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Preparation and Characterization of Nisoldipine Nanoparticles by Nanoprecipitation Method

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Abstract

The purpose of this study is to develop Nisoldipine nanoparticles with Eudragit RLPO. In this study, nanoparticles were prepared by nanoprecipitation method. The morphological structure was investigated by Transmission electron microscope (TEM) The drug loaded nanoparticles found to exhibit a spherical shape. The mean particle size of the nanoparticles found to 400-600 nm with narrow size distribution with zeta potential of about -25mV. Fourier transform infrared spectroscopy and Differential scanning colorimetry (DSC) indicated that no possible interactions between the drug and polymer. Nisoldipine was selected as a model hydrophobic, poorly water soluble drug. The *in vitro* release from Nisoldipine loaded Eudragit RLPO nanoparticles showed 11% at pH 1.2, 55%at pH 5.0 and 90%at pH 6.8, within 24 h.

Key Words: *Nisoldipine, Eudragit RLPO, nanoparticles, nanoprecipitation.*

INTRODUCTION

The objective of this investigation is to develop and evaluate the physiochemical properties, drug loading and entrapment efficiency, *in vitro* release of polymeric nanoparticulate formulations containing Nisoldipine. Nisoldipine is a calcium antagonist of the 1, 4-dihydropyridine class. Nisoldipine has low oral bioavailability (3.9 – 8.4%), due to its first pass metabolism in the liver and gut.[1-2] It is extensively metabolized by the cyto-chrome P450 (CYP) system, with the isoenzyme CYP 3A4 catalyzing the dehydrogenation of the dihydro-pyridine ring. The absorption of Nisoldipine occurs across the entire gastrointestinal tract with an increase in bioavailability in the colon because of the lower concentrations of metabolizing enzyme in the distal gut wall. Nisoldipine also has a high potential of protein binding of more than 99% because of this, the level of unbound drug concentration is very low, which fails to achieve required therapeutic drug concentration in plasma. [3]

Eudragits are biocompatible co-polymers synthesized from acrylic and methacrylic acid esters. These polymers commonly used for the enteric coating of tablet and the preparations of controlled release. Eudragit RLPO is a copolymer of poly (ethyl acrylate, methyl-methacrylate and chloroethyl ammonio ethyl methacrylate) containing an amount of quaternary ammonium groups between 8.8-12 %. The aim in the present study was to prepare Nisoldipine loaded nanoparticles with pH independent Eudragit RLPO polymer[4-5]. The resulting Nisoldipine nanoparticles were characterized with regard to morphology, size, drug loading and *in vitro* release.

MATERIALS AND METHODS

Materials

Nisoldipine, Eudragit RLPO, were obtained as a gift sample from Orchid Pharma, Chennai. The surfactant sodium lauryl sulphate was procured from Ranbaxy, India. All other chemicals and reagents used in the study were of AR grade.

Methods

Preparation of Nisoldipine loaded Eudragit RLPO nanoparticles.

The Eudragit nanoparticles were prepared by nanoprecipitation method[7-9]. Briefly the drug and polymer were dissolved in 10 ml of ethanol then slowly added to 50 ml of water, containing 0.02ml of Tween 80. The solution was stirred at 1400 rpm, until the complete removal of ethanol. The suspension was centrifuged at 17000 rpm and particles were collected, washed with water and freeze dried. The supernatant liquid was collected and analyzed by HPLC for free drug content.

Table 1 Formulation of Nisoldipine Eudragit nanoparticle

Formulation code	Nisoldipine (mg)	Eudragit RLPO (mg)
IF1	75	75
IF2	75	150
IF3	75	225
IF4	75	300

Characterization of drug loaded nanoparticles.

***Transmission Electron Microscope* (TEM)**

The surface morphology of the prepared nanoparticles was evaluated by Transmission Electron Microscope (TEM).

Particle Size Analysis

Mean particle size of nanoparticles was determined by Malvern Zetasizer (Malvern instruments UK).The The measurements were realized in triplicate at 25ºC under suitable dilution conditions.

Zeta potential Measurement

Zeta potential of nanoparticle dispersions was measured in mV by Malvern Zetasizer ver 6.20 (Malvern instrument's Malvern UK) to determine the surface charge and the potential physical stability of the nano system. Zeta potential of nanoparticles was measured in an aqueous dispersion.

Table 2 Particle size, polydispersity index and zeta potential of nanoparticle formulations

Formulation code	Average diameter (nm)	PDI	Zeta Potential (mV)
IF1	983.7	0.493	-26
IF2	520.4	0.493	-22.4
IF3	576.2	0.495	-24.8
IF4	496.3	0.496	-21.4

Table 3 Drug content, encapsulation efficiency and loading capacity of nanoparticle formulations

Formulation code	Amount of Nisoldipine per 100 mg of nanoparticles* (mg)	Encapsulation efficiency* (%)	Loading capacity* (%)
IF1	49.51 ± 12.16	66.01 ± 1.2	38.08 ± 1.23
IF2	44.24 ± 0.62	58.99 ± 0.83	22.12 ± 0.86
IF3	52.29 ± 0.01	69.71 ± 0.02	20.91 ± 0.41
IF4	48.03 ± 0.34	64.04 ± 0.45	16.01 ± 0.42

***n = 3; mean±SD**

***Differential scanning calorimetry* (DSC)**

The differential scanning calorimetry experiment was performed on the drug, the polymer and the nanoparticle formulation. The samples were weighed into aluminium pans and heated in an inert atmosphere of nitrogen at a heat rate of 10ºC/min analyzed by DSC analyzer (Shimadzu Japan).

***Fourier transform infrared spectroscopy* (FTIR)**

The drug-polymer compatibility was ascertained by subjecting the drug and polymer to Fourier transform infrared spectrometric study using a Perkin Elmer 1600 spectrophotometer with a resolution of 2 cm^{-1}. The samples were scanned in the spectral region between 4000 and 400 cm^{-1}. Solid powder samples were dried in an oven around 30^0C finely crushed and mixed with potassium bromide (1:10 ratio by weight) and pressed at 15,000 psig to make disc. The detector was purged carefully by clean dry nitrogen gas to increase the signal level and reduce the moisture.

Nanoparticles yield, drug loading content and entrapment efficiency.

Determination of Drug Content

Drug content was determined by using a validated HPLC method. The Nanoparticle formulation was centrifuged and the supernatant was separated. The Nisoldipine concentration in the filtered supernatant and in the drug loaded nanoparticle was determined by a reverse-phase HPLC. The UV detection was carried out at 238 nm. The nanoparticle yield was calculated as the percentage of Nisoldipine in the filtered suspension relative to the theoretical drug amount added. The drug entrapment efficiency was expressed as a percentage of the Nisoldipine difference between the filtered suspension and the supernatant relative to the total amount of Nisoldipine in the filtered suspension. The drug loading was estimated as the ratio of Nisoldipine incorporated to the theoretical carrier.

***In vitro* release experiments of the nanoparticles**

The *in vitro* release experiment was developed to measure the drug release kinetics from the polymeric nanoparticles in a simulated condition. Nanoparticle samples were suspended in 1 ml pH 7.4 buffer then enclosed in dialysis bags, which were hermetically sealed and placed in a dissolution medium; the normal sink condition was maintained[10-11]. Drug release study was carried out using the USP dissolution apparatus under the change over conditions at 37 ± 0.5 ºC and stirred at 100 rpm. The nanoparticle formulations are tested for drug releases for 2 h in 300 ml of hydrochloric acid buffer of pH 1.2 containing 0.5 % SLS. Then the dissolution medium was replaced with 300 ml of phosphate buffer of pH 5.0 containing 0.5 % SLS and tested for drug release for next 3 h, afterwards the dissolution medium was replaced with 300 ml of phosphate buffer of pH 6.8 containing 0.5 % SLS for next 18 h. At each time interval 2.0 ml of sample was collected and replaced with fresh respective buffers, the collected sample was centrifuged using microcentrifuge (6000 rpm) and the supernatant was introduced into HPLC and peaks were then observed at 238nm.

RESULTS AND DISCUSSION

Characterization of the Nanoparticles

Morphological properties of nanoparticles

The morphology of the nanoparticles shown by the TEM image in Fig. 1, which all present spherical shapes without aggregation. The particle size and polydispersity index data are shown in Table 2 had a size in the range of 400 to 900 nm with good dispersity. The surface charge of nanoparticles is negative in the range of -26 to -21 mV as shown in Table 2. The zeta potential increased with an increase of polymer concentration. The DSC thermograms of Nisoldipine, Eudragit RLPO and nanoparticles are shown in Figure 3. The obtained thermograms showed the melting point obtained in pure drug and formulation was in the similar range, which infers that

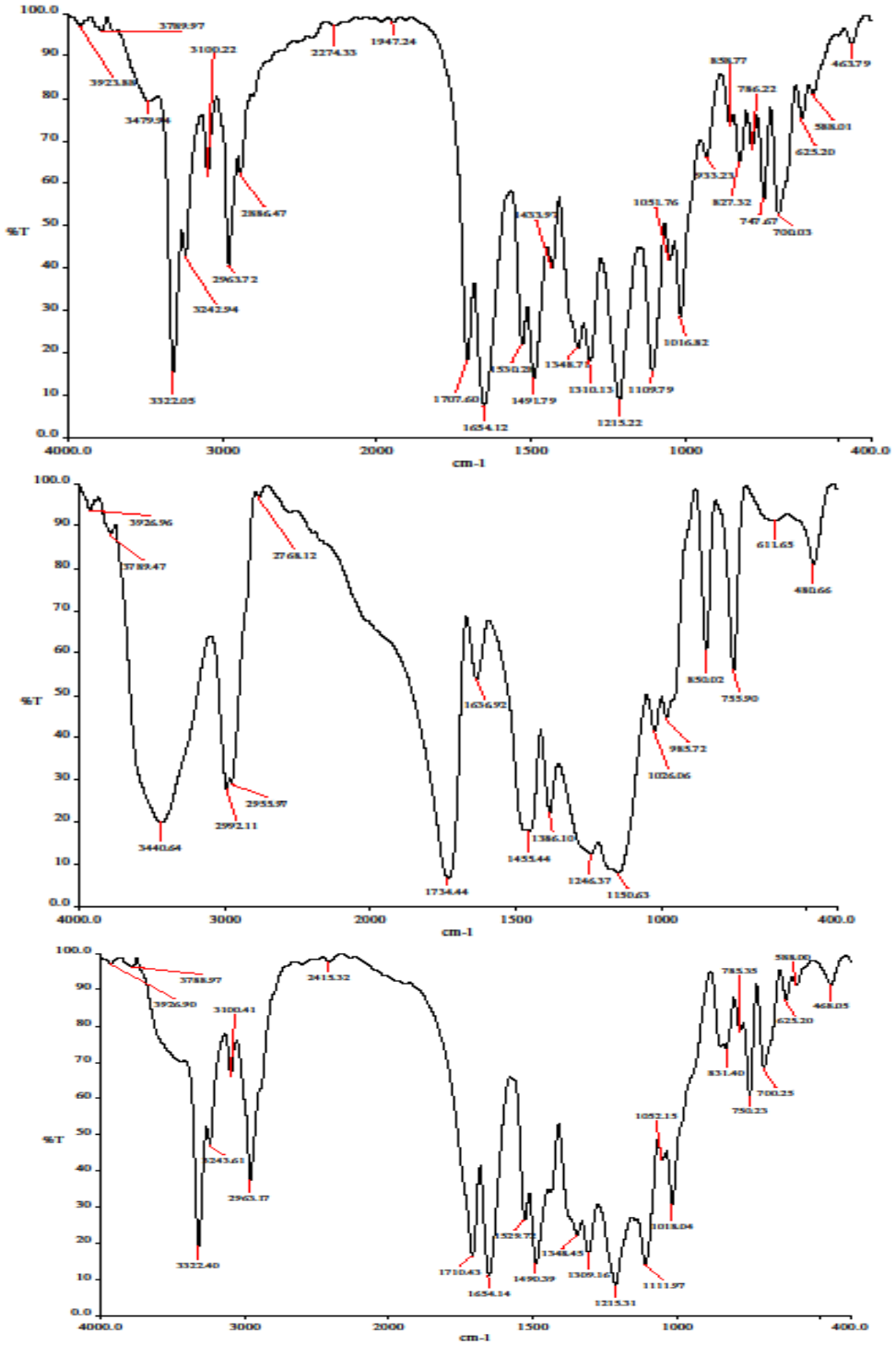

Figure 2 FTIR spectra of (a) Nisoldipine (b) Eudragit RLPO (c) Nanoparticles of Eudragit

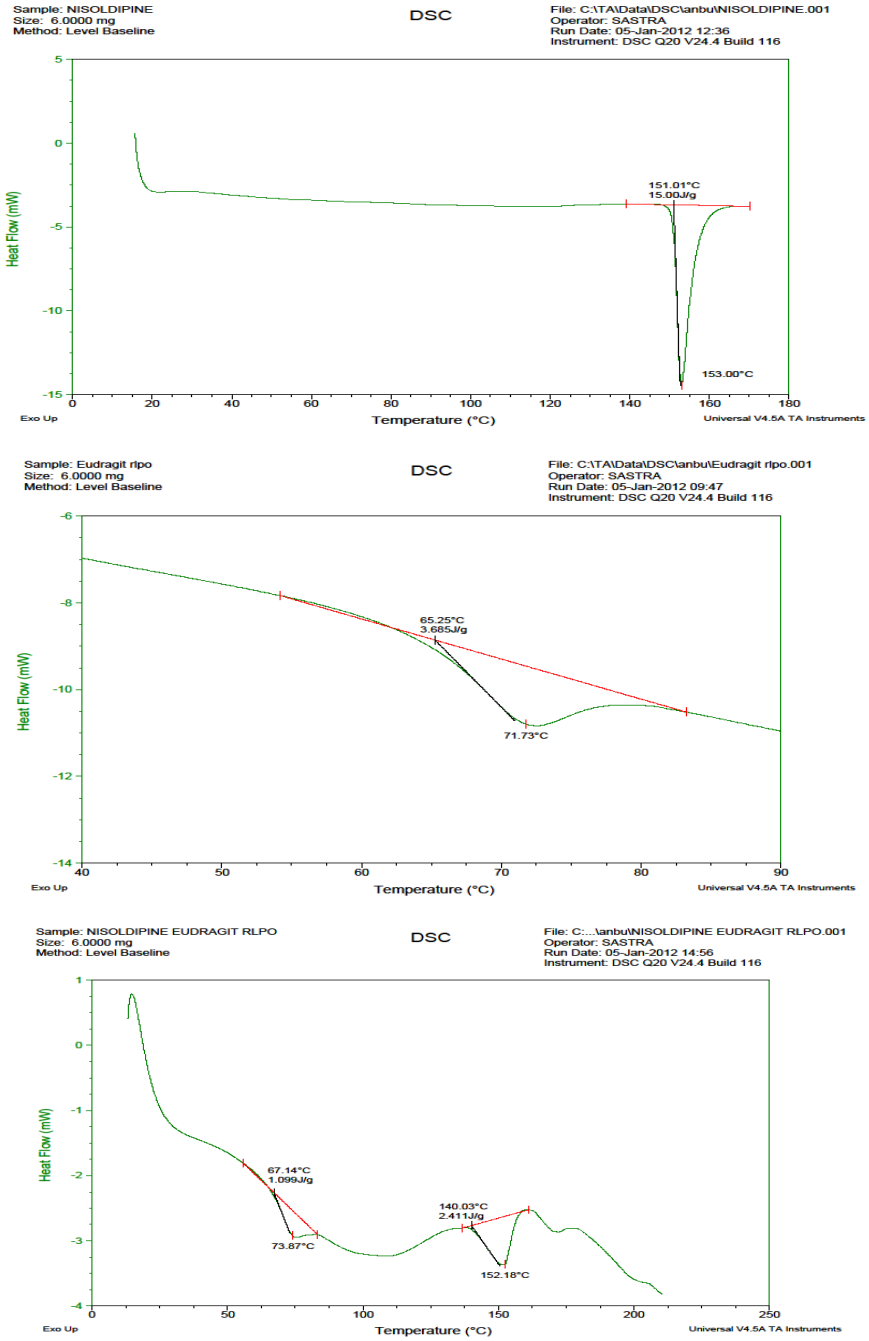

Figure 3 DSC photos of (a) Nisoldipine (b) Eudragit RLPO (c) Nisoldipine loaded nanoparticles

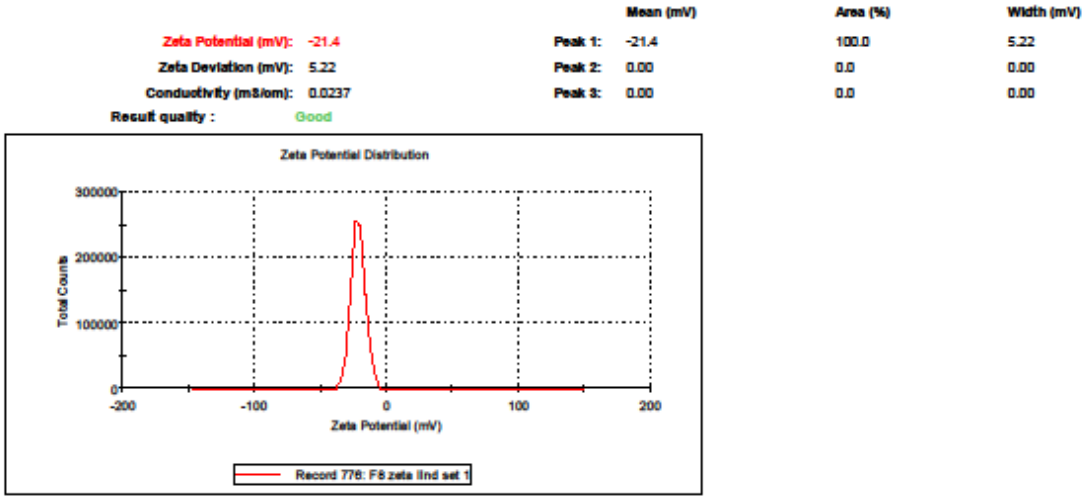

Figure 4 Zeta potential of Nisoldipine nanoparticles

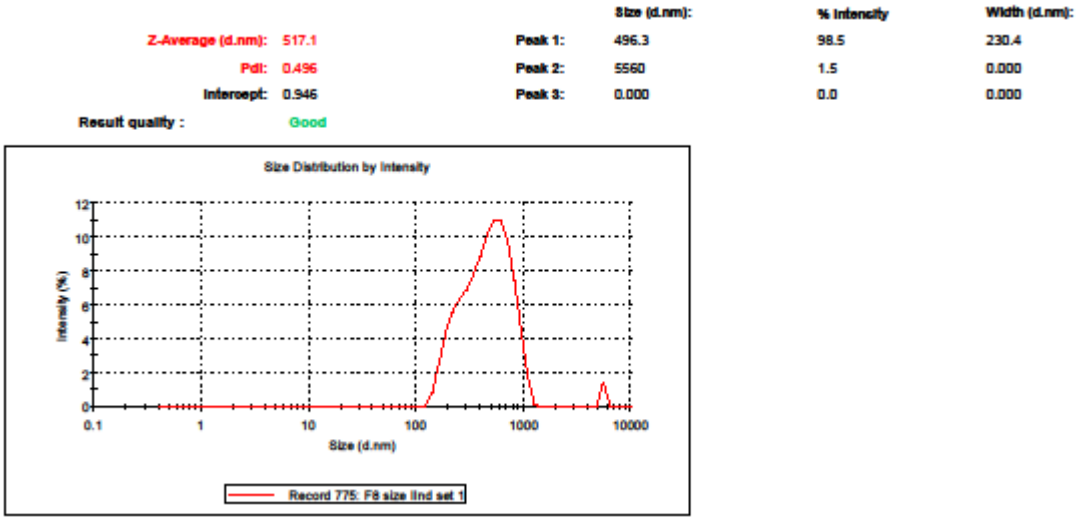

Figure 5 Nisoldipine nanoparticles particle size distribution

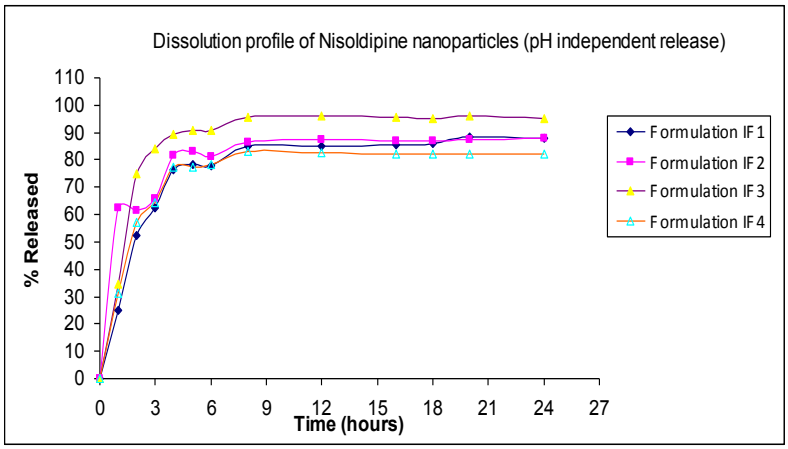

Figure 6 The cumulative release curves of Nisoldipine from Eudragit RLPO nanoparticles.

there was no drug polymer interaction and the drug was compatible with excipients.

FTIR studies

In FTIR study, the characteristic peak of Nisoldipine has appeared in the spectra of nanoparticles without any remarkable change in the position. It was confirmed that there was no chemical interaction between the drug and polymer.

Evaluation of Drug content, drug loading and entrapment efficiency

An important aspect in using nanoparticles as a drug vehicle is the effect of the drug loading levels. Table 3 summarizes the drug content, drug loading and entrapment efficiency of Nisoldipine nanoparticles.

In vitro Drug Release Studies

The Nisoldipine release from the nanoparticle formulations are shown in Figure 6. The *in vitro* release of Nisoldipine from the formulations showed a continuous release as shown in Fig 2 and shows pH independent release of drug from the nanoparticles. In general, *in vitro* release profile suggests that the release of Nisoldipine from nanoparticles was independent of pH.

CONCLUSION

Nanoparticles were prepared with nanoprecipitation technique. It is a rapid and easy technique and nanoparticles were formed spontaneously. Nanoparticles were characterized by particle size distribution, zeta potential analysis, drug entrapment efficacy and *in vitro* release studies. This method can be used to improve the therapeutic efficacy of poorly soluble drugs

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