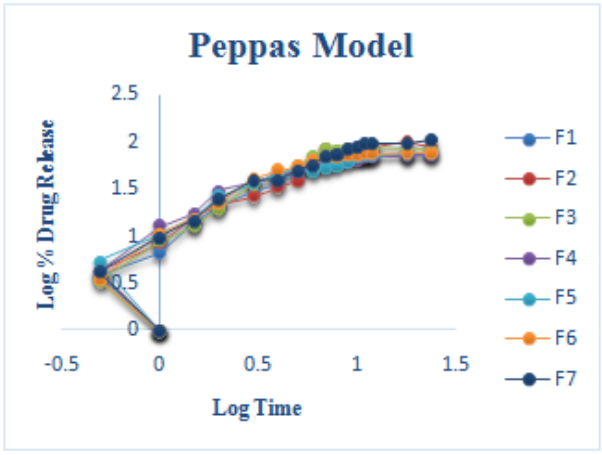

Figure 11: Graph of Peppa's model *in-vitro* drug release of formulation F1 to F 7.

Table 5: Regression co-efficient (R2) values and 'n' values of SNEDDS according to different kinetic models.

Formulation	Zero order		First order		Higuchi	Peppas	
	R^2	n	R^2	n	R^2	R^2	n
SIM1	0.926	3.312	0.648	0.104	0.893	0.875	0.412
SIM2	0.862	3.348	0.638	0.098	0.889	0.879	0.444
SIM3	0.883	3.515	0.717	0.089	0.934	0.962	0.505
SIM4	0.776	3.363	0.675	0.079	0.907	0.957	0.490
SIM5	0.959	3.395	0.771	0.091	0.962	0.984	0.416
SIM6	0.864	3.428	0.719	0.081	0.933	0.966	0.507
SIM7	0.840	3.312	0.713	0.073	0.929	0.965	0.518

Stability Study Report:

The Prepared Nanoparticles were packed in screw capped HDPE bottles and were stored at 40± 2 ^{0}C and 75 % RH for 45 days. After storage for 45 days, the products were tested for drugentrapment efficiency and drug release study as per the methods described earlier. The resultsare given in **Table No. 14 and 15**

Table 6: Stability study report of Drug entrapment efficiency.

Formulation code	Drug entrapment efficiency	
	Before stability test	After stability test
F7	90.28%	89.06%

***In-vitro* Dissolution study:**

Dissolution Study of optimized was studied according to earlier procedure and determined drugrelease rate

Table 7: Stability study report of SIM 9 Formulation Drug release.

Formulation code	% Drug release	
	Before stability test	After stability test
F7	91.26%	90.45%

CONCLUSION

From the results it can be concluded that biocompatible and cost-effective polymers like eudragit RS100 and eudragit RSPO can be used to formulate an efficient nanoparticle of losartan, with good percentage entrapment efficiency and practical yield. The particle size of the Polymeric nanoparticle formulations indicated that the particles were in the size range of 300-500 nm.The prepared nanoparticles were smooth and spherical in shape shown by SEM studies. The *In-vitro* drug release shown that the release from the formulations successfully retarded for 24hrs. Pharmacokinetic studies indicate that the *In-vitro* drug release of formulations were fitted to Peppa's model and the mechanism follows Non Fickian drug release. The formulations found to be stable in short term stability studies. By considering the overall study results obtained from *In-vitro* drug release and stability studies, it can be suggested that there is a further scopefor *In-vivo* and pharmacokinetics study. It can be suggested that there is further scope for the *in-vivo* and the Pharmacokinetic Study.

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REFERENCES

1. Nasir A, Kausar A, Younus A. A review on preparation, properties and applications of polymeric nanoparticle-based materials. Polymer-Plastics Technology and Engineering. 2015;54(4):325-41
2. Paliwal R, Babu RJ, Palakurthi S. Nanomedicine scale-up technologies: feasibilities and challenges. Aaps Pharm SciTech. 2014;(6):1527-34.
3. Reis CP, Neufeld RJ, Ribeiro AJ, Veiga F. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine. 2006;2(1):8-21.
4. Tiruwa R. A review on nanoparticles–preparation and evaluation parameters. Indian journal of pharmaceutical and biological research. 2016;4(2):27-31.
5. Mohanraj VJ, Chen Y. Nanoparticles-a review. Tropical journal of pharmaceutical research. 2006;5(1):561-73.
6. Ealia SA, Saravana Kumar MP. A review on the classification, characterisation, synthesis of nanoparticles and their application. InIOP Conference Series: Materials Science and Engineering 2017;263(3):032. IOP Publishing.
7. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arabian journal of chemistry. 2019;12(7):908-31.
8. Pal SL, Jana U, Manna PK, Mohanta GP, Manavalan R. Nanoparticle: An overview of preparation and characterization. Journal of applied pharmaceutical science. 2011 (6):228-34.
9. http://www.medical newstoday.com/articles/295385.php.
10. Sharma A, Khanijau MR, Agarwal MR. Hyperlipidemia: A Review Article.
11. Singh A, Tuteja V, Nawal CL. Current trends in managing hyperlipidemia- Review article. Int J Curr Adv Res. 2018;7(1):9164-1968
12. Toth PP, Banach M. Statins: then and now. Methodist DeBakey cardiovascular journal. 2019 (1):23.
13. Vaidya A, Jain S, Jain A, Jain A. Simvastatin-Loaded PEGylated Solid Lipid Nanoparticles: Lipid Functionalization to Improve Blood Circulation. BioNanoScience. 2020 (3):773-82.
14. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010 May 1;67(3):217-23.
15. Shaikh HK, Kshirsagar RV, Patil SG. Mathematical models for drug release characterization: a review. World J. Pharm. Pharm. Sci. 2015;4(4):324-338.