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Formulation and Evaluation of Nanosuspension of Atorvastatin Calcium by Nanoprecipitation Method

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ABSTRACT

Enhancement of poorly soluble drug is a challenging task during formulation development. The main objective of the study is to formulate nanosupension of Atorvastatin calcium for the treatment of hypercholesterolemia and to improve the efficacy and patient compliance. In the present work nanosuspension of atorvastatin calcium was prepared by nanoprecipitation Technique by using different polymers, Volatile solvent and Surfactant. The nanosuspension was prepared by using methanol as a solvent, Hydroxyl propyl methyl cellulose E50, Polyvinyl Pyrollidone k-30, Polyethene glycol 400 as polymers Sodium Lauryl Sulphate as surfactant. Several formulations was prepared by having drug concentration in methanol (volatile solvent) with varying concentration of different polymers in water with SLS. The prepared nanosuspension was evaluating for solubility studies, Drug content, In vitro dissolution study, Particle size determination and Zeta potential. From the result of saturation solubility studies it was observed that there was increase in solubility of drug in nanosuspension as compared to pure drug. With increase in the concentration of polymers increased and the nanosuspension containing PVP K 30 and PEG 400 in (F6) and (F9) has increased the solubility up to five times.

Keywords : - Nanosuspension, Nanoprecipitation Technique, Atorvastatin Calcium, Hypercholesterolemia, Solubility studies, In-vitro drug release .

INTRODUCTION

Nano suspensions are aqueous suspensions containing one or several submicronsized drug substances and appropriate stabilizers. Stabilizers include excipients that enable nano grinding of the drug particles, prevent crystal growth or nano particle aggregation during storage, pH-buffering substances, preservatives, and other components that may be needed for further processing (e.g. transforming into a solid form) or administration to patients (e.g., sweeteners, colorants). The term nano sizing, as used in this work, describes the reduction of suspended drug particles down to the submicron size range[1].

A Nano suspension is a submicron colloidal dispersion of drug particles. A pharmaceutical Nano suspension is defined as very finely colloid, Biphasic dispersed, solid drug particles in an aqueous vehicle size below 1µm without any matrix material stabilized by surfactants and polymers, prepared by suitable methods for Drug Delivery applications, through various routes of administration like oral, topical, Parenteral, ocular and pulmonary routes. nanosuspension not only solves the problem of poor solubility and bioavailability but also alters the pharmacokinetics of drug and that improves drug safety and efficacy. Nano suspensions differ from nanoparticles which are polymeric colloidal carriers of drugs (Nano spheres and Nano capsules), and from solid lipid nanoparticles(SLN), which are lipidic carriers of drug. In case of drugs that are insoluble in both water and in organic media instead of using lipidic systems nanosuspensions are used as a formulation approach.The use of nanotechnology to formulate poorly water soluble drugs as nanosuspension offers the opportunity to address nature of the deficiency associated with this class of drugs[2, 3].
Nanosuspension has been reported to enhance absorption and bioavailability it may help to reduce the dose of the conventional oral dosage forms therefore to maintain the therapeutics concentration it may be used as nanosuspension with a nanoparticle size in the nano range typically between 1-1000nm is proposed. The present study is to design metronidazole nanosuspension (MNS) as a novel controlled dosage form that could release the drug in a controlled fashion at the site to have better therapeutic efficiency at a much lower dose[3].

MATERIAL AND METHOD

Material

Atorvastatin was received as a gift sample from Glenmark (Pithampur). Poly vinyl pyrrolidone (PVP 30) was procured from Molychem (New Delhi). Sodium lauryl sulphate, HPMCK15M , Methanol , octanol, chloroform, Hcl from Himedia. Sodium lauryl sulphate From SdFine-chem limited (Mumbai). All other solvent nd reagent are used was of analytical grade.

Method of preparation

Nano suspensions were prepared according to nano precipitation method . Precipitation has been applied for years to prepare submicron particles within the last decade especially for the poorly soluble drugs. The nanosuspension of atorvastatin calcium was prepared by nanoprecipitation Technique. Formulation of nanosupension includes the selection of different polymers, Volatile solvent and Surfactant. The nanosuspension was prepared by using methanol as a solvent, Hydroxyl propyl methyl cellulose E50, Polyvinyl Pyrollidone k-30, Polyethene glycol 400 as polymers Sodium Lauryl Sulphate as surfactant. Several formulations was prepared by having drug concentration in methanol (volatile solvent) with varying concentration of different polymers with SLS.

Table 1: Composition of Nanosuspensions of Atorvastatin Calcium

Ingredients (Mg)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Atorvastatin Calcium	10	10	10	10	10	10	10	10	10
HPMCK15M	20	40	60	-	-	-	-	-	-
PVPK 30	-	-	-	20	40	60	-	-	-
PEG 400	-	-	-	-	-	-	20	40	60
Methanol	10	10	10	10	10	10	10	10	10
Water	40	40	40	40	40	40	40	40	40
SLS	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3

EVALUATION OF OPTIMIZED NANOSUSPENSIONS

Determination of saturation solubility

Solubility study was performed according to method reported by Higuchi and Connors. The formulated nanosuspensions batches F1, F2, F3, F4, F5, F6, F7, F8 and F9 were added in 10 ml distilled water taken in stoppered conical flask and were shaken for 24 hrs at $37^0C \pm 1$ in orbital shaker. Two ml aliquots were withdrawn at 1 hr intervals and filtered through whatman filter paper. The filtered solution were analyzed spectrophotometrically at 246 nm against blank[76]. The soubility of all the forumations are shown in the table 4.

Determination of drug content in formulations

The nanosuspension equivalent to unit dose of drug was weighed accurately and dissolved in 100 ml of methanol. The solutions were filtered through whatman filter paper and analysed by UV spectrophotometer at 246 nm[6]. The drug content calculated accordingly. The drug content of all the formulations are shown in the table no 5.

Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared was conducted using shimadzu 8300 spectrometer and the spectrum was recorded in the region of 4000-400 cm^{-1}. The procedure consisted of placing a sample powder dispersed in KBr (200-400 mg) and compressed into a disc by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained. The drug and excipients were scanned individually as well as combined from in order to find the drug – excipient interaction[7]. The IR Spectra of Pure drug, Drug-Excipient sample are shown in the fig. 3and 4.

In vitro Drug Release Studies

In vitro drug release of nanosuspensions was determined by using a dialysis tube (doner compartment) containing the known sample of quantity (10 ml) of nanosuspension in a water-jacketed beaker containing 900 ml of phosphate buffer pH 7.4 at 37^0 C for 1 hr. The sample were withdrawn 5ml for 5, 10, 15, 30, 45, 60 min. and replaced with 5 ml volume of fresh phosphatebuffer 7.4. The sample was filtered through whattman filter paper and assayed by measuring the absorbance at 246 nm using the UV-visible spectrophotometer. .

Particle Size determination

Mean particle size and size distribution analysis was carried out by using Malvern zeta sizer Nano- S90.Which follows principle of LAZER light diffraction or also called photon correlation spectroscopy(pcs). It is based on measurement of Brownian motion of particles[9].The prepared nanosuspension of 100 µl was diluted to 5 ml with double distilled water and diluted dispersion was measured by using Malvern Zetasizer[9]. The particle size of best 2 formulation F6 and F9 are shown in the fig.8 and 9.

Zeta potential

The zeta potential of all the batches was measured using zeta sizer Nano series Nano-ZS (Malvern instrument, the sample was diluted 100 times with

distilled water[80]. Zeta potential of best two formulation F7 and F9 are shown in the fig.10 and 11.

RESULT AND DISCUSSION

Preformulation Studies

Organoleptic properties

The organoleptic properties of drug were found like white to offwhite crystalline powder, odorless, bitter taste and crystallinity.

Melting point:

The melting point of drug sample was determined by using melting point apparatus. The melting point was found to be in the range of 158^0- 161^0C, which is found to be similar as given in the reference[80]. The melting point of Atorvastatin Calcium is 159.2-160.7°C .

Partition coefficient

The Log P value of Atorvastatin Calcium was found be 4.2 which show the lipophilic nature of drug.

Determination of λmax by UV Spectroscopic

Characterization:

The λ max of Atorvastatin Calcium was obtained at 246 nm. This found to be similar as given in the reference[81]. Which shows that drug is pure. The UV spectrum of Atorvastatin Calcium is shown in the fig.1.

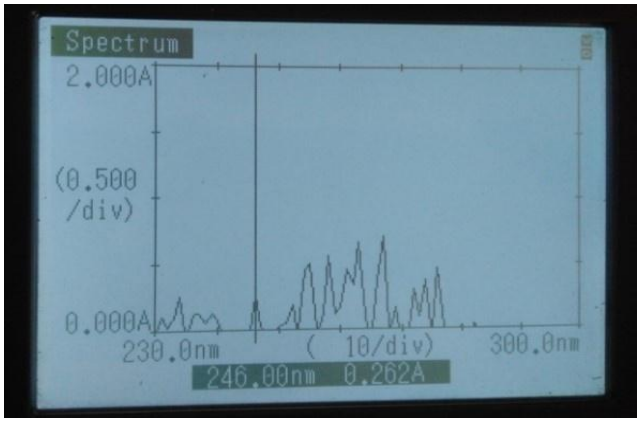

Fig 1 : Spectrum of Atorvastatin Calcium by UV Spectroscopy

Preparation of standard Calibration curve of Atorvastatin Calcium in methanol (λmax 246 nm)

Calibration curve of Atorvastatin Calcium was prepared in methanol at 246 nm. The absorbance values (mean of three determinations) with their standard deviation at different concentration in the range of 5-25 µg/ml for methanol are tabulated. The drug obeys Beer's Lambert law in the concentration range. Linear regression analysis for all calibration curves of atorvastatin calcium is given in Table. So, this equation was used for the calculation of the solubility of the drug in different solvent, drug content and drug release. The calibration curve of Atorvastatin Calcium is shown in fig. 2 .

Table 2: Data of standard calibration curve of Atorvastatin Calcium in Methanol

S.No.	Concentration (µg/ml)	Absorbance (nm)
1.	0	0
2.	5	0.127
3.	10	0.231
4.	15	0.327
5.	20	0.439
6.	25	0.533

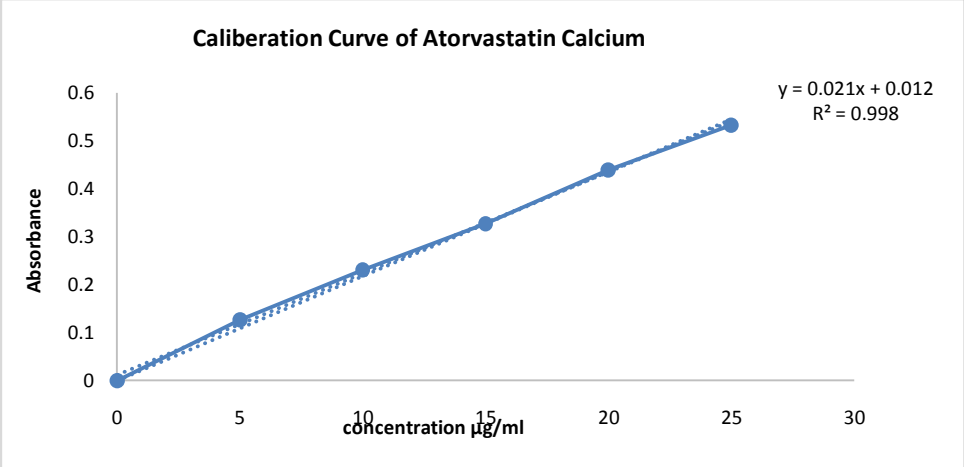

Fig 2: Calibration curve of Atorvastatin Calcium in Methanol

Determination of solubility of Atorvastatin Calcium in various medium :

The solubility of Atorvastatin Calcium in various mediums was studied and the results of study are shown in below table no.3.

Table no 3: Solubility data of Atorvastatin Calcium in different mediums:

S.NO	Solvent	Solubility(mg/ml)	Inference
1.	Water	0.578	Very Slightly soluble
2.	Methanol	30.106	Soluble
3.	Phosphate buffer PH 7.4	0.084	Practically insoluble
4.	PEG 400	0.675	Slightly Soluble
5.	PVP K30	0.723	Slightly Soluble

EVALUATION OF OPTIMIZED NANOSUSPENSIONS

Determination of saturation solubility

Saturation solubility studies were carried out for pure drug, as well as for prepared nanosuspension. From the result of saturation solubility studies it was observed that there was increase in solubility of drug in nanosuspension as compared to pure drug. With increase in the concentration of polymers increased and the nanosuspension containing PVP K 30 and PEG 400 in (F6) and (F9) has increased the solubility up to five times. The saturation solubility of all the formulations are given in the table 4.

Table.4 : Saturation solubility study of various formulation:

S.NO	Formulation Code	Saturation Solubility (mg/ml) at 37^0 C in water	Percentage Solubility Enhancement%
1	Pure Atorvastatin Calcium	0.578	-
2	F1	0.724	14%
3	F2	0.822	24%
4	F3	0.912	33%
5	F4	0.814	23%
6	F5	0.901	32%
7	F6	0.913	33%
8	F7	0.822	24%
9	F8	0.876	29%
10	F9	0.921	34%

Drug Content

The drug content of all the formulation were found to be in the range of 95.2 to 99.2, Which is within the specified limit as per Indian Pharmacopoeia 1996 (i.e. 90-110% w/w). The results are shown in the table 5.

Table 5: Drug content of various formulations.

S.NO.	Formulation Code	% Drug content
1.	F1	95.2
2.	F2	97.2
3.	F3	98.3
4.	F4	95.3
5.	F5	96.8
6.	F6	98.5
7.	F7	99.2
8.	F8	98.5
9.	F9	99.3

FTIR Spectrophotometry:

Drug and excipients compatilibility study is done by the fourier transform infrared (FT-IR) spectra were obtained by using FT-IR spectroscopy.The compatibility studies provide the frame work for drug combination with the excipients in the fabrication of the dosage form The study was carried out to establish that the therapeutically has not undergone any in changes, after it has been subjected to processing steps during formulation of tablets. It showed that there were no change of any characteristic peaks of pure durg Atorvastatin calcium and excipients which confirmed that absence of chemical interaction between pure drug and excipients**.** The above changes in the FTIR spectra of atorvastatin calcium and HPMC K50 are significant.The result shows that there is no interaction between the drug and excipients

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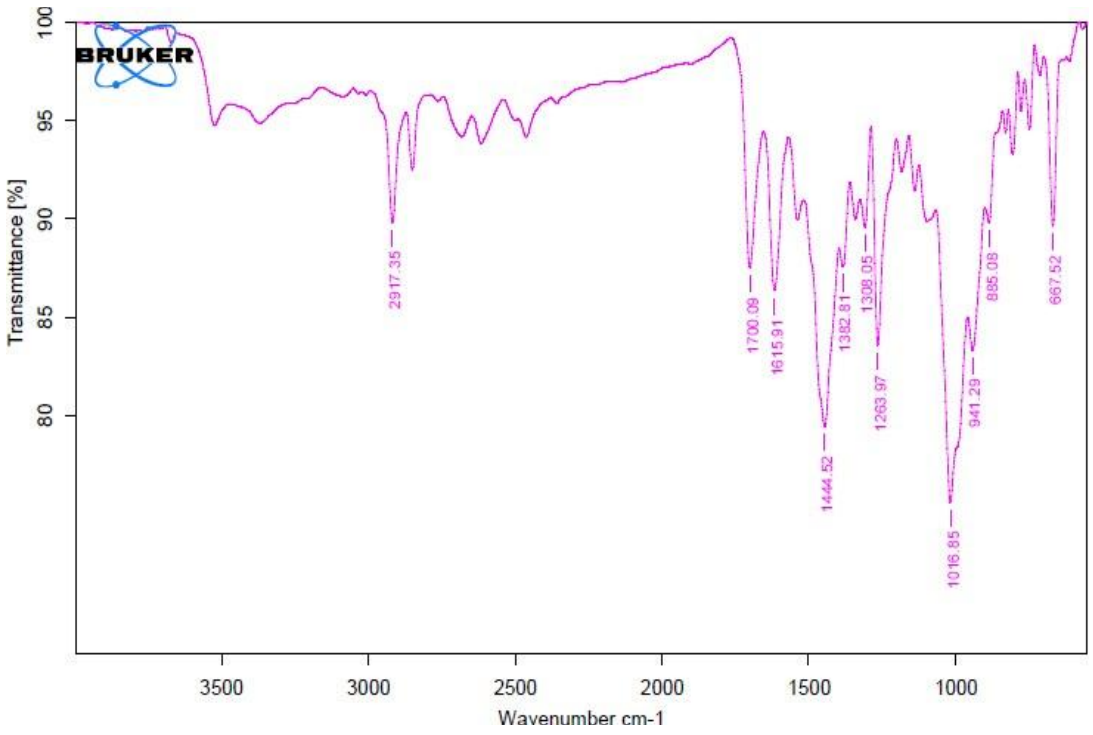


Fig.3: FTIR of Atorvastatin Calcium

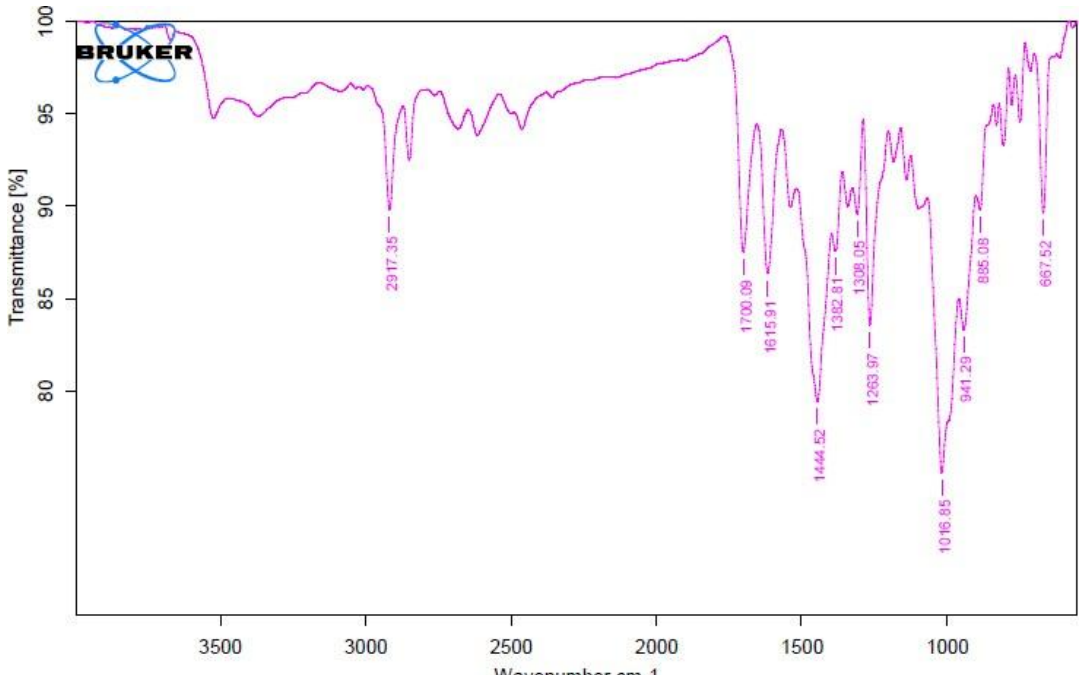


Fig.4 : FTIR of Atorvastatin Calcium with HPMCK15)

In vitro dissolution studies:

The in vitro drug profiles of pure drug Atorvastatin Calcium and nanosuspensions in dissolution medium are shown in figure (5, 6, 7). The formulated nanosuspensions of Atorvastatin calcium showed a significant increase in the dissolution rate as compared with pure Atorvastatin calcium. In the nanosuspension's formulations the drug released from formulation F1 to F4 were found to be 92.2, 93.3, 98.2 and 93.1 %.The drug released from formulation F5 to F9 were found to be 94.3, 99.1, 94.4, 95.1and 98.6 % respectively. All the formulation showed improves drug release rate as compared to pure Atorvastatin Calcium. The in-vitro drug release of all the formulations are given in the table no 6. The release rate of F6 and F9 was found to be higher when compared to other formulations this is due to increase in the concentration of PVP K30 and PEG 400 (Polymers).These results are indicating that has higher drug retarding ability for long duration. The results are shown in the table 6.

Table.6: In-Vitro drug dissolution rate:

S.No	Time (min)	Drug	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	5	6.2	18.71	18.95	19.22	19.16	19.30	20.69	19.20	19.36	20.4
2	10	9.07	37.2	39.5	37.0	39.4	38.6	42.7	38.5	39.4	40.5
3	15	15.1	49.7	45.5	48.6	48.4	43.5	55.5	46.4	49.4	54.5
4	30	26.8	55.7	56.2	58.2	59.5	62.2	67.0	59.5	63.5	68.2
5	45	36.19	74.5	84.2	83.5	86.9	85.1	88.2	89.9	84.9	84
6	60	48.11	92.2	93.3	98.2	93.1	94.3	99.1	94.4	95.1	98.6

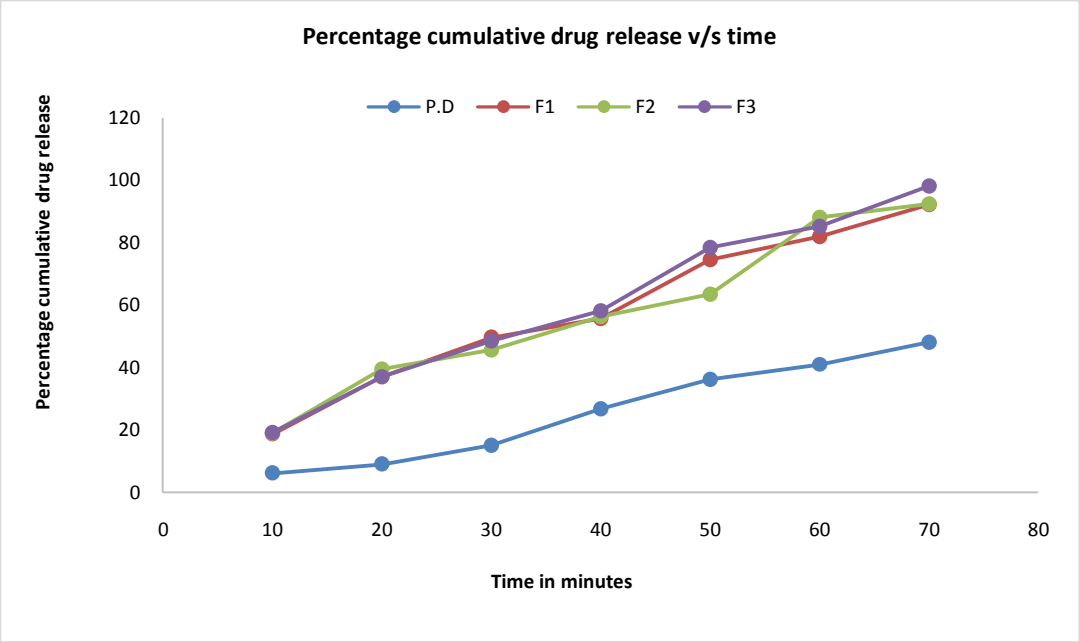


Fig.5 : Comparison of In- vitro release of pure drug & F1,F2 and F3 Batches

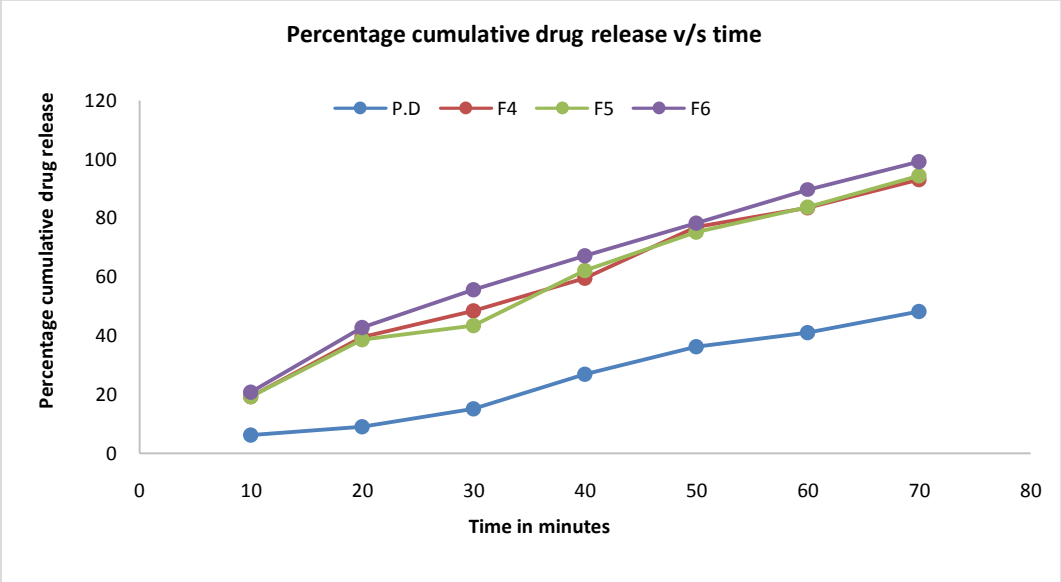


Fig.6 : Comparison of In- vitro release of pure drug & F4, F5 and F6 Batches

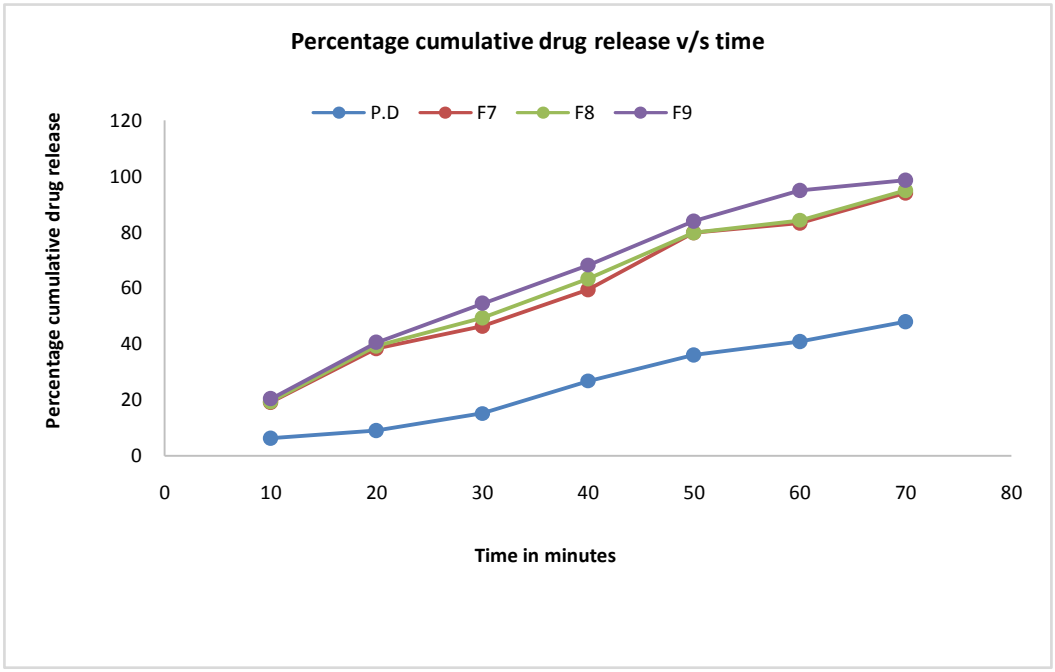
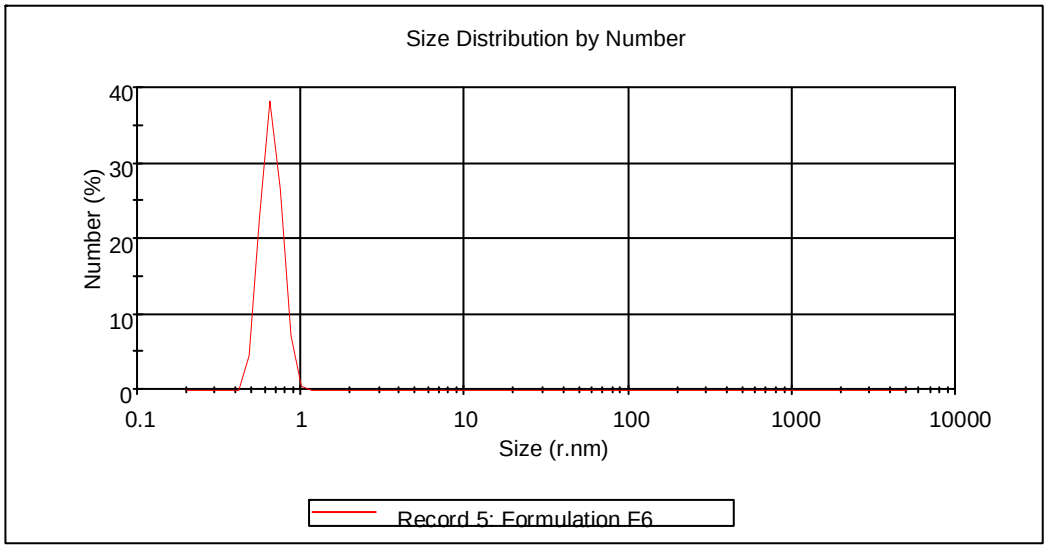


Fig.7 : Comparison of In- vitro release of pure drug & F7, F8 and F9 Batches

Particle Size

Particle size determination is the most important evaluation test to check the dispersibility and homogeneity of nanosuspension. The batch F6 and F9 had particle size 241.2 and 489.1. Therefore can be considered as physically stable nanosuspension as given in the refrence[82]. The particle size of F6 and F9 batches are shown in fig. 8 and 9.



Fi g 8 : particle size of F6 batch

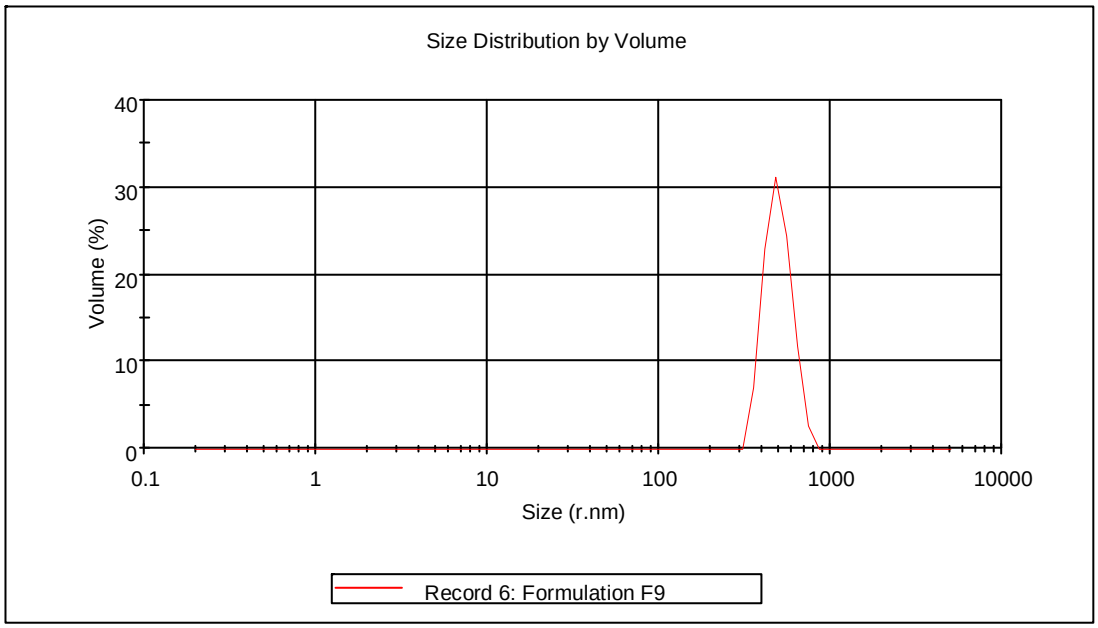


Fig 9 : particle size of F9batch

Zeta Potential

The zeta potential of optimized formulation batch F6 and F9 was found to be -11.56 and -10.18 indicating good stablilty of formulation as given in the refrence[8]. The zeta potential of F6 and F9 are shown in fig 10and 11 .

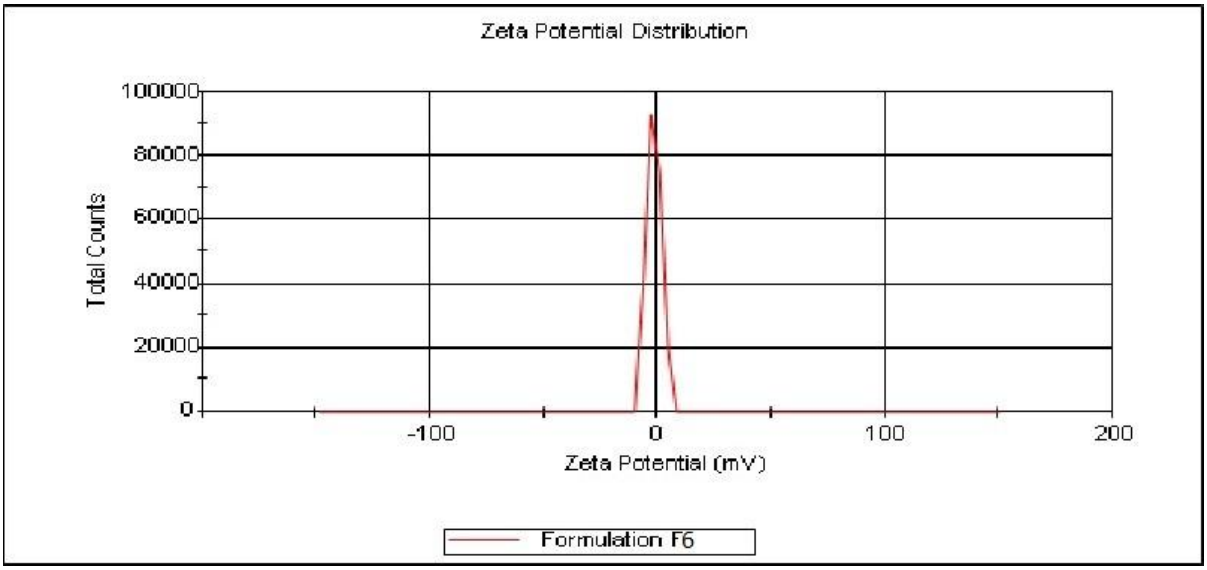


Fig 10 : Zeta potential of F6 Batch

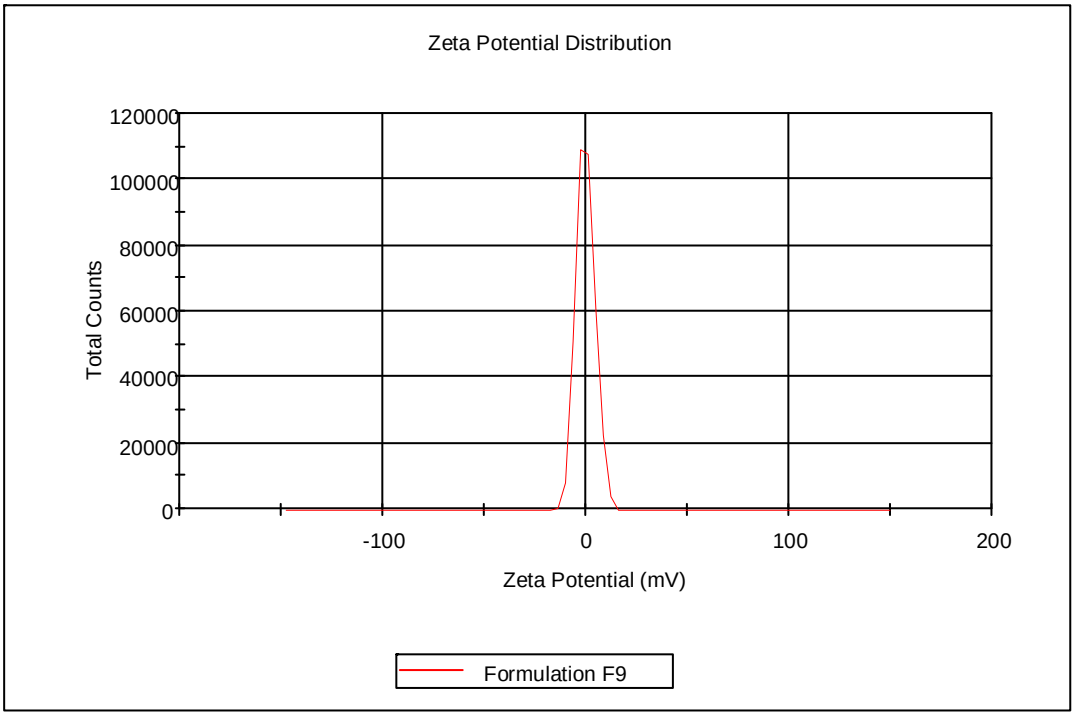


Fig. 11: Zeta potential of F9 Batch

CONCLUSION

It can be concluded that by the use of the designed experimental technique solubility of poorly soluble drug can be improved by using novel nanosuspension formulation. This technique is effective for the formulation of BCS Class II and BCS Class IV drug which are poorly soluble drugs. This method is simple and effective and can be used on industrial scale. This is an economical technique and very effective tool for enhancement of dissolution rate of poorly soluble drugs. It is a very successful and simple method to enhance the aqueous solubility and dissolution rate. Nature of stabilizers used played important role in enhancement of dissolution rate. The use of
different stabilizers in the formulation of nanosuspension at different ratios cause increased wettability of water insoluble drug Atorvastatin calcium. Drug release data reveals due to nanosuspension formulation there is increase in drug release profile and enhance solubility compare to the pure drug

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.REFERENCES

1. Liversidge GG and Conzentino P, 1995, Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats, International Journal of Pharmaceutics, 125(2), 309–313, ISSN: 0378-5173.
2. Muller RH, Jacobs C and Kayer O (2000) Nanosuspensions for the formulation of poorly soluble drugs. In: F Nielloud, G Marti- Mestres (ed). Pharmaceutical emulsion and suspension, Marcel Dekker, New York, pp. 383-407.
3. Müller RH and Peters K, 1998, Nanosuspensions for the formulation of poorly soluble drug I: Preparation by size reduction technique, International Journal of Pharmaceutics, 160(2), 229-237, ISSN: 0378-5173.
4. Dhiman S and Thakur GS, 2011, Nanosuspension: A recent approach for nano drug delivery system, International Journal of Current Pharmaceutical Research, 3(4), 96101, ISSN: 0975-7066.
5. Chandra A, Soni RK, et al, 2013, Nanosuspension: an Overview, Journal of Drug Delivery and Therapeutics, 3(6), 162-167, ISSN: 2250-1177.
6. Mura P, Faucci MT, and Bettinetti GP, 2001, The influence of polyvinylpyrrolidone on naproxen complexation with hydroxyl propyl-β-cyclodextrin, European Journal of Pharmaceutical Sciences, 13(2), 187-194, ISSN: 0928-0987.
7. Mura P, Faucci MT, and Bettinetti GP, 2001, The influence of polyvinylpyrrolidone on naproxen complexation with hydroxyl propyl-β-

cyclodextrin, European Journal of Pharmaceutical Sciences, 13(2), 187-194, ISSN: 0928-0987.

8. Otsuka M and MatsudaY, 1995, Effect of co-grinding with various kinds of surfactants on the dissolution behavior of phenytoin, Journal of Pharmaceutical Sciences, 84(12), 1434-1437, ISSN: 1520-6017.
9. Mura P, Faucci MT, and Bettinetti GP, 2001**,** the influence of polyvinylpyrrolidone on naproxen complexation with hydroxyl propyl-β-cyclodextrin, European Journal of Pharmaceutical Sciences, 13(2), 187-194, ISSN: 0928-0987.