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Review Article

FORMULATION AND INVITRO EVALUATION OF NANO SUSPENSIONS OF NATEGLINIDE USING POLOXOMER AS POLYMER

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ABSTRACT

Several pharmaceuticals are lipophilic today, and many of them are soluble in water only in small amounts, especially anti-diabetic pharmaceuticals like Nateglinide. As a result of the low water solubility of this medicine, its bioavailability and effectiveness are significantly reduced. A new class of oral hypoglycemic drugs is being developed to control blood glucose fluctuations following meals. Even though it doesn't work like sulfonylureas, a lot of metabolic processing occurs during its first passage through the body, limiting its bioavailability. Drug particles are dispersed in colloidal dispersion with surfactants to make nanosuspensions. Low soluble and high permeable chemicals present significant challenges to formulators. In our study, we examined the spectra of medication, pure polymers, and nanosuspension formulations for their biological properties. There was no significant interaction between the medication and polymer. In vitro drug release, penetration efficiency, amount of drug, surface morphology, and yield were examined in the formulations. It was found that drugs are distinctive, round, and smooth in nanosuspensions. A very rapid release of Nateglinide was observed in vitro, followed by a very slow release of the drug during a later period of time. When the nanoparticle is first released, some of the drug remains on the surface of the nanoparticle in a rapid first release situation. When compared to other formulations, NNF4 is found to have the best performance regarding the release of a drug than those other formulations. There is a clear preference for Peppa's and Higuchi's models regarding drug release, while R2 values tend to indicate a greater release rate in all formulations. We found the 'n' value to be less than 0.50 for all formulations. This indicates a Fickian diffusion mechanism is likely to be involved.

Keywords Nateglinide, Nanosuspension, Fickian Diffusion, Oral Hypoglycemic Drugs.

INTRODUCTION

Capillary basement membrane thickening is associated with hyperglycemia and negative nitrogen balance. It occurs when vessels wall matrix is abnormal and cells proliferate, causing ketoacidosis [1]. Atherosclerosis and glomerular capillary sclerosis can also result as well as retinopathy, neuropathy, and peripheral arterial insufficiency [2,3]. Ketosis is more prevalent in patients with type I diabetes who have low insulin levels. This type is less common and has low genetic predisposition [4]. 10% of diabetics have type II diabetes. Peripheral tissues become less sensitive to insulin due to reduction in insulin receptors [5]. Insulin resistance is common in hypertensive patients [6,7], but hypoglycemia does not occur. Blood sugar levels are effectively lowered with these oral drugs. It became possible to exploit this advance by developing a sulfonylurea tolbutamide [8]. It was soon followed by many others. Meglitinides, -glucosidase inhibitors, and drugs targeting thiazolidinedione acidosis have been introduced since then.

When repaglinide is taken, insulin is released rapidly and for a short time. Short-acting medications

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may cause less hypoglycemia since they are shorter acting [9]. There is a connection between dyspepsia, arthralgia, and weight gain with mild headaches.

The hypoglycaemic effects of natalieglinide are more rapid and shorter than those of Repaglinide because it stimulates insulin secretion in the first phase [10]. When combined with other antidiabetics, it helps those with type II diabetes reduce blood sugar spikes after eating. In the gastrointestinal tract, many factors impact drug absorption [11], which affects bioavailability. Examples: drug solubility in GI fluids, dissolution rate, permeation of the drug etc.,

Surfactant-stabilized colloidal dispersions are used to make nanomedicine. Both aqueous and lipid environments are incompatible with some medications [12]. Nano suspensions increase their solubility. It may be useful to formulate nano suspensions when a molecule has poor solubility or poor permeability in contrast to solid-lipidic nanoparticles (SLN) [13]. Molecular carriers such as nano suspensions may be effective for soluble or poorly permeable molecules. Known for their unique dispersibility, nanosuspensions are administered orally, topically, parenterally, or by inhalation [14]. The average particle size in nano suspensions is between 200 and 600 nanometers, with solid particles ranging from 200 to 600 nanometers. Nano suspensions have many advantages over conventional delivery methods [15].

Due to the crystallization of the medication and the smaller particle size, nanosuspension technology increases dissolving rate and bioavailability [16]. Oswald ripening cannot occur in nanosuspensions due to large particle size differences, preventing different saturation solubilities and concentration gradients. A dense concentration of molecules diffuses to a lower concentration area. This causes the particles to crystallize due to large particles surrounding them and a saturated environment.

Manufacturing process types [17]

1. Precipitation process
2. Hyperbaric homogenization
3. Superfluid technique
4. Micromilling
5. Grinding in dry conditions

Methodology

Procurement of active pharmaceutical ingredient and excipients

Nateglinide were procured as gift sample from Corporation of India Limited, Mumbai. The polymer (Poloxamer 188) were purchased from Pharma (Azing).

Preformulation study

To develop a stable, safe, and effective dosage form, new therapeutic molecules are studied individually and in combination with excipients during preformulation.

1. Solubility profile

To evaluate drug solubility, excess nateglinide was added in triplicate to water and buffer solutions of varying pH (1.2, 4.5, and 7.2). A rotary shaker was used to stir the mixture for 24 hours. A UV spectrophotometer at 247 nm was used to analyze the solutions after 24 hours, determine the absorption maxima, and estimate medication concentrations.

2. Compatibility profile by FTIR

In order to create the FTIR spectrum, a PerkinElmer 1600 spectrophotometer was used. A total of 8 scans were performed on each sample in the spectral range of 4000 to 400 cm^{-1}. We purged the detector with dry nitrogen gas to boost the signal and eliminate moisture [15]. Analysis of the data was carried out using the spectrum GX series model software. Many drugs (200-400 nm) absorb UV light because they are aromatic or contain double bonds.

Stock solution preparation

Methanol was dissolved in 100 mL of 100 mg Nateglinide on an electronic scale. Stock solution-I was diluted with methanol to 100 ml using 10ml of stock solution-I. Dilution was needed to reach 20g/ml. Using a UV scanner between 200-400 nm, the solution was scanned. Pure drug is taken at its maximum value from the graph [11].

Standard Plot (0.5N HCl)

0.5 N HCl buffer solution was used to make a 100 g/ml stock solution by weighing and diluting 10 mg of Nateglinide. A series of 50 ml volumetric flasks were filled with aliquots of 2.5 ml, 5 ml, 7.5 ml, 10 ml, and 12.5 ml of this stock solution to provide a concentration that ranged from 5 to 25 grams. As a blank, the absorbance at 247nm of the resultant solution was measured using a UV spectrophotometer. The standard curve is plotted as absorbance vs. concentration.

Phosphate buffer (pH- 1.2 & 7.2)

A stock solution of 100 g/ml was created by accurately weighing 10 mg of Nateglinide and diluting it in 100 ml of buffered 1.2 & 7.2 pH solution separately. As a blank, we measured the absorbance of the resultant solution at 247nm using a UV spectrophotometer. In order to obtain the standard curve, absorbance was plotted against concentration in g/ml [11].

Formulation of Nanosuspension

We made the nanosuspension using solvent evaporation. 6 mL of methanol was added to nateglinide

at room temperature. To evaporate the volatile solvent, it was placed in 20 ml water with various concentrations of Ploxamer F-68 and mixed for 1 hour at various agitation speeds (Remi, High speed stirrer, India). Water containing surfactant is added directly from a syringe containing organic solvents. Nanosuspension was magnetically stirred slowly for 2 hours while organic solvents evaporated at room temperature [18].

Evaluation of Nateglinide Nanosuspension

1. Scanning Electron Microscopy

We evaluated particle surface morphology and shape using scanning electron microscopy (SEM). Vacuum drying was done using a concentrated aqueous suspension. Shade was provided in an evaporator by a 20-nanometer thick gold layer. The photos were taken using a 10KV scanning electron microscope (JSM-5200, Tokyo, Japan).

2. Determination of % entrapment efficiency

An amount of medication (10mg/20ml) was added to the Nanosuspension and centrifuged for 15 minutes. In addition to a blank solution of 2 percent w/v tween 80 solution, 5ml of supernatant was mixed with 100ml of 2 percent w/v tween 80 solution for measuring absorbance at 247 nm. It was calculated how much medication was not trapped in the supernatant. We calculated the percentage entrapment and the amount of drug entrapped based on the amount of drug unentrapped. Three trials were analyzed to calculate the standard deviation.

3. *In vitro* drug release profile

The nanosuspension contained medication in a 10 mL quantity, which provides sink conditions for the experiment. 0.2M Phosphate buffer solution with a pH of 7.4 was kept at 37°C in the receptor compartment using a magnetic stirrer. A 1mL aliquot was removed and replaced with fresh phosphate buffer at predetermined intervals. Using a Thermo Electron Corporation, USA, Genesis 10 UV spectrophotometer, the amount of medication released at 247 nm was measured.

RESULTS AND DISCUSSION

Preformulation study

Solubility profile

In the table below, the results of testing pure medication's solubility are shown

Standard curve and absorption maxima determination

To generate a calibration curve for Nateglinide, the UV absorption spectrum has a peak at 247 nm. Table 3 summarizes the results of the Nateglinide standard plot. Conventional plots are shown in figure 1

FTIR COMPATIBILITY STUDY

The FTIR spectra of Nateglinide and Polymer exhibited several peaks related to different bonds. For instance, Nateglinide showed peaks at 1737.84 cm-1 for C=O stretching, 2931.53 cm^{-1} for C—H stretching, 1221.13 cm^{-1} for —CH3 stretching, and 3313.87 cm^{-1} for N—H stretching. Polymer also displayed similar peaks, such as 1109.52 cm^{-1} for C=O stretching, 2883.57 cm^{-1} for C—H stretching, and 1339.81 cm^{-1} for O—H stretching. However, the C=O stretching peak of the drug at 1741.21 cm^{-1} was shifted to 1725.17 cm^{-1}, and the —CH3 peak at 1214.38 cm^{-1} was shifted to 1219.07 cm^{-1}, indicating a strong bond between the drug and polymer. Nonetheless, no new peaks were detected, indicating that there was no chemical interaction between the drug and polymer.

EVALUATION OF NANOSUSPENSION

Drug Entrapment, % Yield And % Of Drug Content

Nano-sized drug particles were used in nanosuspension formulations. Formulation process did not result in drug loss, so formulation can theoretically be considered 100% drug-free. We calculated the percentage drug content, drug entrapment efficiency, and percentage yield of each formulation, and presented the results in a table. Drug content for Formulation F4 was 99.43 percent, while drug content for Formulation F7 was 98.7%. Nateglinide assays can be performed with a pure drug suspension of 99.93%.

Compared to other formulations, NNF4 exhibited a high drug entrapment efficiency. The drug entrapment efficiency improved with an increase in polymer concentration for formulations NNF1, NNF2, and NNF3. However, NNF4, NNF5, and NNF7 showed different results, possibly because the medication was trapped by the polymer and tween. This could have led to smaller drug molecules that were ionised in water. NNF1, NNF2, and NNF3 had low quantities of tween80, which prevented the drug from being reduced to smaller particle sizes or high polymer ratios, resulting in the capture of drug molecules.

The percentage yield was highest for NNF4 (78.5%), followed by NNF5, NNF3, and NNF2, indicating that NNF4, with a maximum polymer concentration and a safe level of tween concentration, is the most effective formulation. The yield decreased as the concentration of tween decreased.

SEM ANALYSIS

SEM micrographs (Figure. 3) revealed noticeable differences between pure Nateglinide and the optimised nanosuspension formulation. The Nateglinide particles were large and irregular in shape (Figure. 3). However, after formulation, the particles disappeared, and the drug became small and uniform. This may be due to the hydrophobic attachment of the surfactant to the crystal surface, which helped to stabilize the drug

particles. Therefore, it can be concluded that the solubility-enhancing strategy used was effective.

Table: 5.3 Nanosuspension of Nateglinide Formulation

Ingredients	NNF1	NNF2	NNF3	NNF4	NNF5	NNF6
Nathanglinide (mg)	11	11	11	11	11	11
Methyl Ethanol (ml)	9	9	9	9	9	9
Poloxam (% w/v)	0.26	0.6	0.76	0.26	0.6	0.76
Methylene chloride (ml)	2	2	2	1	1	1
Milliliters (ml) of distilled water	21	21	21	21	21	21

Table: 2 Solubility profile of nateglinide

S.No	Solvent	Solubility
1	Dissolved water	0.039%
2	Buffer of pH 1.2	0.10 mg/ml
3	Phosphorus buffer pH 4.5	1.197 mg/ml
4	Buffer pH 7.2	0.424 mg/ml

Table: 2 Calibration curves of Nateglinide by using various solvents

S.No	Concentration (µg/ml)	Absorbance		
		pH-1.2	pH-7.2	0.5 N HCL
1	5	0.0928	0.1553	0.0759
2	10	0.1873	0.2103	0.1389
3	15	0.2922	0.3598	0.2172
4	20	0.3838	0.4027	0.2758
5	25	0.4709	0.5328	0.3332

Table:3 The drug entrapment efficiency and percentage yield of nanosuspension vary based on the percentage of drug content.

Batches	% of drug content	Capture amount	% yield
NNF1	99.49±0.55	74.25±2.82	53.71±2.82
NNF2	98.77±0.92	77.91±4.57	74.81±2.17
NNF3	99.17±0.45	79.81±4.57	71.47±2.09
NNF4	99.59±0.27	87.19±3.15	79.25±3.75
NNF5	98.83±0.77	80.19±4.09	73.18±2.48
NNF7	98.59±0.55	75.47±2.57	71.47±2.89

Table: 4 Preparation of nanosuspensions for drug release.

S. No.	Time (min)	% drug release (Mean± S.D)					
		F1	F2	F3	F4	F5	F7
1.	0	0	0	0	0	0	0
2.	5	21.38±0.75	23.97±5.48	18.57±2.38	27.84±3.97	24.92±3.18	21.24±3.18
3.	15	34.42±0.78	37.17±5.17	31.08±3.88	38.17±3.29	37.45±3.08	30.17±2.05
4.	30	78.97±0.29	54.71±4.12	54.77±3.45	59.82±2.48	55.73±2.48	47.91±2.45
5.	70	82.38±3.77	71.85±4.43	74.52±2.74	78.17±1.75	74.81±3.82	78.27±2.57
7.	90	95.28±7.08	77.28±3.87	78.17±3.25	87.91±1.98	81.77±4.17	81.22±1.98
7.	120	97.29±7.19	79.47±3.75	79.71±5	92.78±2.48	85.80±4.78	85.07±2.74

Table: 5 Nateglinide Nanosuspension Fitting Results.

FORMULATION	ZERO VALUE	FIRST LEVEL	HIGUCHI	STUDENTS t TEST	P Value
NNF1	0.8428	0.8383	0.8897	0.9288	0.3715
NNF2	0.8478	0.9987	0.9973	0.9998	0.4588
NNF3	0.8375	0.9741	0.9894	0.9897	0.5072
NNF4	0.8243	0.9814	0.9928	0.9974	0.4304
NNF5	0.8257	0.9975	0.9928	0.9983	0.4505
NNF7	0.8848	0.9989	0.9978	0.9978	0.4875

Figure:1 Calibration curves of nateglinide in pH 7.2,1.2 and 0.5N HCL acid

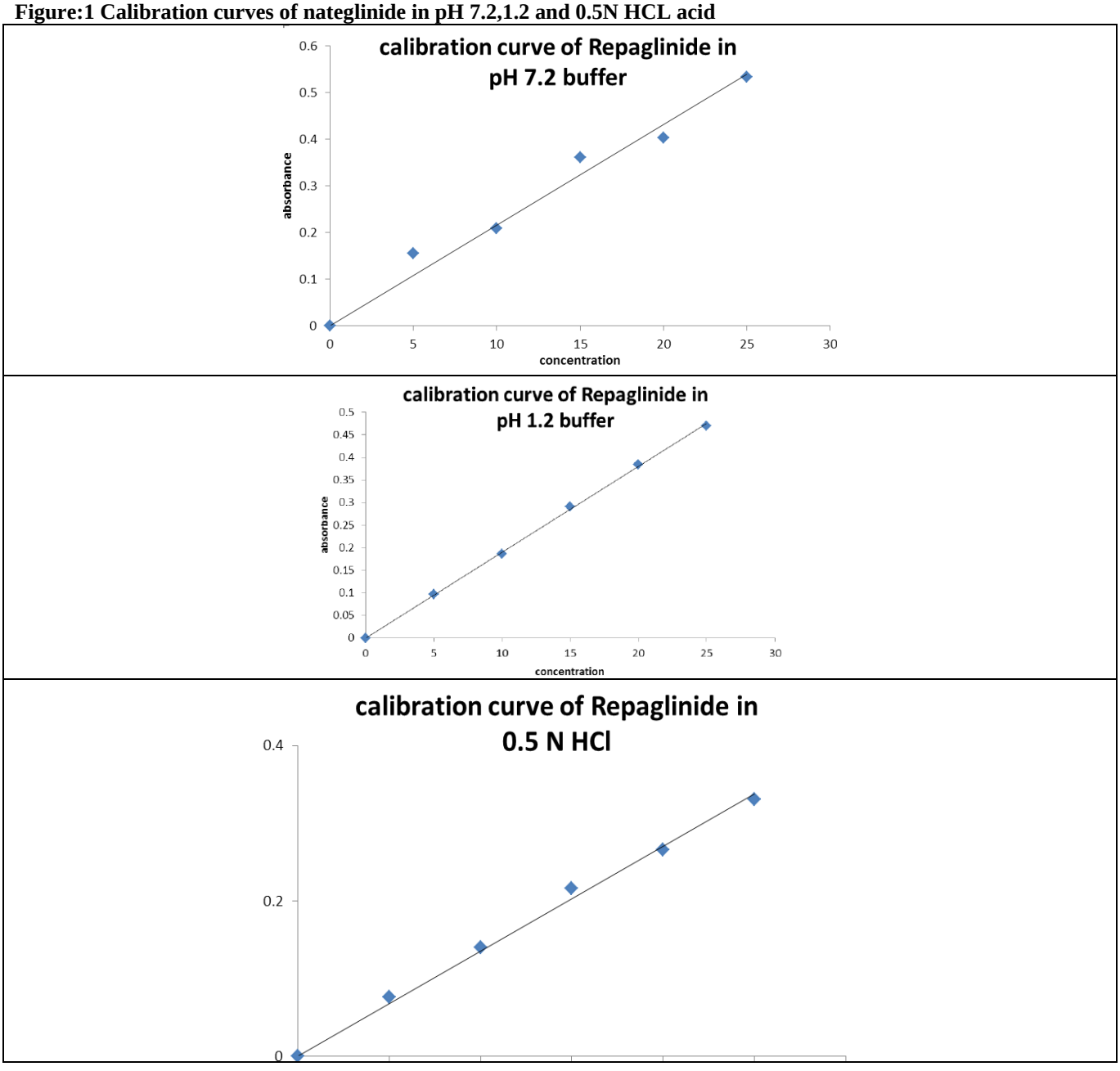


Figure: 2 FTIR Spectrum of Nateglinide (A), Polymer (B), Formulation (C).

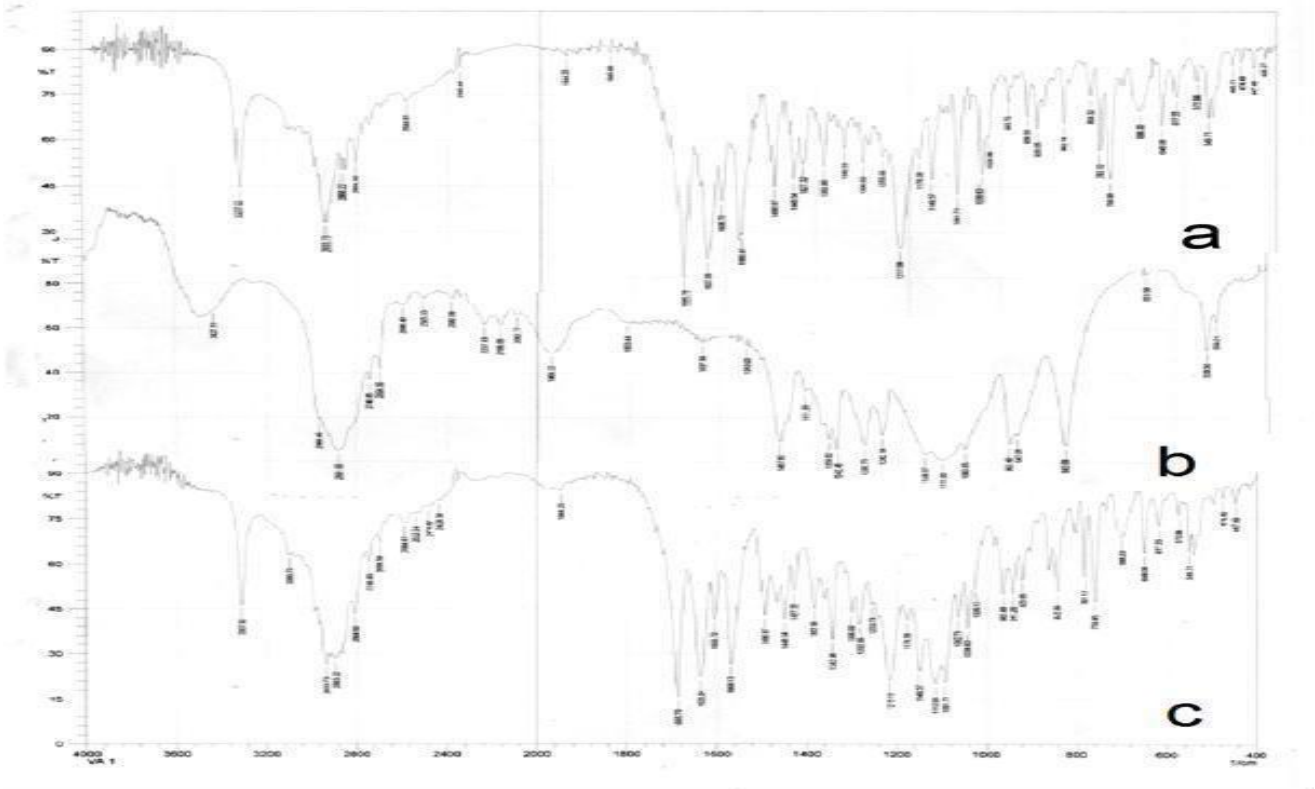


Figure:3 SEM images of a pure drug and nanosuspensions.

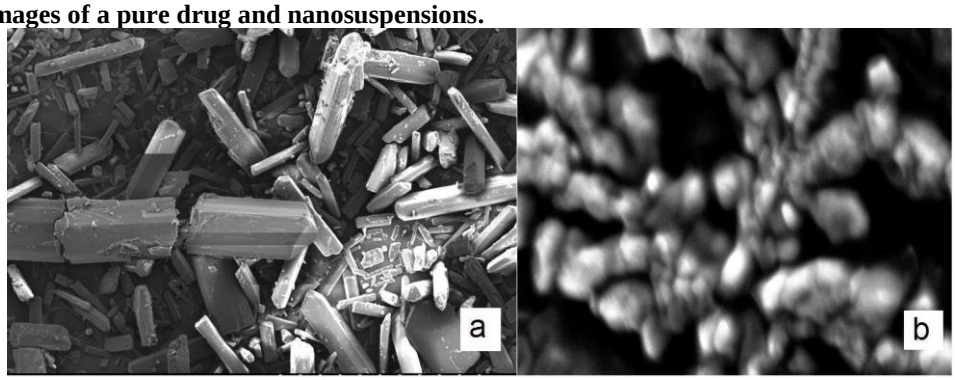
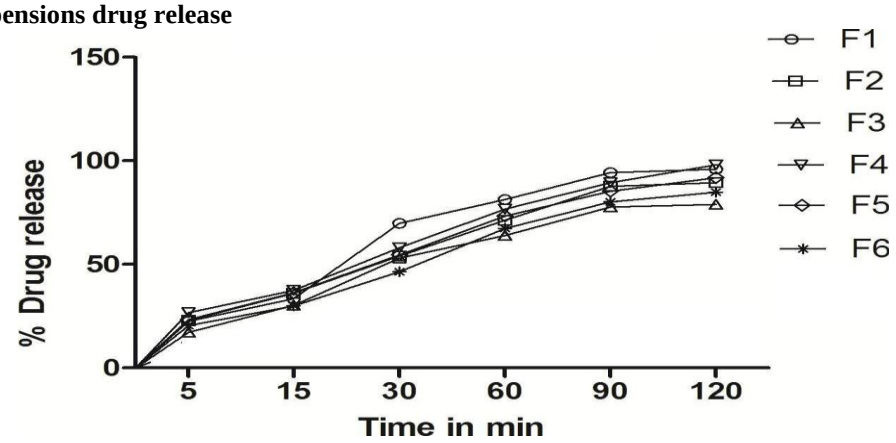


Figure:4 Nanosuspensions drug release



In vitro drug release profile

The study examined the in vitro drug release profile of Nateglinide nanosuspension using various graphical models. The percentage of drug released over time was measured for NNF1, NNF2, NNF3, NNF4, NNF5, and NNF7 formulations, and the data were tabulated. The results were plotted in a graph that shows the percentage of drug released as a function of time for all the formulations. The analysis of the drug release data revealed that the formulations differed in their drug release profiles. NNF4 showed the slowest drug release rate, followed by NNF5, NNF7, NNF2, NNF1, and NNF3. The release rate of NNF4 was significantly different from that of the other formulations. Additionally, the release rate of NNF1 and NNF3 was found to be similar, indicating that they have similar drug release profiles. Overall, the drug release results suggest that the Nateglinide nanosuspension formulation is effective in releasing the drug over an extended period. The findings of this study could be useful in developing drug delivery systems that are more efficient and effective in delivering drugs to the targeted areas in the body.

It seems like you are referring to a specific study or experiment that investigated the release profile of nateglinide from different formulations of nanoparticles. The study appears to have used four different models to fit the release data: zero order, first order, Higuchi, and Peppas. The results of the study showed that the release of nateglinide had a rapid initial burst, which may suggest that some of the drug was localized on the surface of the nanoparticles. Additionally, the release of the drug was observed to be gradual over time. Among the different formulations tested, NNF4 showed the best release profile and was considered to be the best formulation. However, without additional context or data, it is difficult to provide further interpretation or analysis of the results.

Based on the information provided, it seems that the study involved the evaluation of the release kinetics of six formulations using various kinetic release models. The results showed that the drug release from all formulations followed the Peppas release and Higuchi model, with greater R2 values, indicating that the release mechanism was anomalous diffusion. Furthermore, the diffusion exponent (n) values for all batches were within 0.5, indicating that the drug release mechanism was pure Fickian diffusion. The Peppas model was used to determine the release mechanism as either Fickian diffusion, non-Fickian diffusion, or zero-order diffusion, and the 'n' value was used to describe various release mechanisms. Overall, the study suggests that the drug release from the formulations followed the Peppas model and the Higuchi model, with a pure Fickian diffusion mechanism. These findings could be useful in developing and optimizing drug delivery systems for various applications.

CONCLUSION

A new method for synthesizing Nateglinide nanosuspension with poloxamer as a stabilizer has been developed using nanoprecipitation techniques. The size of Nateglinide particles can be adjusted by altering the operational parameters such as stabilizer concentration, surfactant concentration, and mixing rate during the process. This research suggests that nanosuspensions of poorly soluble drugs like Nateglinide can be easily formulated and may be a promising approach for delivering diabetic medication. In vitro testing of the nanosuspension formulation in PH 1.2 phosphate buffer revealed that it had a higher drug release than a pure drug formulation. This suggests that nanosuspensions could be a viable alternative to traditional drug delivery systems for low-water-solubility drugs, ultimately improving the biopharmaceutical efficacy. This study sets the foundation for further research to investigate the bioavailability and bioequivalence of medication in vivo, as well as their biological profiles in blood serum. The nanosuspension technique is a promising approach to increase the solubility of various drugs, as demonstrated by the success in improving the solubility of Nateglinide in this study. The findings of this study may have broad implications for improving drug delivery methods for drugs with low water solubility. Overall, the development of a nanoprecipitation procedure for synthesizing Nateglinide nanosuspension using poloxamer as a stabilizer has proven to be effective in adjusting the particle size and increasing drug release. The results suggest that this approach may be applicable for other drugs with low water solubility and could have significant implications for drug delivery and efficacy. Future studies may focus on assessing the bioavailability and biological profiles of nanosuspensions, as well as optimizing the process parameters for different drug molecules.

REFERENCE

1. RH Müller, C Jacobs and O Kayer. Nanosuspensions for the formulation of poorly soluble drugs. In: F Nielloud, G Marti-Mestres (ed). Pharmaceutical emulsion suspension. New York, Marcel Dekker. 2000; 383-407
2. JE Kipp, JCT Wong, MJ Doty and CL Rebbeck. Microprecipitation method for preparing submicron suspensions. US Patent 6,607,784 2003.

3. X Zhang, Q Xia and N Gu. Preparation of all-trans retinoic acid nanosuspensions using a modified precipitation method. Drug Dev Ind Pharm. 2006; 32: 857-63.
4. P Kocbek, S Baumgartner and J Kristl. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drug. Int JPharm. 2006; 312: 179-86.
5. J Möschwitzer, G Achleitner, H Pomper and RH Müller. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. Eur J Phar Biopharm. 2004; 58: 615-9.
6. VB Patravale, AA Date and RM Kulkarni. Nanosuspension: a promising drug delivery strategy. J Pharm Pharmacol. 2004; 56: 827-40.
7. K Itoh, APongpeerapat, Y Tozuka, T Oguchi and K Yamamoto. Nanoparticle formation of poorly water-soluble drugs from ternary ground mixtures with PVP and SDS. Chem Pharm Bull. 2003; 51: 171- 4.
8. A Wongmekiat, Y Tozuka, T Oguchi and K Yamamoto. Formation of fine drug particles by co-grinding with cyclodextrin. I. the use of β- cyclodextrin anhydrate and hydrate. Pharm Res. 2002; 19: 1867-72.
9. HM Abdou, Dissolution. AR Gennaro, JB Schwartz. Eds. In: Remington'sPharm. Sci., Mack Publishing Company: Easton, PA, 1990; 18: 589-602.
10. DF Evans and H Wennerström. The Colloidal Domain: Where Physics, Chemistry, Biology and Technology Meet, 2nd ed.; VCH Publishers Inc.: NewYork, 1994.
11. MN Mosharra. The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs. Int J Pharm.1995; 122: 35-47.
12. D Duchêne and G Ponchel. Bioadhesion of solid oral dosage forms, why and how. Eur J Pharm Biopharm.1997; 44: 15-23. 17.
13. CJ Van Oss, DR Absolom and HW Neumann. Interaction of phagocytes with other blood cells and with pathogenic and nonpathogenic microbes. Ann NY Acad Sci.1984; 416: 332-350.
14. KH Wallis and RH Müller. Determination of the surface hydrophobicity of colloidal dispersions by mini-hydrophobic interaction chromatography. Pharm Ind.1993; 55: 1124-1128.
15. CM Keck and RH Müller. Drug nanocrystals of poorly soluble drugs produced by high pressure homoginisation. Eur J Pharm Biopharm. 2006; 62: 3-16.
16. F Kesisoglou, S Panmai and Y Wu., Nanosizing-Oral formulation development and biopharmaceutical evaluation, Adv Drug Deliv Rev. 2007; 59, 631-44.
17. PU Xiaohui, Jin Sun, Mo Li and Zhonggui He. Formulation of Nanosuspensions as a New Approach for the Delivery of Poorly Soluble Drugs, CurrNanosci. 2009; 5: 417-427.
18. Mahendranakarani, Cyclosporine A-Nanosuspension: Formulation, Characterization and In Vivo Comparison with a Marketed Formulation, Sci Pharm. 2010; 78: 345–361

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