e-ISSN: 2248-9126
Vol 12|Issue 1| 2022|1-6. Print ISSN: 2248-9118

Indian Journal of Pharmaceutical Science & Research

www.ijpsrjournal.com

DESIGN OF NANOSUSPENSIONS OF NATEGLINIDE USING POLOXAMER AND INVITRO DRUG RELEASE

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ABSTRACT

Nano suspensions are colloidal dispersions containing nanoparticles of drug that have been stabilised with surfactants and are used in the manufacture of nanomedicine. Additionally referred to as biphasic systems, they consist of pure drug particles dispersed in an aqueous medium with a diameter of less than 1 micron and a suspended particle with a diameter of less than 1 micron. The majority of drugs used today are lipophilic, and many are insoluble in water owing to their functional groups, particle size, chemical composition, and other variables, notably anti-diabetic pharmaceuticals such as Nateglinide. Thus, the purpose of this work is to create nano-suspensions of nateglinide utilising poloxamer as a polymer and to evaluate their increased bioavailability. Physical criteria such as drug entrapment efficiency, drug content, yield, surface morphology, and in vitro drug release studies were tested on the produced formulations. In vitro testing in a PH 1.2 phosphate buffer revealed that the nanosuspension formulation provided more drug release than the pure drug. As a result, nanosuspensions may represent a viable alternative to traditional delivery systems for medications with low water solubility, with the potential to enhance their biopharmaceutical performance.

Keywords: Nanosuspensions, Nateglinide, Bioavailability, Drug Release.

INTRODUCTION

One of the most persistent problems faced by drugs with poor aqueous solubility is that their oral delivery is frequently associated with implications of low bioavailability and lack of dose proportionality. Efforts are going on to enhance the oral bioavailability of such lipophilic drugs in order to increase their clinical efficacy. Oral bioavailability of drugs is affected by a variety of factors, which influence their absorption from gastrointestinal tract. One determinant factor for absorption is drug dissolution, which is influenced by solubility of drug in GI fluids. Variety of methods has been developed over the years to improve the release and dissolution of such drugs [1].

Nano suspensions are colloidal dispersions containing nano-sized drug particles stabilised by surfactants that are used in the production of nanomedicine. Also known as biphasic systems, they are comprised of pure drug particles distributed in an aqueous medium with a diameter of less than 1 micron, and the diameter of the suspended particle is less than 1 micron in size. Nano suspensions can be used to improve the solubility of medications that are poorly soluble in both aqueous and lipid environments, according to the National Institutes of Health [2].

Most of the medications given today are lipophilic, and many of them are poorly soluble in water due to their functional groups, particle size, chemical nature, and other factors, particularly when it comes to anti-diabetic treatments such as Nateglinide [web 1], which are poorly soluble in water. Because of this drug's low water solubility, its bioavailability and effectiveness are severely restricted. It has now been discovered that decreasing the particle size of any medicine increases its solubility, which in turn increases its bioavailability. Many other strategies, such as nano suspension, micronization, surfactants, complexation, and so on, have been used to accomplish this. For this reason, nano suspension technology is taken into consideration for the procedure. The use of nano suspensions was used to increase the solubility of Nateglinide, which in turn increased the rate of dissolution and absorption. So the aim of the current work is to prepare the Nano suspensions of Nateglinide using poloxamer as polymer and evaluation of enhanced bioavailability.

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MATERIALS AND METHODS

Drug Solubility

Drug solubility studies were performed in triplicate by adding excess amounts of Nateglinide to water and buffer solutions having different pH (1.2, 4.5, and 7.2) buffers. The solutions containing flasks were kept on a rotary shaker for 24 h. After 24 h, solutions were analyzed using UV spectrophotometer at 247 nm, which was the absorption maxima determined earlier and drug concentrations were calculated.

Compatibility Studies

The Fourier transform infrared analysis was conducted to verify the possibility of interaction of chemical bonds between drug and polymer. The FTIR spectrum was 93 performed by using a PerkinElmer 1600 spectrophotometer with a resolution of 2 cm^{-1}. The samples were scanned in the spectral region between 4000 and 400 cm^{-1} by taking an average of 8 scans per sample. Solid powder samples were oven dried at around 300C, finely crushed, mixed with potassium bromide (1:10 ratio by weight) and pressed at 15000 psig (using a Carver Laboratory Press, Model C, Fred S. carver Inc., WIS 53051) to make disc. The detector was purged carefully by clean dry nitrogen gas to increase the signal level and reduce moisture. For the analysis of the data, the spectrum GX series model software was used.

Preparation of Nateglinide Nanosuspension by using nano-precipitation method [3]

Nanosuspension was prepared by the solvent evaporation technique. Nateglinide was dissolved in a methanol (6 ml) at room temperature. This was poured into 20 ml water containing different amounts of Ploxamer F-68 maintained at a temperature of 30–40°C and subsequently stirred at ranging agitation speed for 1 hr to allow the volatile solvent to evaporate (Remi, High speed stirrer, India.). Addition of organic solvents by means of a syringe positioned with the needle directly into surfactant containing water. Organic solvents were left to evaporate under a slow magnetic stirring of the nanosuspension, at room temperature for 2 hours.

Evaluation of Nateglinide Nanosuspension

Scanning Electron Microscopy

In order to examine the particle surface morphology and shape, Scanning Electron Microscopy (SEM) was used. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in an evaporator with a gold layer 20 nm thick. Photographs were taken using a JSM-5200 Scanning Electron Microscope (Tokyo, Japan) operated at 10 kV.

Determination of Nanosuspension Process Yield [5]

The nanosuspension production yield was calculated by gravimetry. Fixed volumes of nanoparticles suspension were centrifuged (16,000×g, 30 min, 15ºC) and sediments were dried.

The percentage process yield (% P.Y.) was calculated as follows:

$$\% \text{ P.Y.} = \frac{\text{Nanoparticles weight}}{\text{Total solids weight}} \times 100$$

Determination of % Entrapment Efficiency [6]

The Nanosuspension with known amount of drug (10mg/20ml) incorporated was centrifuged at 5000 rpm for 15 minutes. The supernatant solution was separated. 5ml of supernatant was distributed with 100 ml of 2% w/v tween 80 solutions and the absorbance was measured using UV spectrophotometer at 247 nm using 2% w/v tween 80 as blank. The amount of drug unentrapped in the supernatant was calculated. The amount of drug entrapped and percentage entrapment was determined from drug unentrapped. Standard deviation was determined for 3 trials.

$$\text{Loading efficiency} = \frac{\textit{Total amount of drug} - \textit{Amount of unbound drug}}{\textit{Nanoparticles weight}} \times 100$$

In vitro drug release study [7, 8]

A 10 mL portion of the nanosuspension containing drug, sufficient for establishing sink conditions for the assay was placed into the donor compartment. The receptor compartment contained 20 mL of 0.2M Phosphate buffer solution of pH 7.4 maintained at 37°C under mild agitation using a magnetic stirrer. At specific time intervals, aliquots of 1mL were withdrawn and immediately restored with the same volume of fresh phosphate buffer. The amount of drug released was assessed by measuring the absorbance at 247 nm using a single beam UV spectrophotometer (Genesis 10 UV, Thermo electron Corporation, USA).

RESULTS AND DISCUSSION

Nateglinide nanosuspension was formulated using different drug polymer ratios, the composition of which was shown in Table 1. The formulations were evaluated for process yield, surface morphology, particle size, drug entrapment, zeta potential, in vitro drug release and release kinetic data.

FTIR studies

FTIR spectrum of Nateglinide was characterized with various peaks corresponding to various bonds like 1636.84 cm^{-1} for C=O stretching, 2931.53cm^{-1} for C—H stretching, 1221.13 cm^{-1} for --CH_3, 3313.87 cm^{-1} for N—H stretching. Similarly corresponding peaks for the polymer,

Polymer had been obtained and infers as 1109.52 cm^{-1} for C=O stretching, 2883.56 cm^{-1} for C—H stretching and 1339.81 cm^{-1} for O—H stretching. The peaks that correspond to C=O at 1641.21 of the drug had been shifted to 1625.17 $cm^{-1}$and $–CH_3$ at 1214.38 cm^{-1} had been shifted to 1219.07 cm^{-1} indicating that there are strong bonds between drug and polymer but there was no other distinctive new peaks seen indicating that there is no chemical interaction between them.

Percentage drug content, Drug entrapment efficiency and percentage yield

In nanosuspension formulation the drug particles were reduced to nano sized. During the formulation process there was not any drug loss step involved, so theoretically the formulation was considered as being 100% drug content. The Percentage drug content, drug entrapment efficiency and percentage yield of all the formulations were calculated and the results were tabulated in table (2). Of all the formulations, formulation F4 gave the highest percentage drug content with 99.43% and least percentage content was found in F6 that is 98.6%. But the pure drug suspension gave the yield of 99.93% which can be considered as an assay of Nateglinide.

The drug entrapment efficiency of NNF4 was high when compared to other formulations. This may be due to the presence of optimum polymer and optimum tween 80 concentrations, comparing the formulations NNF1, NNF2, NNF3 it is clear that increase in polymer concentration increased the drug entrapment efficiency. Interestingly it is not similar in case of NNF4, NNF5, and NNF6. This might be due to reason that drug might have got captured into the polymer and the tween making the drug molecules to lower particle size and is ionized in water. Considering the formulations NNF1, NNF2, NNF3 tween80 is in minimum concentrations and the drug cannot be reduced to lower particle size and high polymer ratios causing the capture of drug molecules.

The percentage yield of formulation NNF4 leads the race with 78.5% followed by NNF5, NNF3. This indicates that NNF4 can be considered as best formulation, where the polymer concentration is optimum and tween concentration is to sufficient limit. Lower the tween concentration lowers the yield.

SEM analysis

SEM micrographs clearly showed great differences between pure Nateglinide (Fig. 2) and optimized nanosuspension formulation The particles of Nateglinide were found to be large and especially irregular. However after formulation, particles disappeared and drug became small and uniform. This might be due to the surfactant which was used to stabilize the drug particles could be adsorbed to crystal surface by hydrophobic interaction. So we can say the method adopted to enhance the solubility is appropriate.

Invitro drug release studies

By plotting various graphical models the in vitro drug release profile of the prepared Nateglinide nanosuspension were studied. The release data obtained for formulations NNF1, NNF2, NNF3, NNF4, NNF5 and NNF6 shows plots of percent drug released as a function of time for all formulations. It was apparent that in vitro release of Nateglinide showed a very rapid initial burst, and then followed by a very slow drug release. An initial, fast release suggests that some drug was localized on the surface of the nanoparticles. NNF4 was showing good release compared to other formulations and it was considered as best formulation.

In order to describe the release kinetics of all six formulations the corresponding dissolution data were fitted in various kinetic release models like zero order, first order, Peppas and Higuchi respectively. These values were compared with each other for model and drug equation. As indicated by higher R^2 values, the drug release from all formulations follows Peppas release and Higuchi model. Since it was confirmed as Peppas model, the release mechanism was anomalous diffusion. The diffusion exponent (n) values for all batches were within 0.5 which indicated that drug release mechanism followed pure Fickian diffusion. The Peppas model is widely used to confirm whether the release mechanism is Fickian diffusion, non-Fickian diffusion or zero order. ‘n’ value could be used to characterize different release mechanisms.

TABEL 1: Formulation of Nateglinide nanosuspension

Ingredients	NNF1	NNF2	NNF3	NNF4	NNF5	NNF6
Nateglinide (mg)	10	10	10	10	10	10
Methanol (ml)	8	8	8	8	8	8
poloxamer (%w/v)	0.25	0.5	0.75	0.25	0.5	0.75
Tween 80 (ml)	1	1	1	2	2	2
Distilled water (ml)	20	20	20	20	20	20

Table: 2. Percentage drug content, Drug entrapment efficiency and percentage yield of Nanosuspension

Formulation batches	Percentage drug content (%)	Entrapment efficiency	Percentage yield
NNF1	99.49±0.54	64.25±2.81	53.61±2.81
NNF2	98.67±0.91	67.91±4.55	64.81±2.16
NNF3	99.16±0.46	69.81±4.58	71.46±2.08
NNF4	99.59±0.27	86.19±3.14	79.25±3.64
NNF5	98.83±0.76	80.19±4.08	73.18±2.47
NNF6	98.59±0.56	75.47±2.56	71.46±2.88

Table: 3. Drug release from the prepared nanosuspensions

Time (min)	% drug release (Mean± S.D)					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
5	21.38±0.74	23.96±5.47	18.57±2.37	27.84±3.96	24.92±3.17	21.24±3.17
15	34.42±0.69	37.16±5.16	31.08±3.87	38.17±3.28	37.45±3.07	30.16±2.06
30	68.96±0.28	54.61±4.11	54.66±3.46	59.82±2.47	55.63±2.47	47.91±2.44
60	82.38±3.65	61.85±4.42	64.52±2.63	78.17±1.76	74.81±3.81	68.27±2.56
90	95.28±6.09	76.28±3.85	78.16±3.24	86.91±1.99	81.77±4.18	81.22±1.97
120	96.29±6.18	79.46±3.74	79.61±2.84	92.78±2.49	85.80±4.69	85.06±2.75

Table: 4. Results of Model Fitting of Nateglinide Nanosuspension

Formulation	Zero order	First order	Higuchi	Peppas	'n' values
NNF1	0.8429	0.8382	0.8896	0.9287	0.3614
NNF2	0.8479	0.9986	0.9962	0.9997	0.4589
NNF3	0.8374	0.9740	0.9893	0.9895	0.5061
NNF4	0.8242	0.9815	0.9929	0.9963	0.4305
NNF5	0.8257	0.9964	0.9929	0.9982	0.4506
NNF6	0.8847	0.9985	0.9979	0.9967	0.4874

Fig: 1. FTIR Spectrum of a. Nateglinide, b. Polymer, c. Formulation

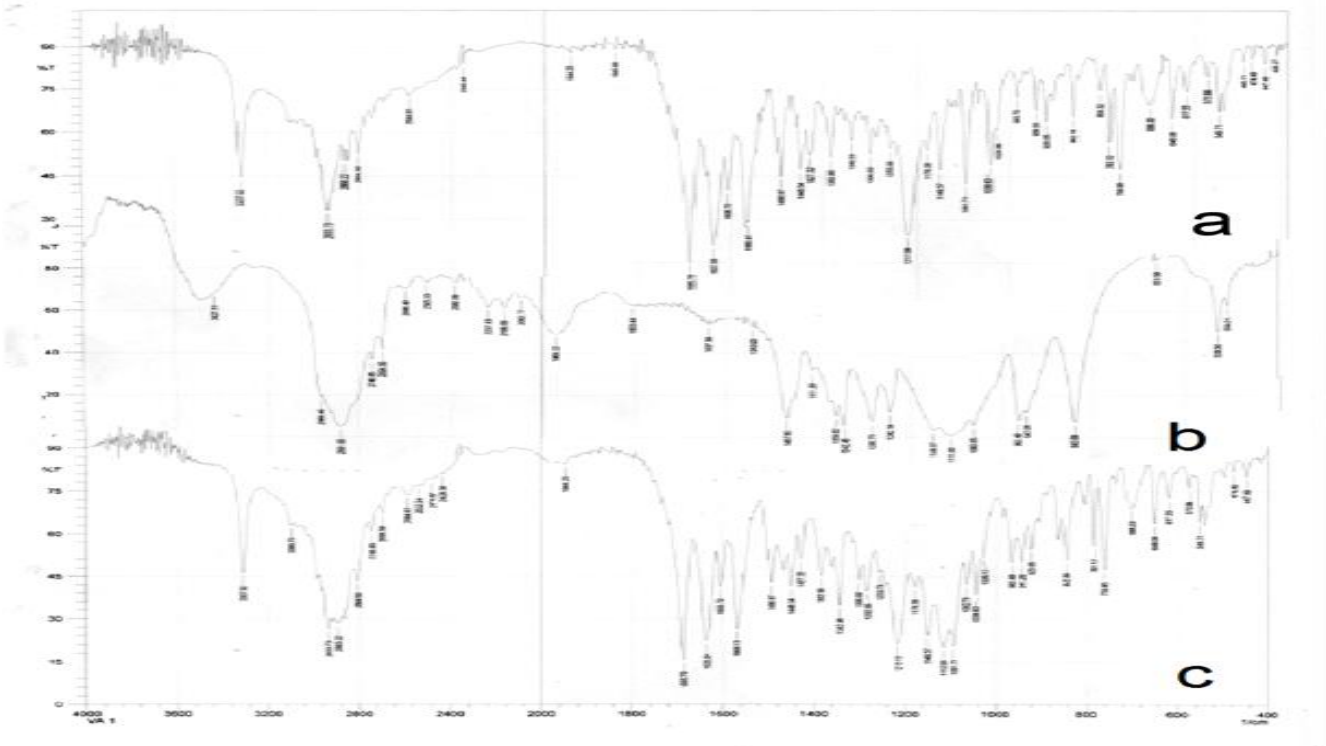
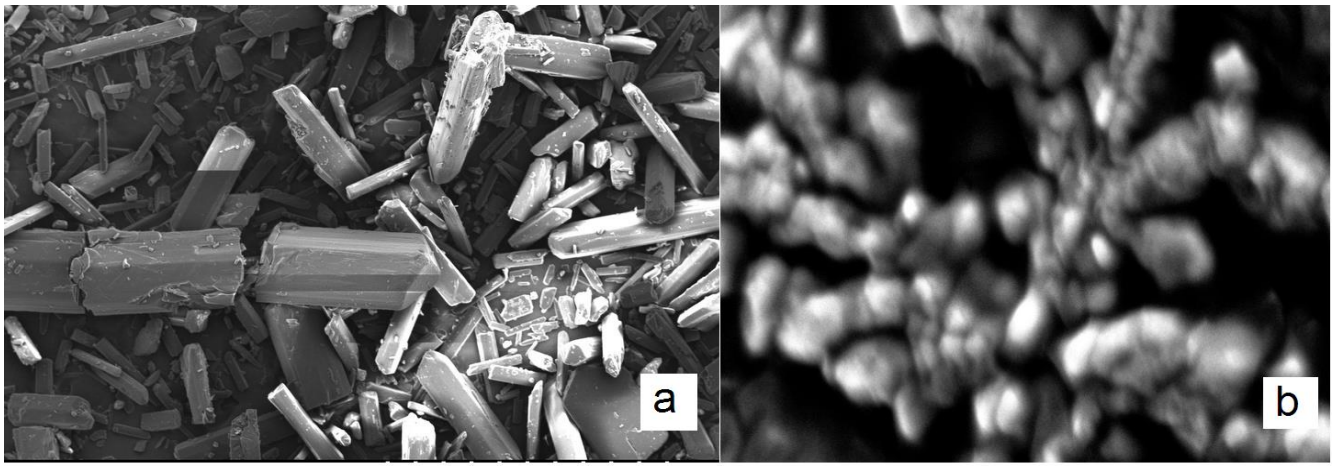


Fig: 2. SEM photographs a. pure drug; b. Nanosuspensions



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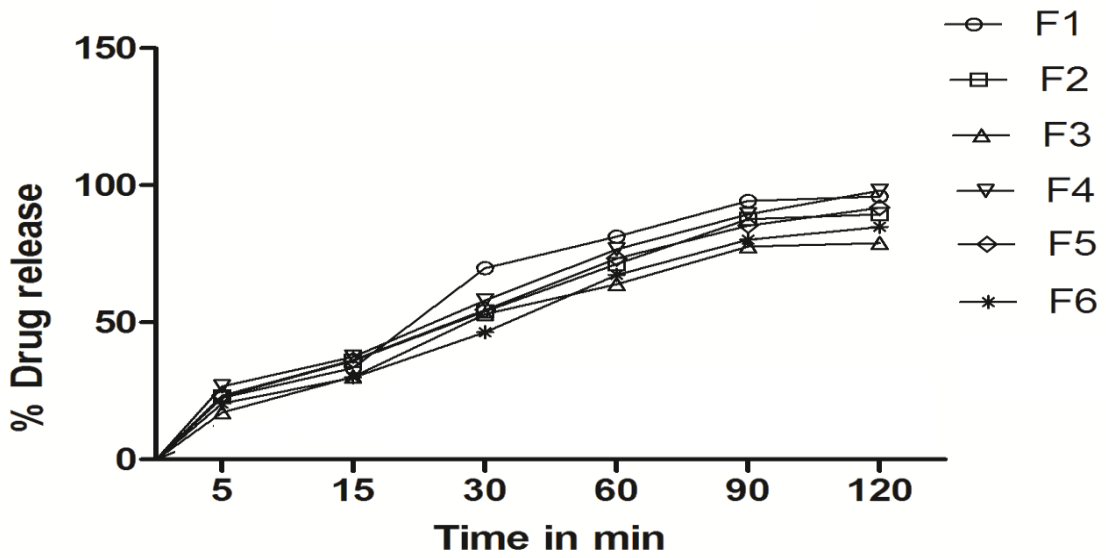

CONCLUSION

Nanosuspensions constitute a possible alternative to conventional delivery strategies for medicines with limited water solubility, with the potential to increase their biopharmaceutical efficacy. This analysis provides a foundation for future research aimed at determining the biological profiles of drugs in blood serum, as well as their bioavailability and bioequivalence in vivo. With the use of the nanosuspension approach, the solubility of other medications may be improved, which is the goal of this research. It was decided that the procedure used to increase the solubility of Nateglinide was effective and gave a favourable outcome throughout the course of the investigation.

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