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FORMULATION AND EVALUATION OF SOFT GELATIN CAPSULES OF NELFINAVIR MESYLATE

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ABSTRACT The present investigation was undertaken with the objective of enhancing the bioavailability of nelfinavir mesylate (NLF), a HIV protease inhibitor through the preparation of liquid fill formulations for soft gels. Liquid fill formulations for NLF (250mg) soft gels were prepared using excipients such as solubility enhancer (Tween 80), viscosity modifier, polyvinylpyrrolidine (PVP K -30), p-gp efflux inhibitors and hydrophobic vehicle, poly ethylene glycol 400 (PEG 400),hydrophilic vehicle(9:1 ratio of ethyl alchohol and water).The prepared formulations were evaluated for appearance, pH, content uniformity, water migration, viscosity, drug-excipient compatibility and *in vitro* drug release parameters. Optimized formulation was further subjected to bioavailability studies in rabbits. Stability of the optimized formulation was evaluated by storing for six months, at 40^0 C and 75% RH. Among all the prepared formulations, formulation F2 containing 10% PVP k-30 and 10% Tween 80 showed superior % drug release (99.94% ± 0.64 within 4 min) with appreciable physical and chemical stability. Comparative bioavailability studies were carried out on formulation F2 and NLF suspension. Formulation F2 showed better relative bioavailability than that of NLF suspension. The results of this study provide guidance for developing soft gel capsule of NLF that give greater bioavailability, than existing dosage forms, and provide protection against p-gp efflux. The proposed techniques are economical, convenient and safe.

KEYWORDS: *In vitro* dissolution, *in vivo* bioavailability, P-gp efflux, Soft gels, stability, viscosity.

INTRODUCTION

Nelfinavir mesylate (NLF), a HIV protease inhibitor is currently used alone or in combination with reverse transcriptase inhibitors for the management of HIV infection. NLF shows site specific absorption window in the stomach and the likelihood of precipitation in the small intestine. NLF is soluble in the acidic environment of the stomach since it is a weak basic drug and its therapeutic efficacy is limited by its poor aqueous solubility. The objective was to enhance its solubility through the preparation of soft gels. Liquid fill formulations for soft gels have excipients that inhibit P-glycoprotein efflux mechanism.

High active antiretroviral therapy (HAART) is a combination therapy with two kinds of reverse transcriptase inhibitors and a HIV protease inhibitor (PI), that prolongs the survival of AIDS patients.[1] All the protease inhibitors (PIs) including NLF are metabolized via CYP3A and are substrates and/or inhibitors of the membrane efflux transporter, P-glycoprotein (P-gp).[2,3,4] NLF is a white to off-white powder, freely soluble in methanol, ethanol, isopropyl alcohol and propylene glycol, and is practically insoluble in water.[5]

The oral absorption of NLF is dissolution rate limited because of which it has low and variable oral bioavailability (20-80%).[6,7,8] NLF belongs to BCS class-II and IV.[6,7,8,9] It is the most lipophilic of the PIs, having a partition coefficient log P value of 4.1.[10,11] NLF shows low and variable bioavailability because of P-glycoprotein efflux system, which can be avoided by modifying its release to occur more in the stomach where P-glycoprotein content is lowest.[12] Presently, NLF is available as immediate release (IR) tablets and powder sachets [(Nelfin-625mg FC tab (Hetero HC), Retronel-250 mg (Alchemy), Nelvir-250mg (Cipla), Viracept-250 mg (Agouron)].

The pharmaceutical industry manufactures approximately 75% of its solid dosage forms as compressed tablets, 23% as hard gelatin capsules and 2% as soft gelatin capsules. A market survey on consumer preferences has indicated that 44.2% consumers prefer soft gelatin capsules, 39.6% prefer tablets and 19.4% prefer hard gelatin capsules.[13]

A challenge faced by many orally administered drugs is effluxation by P-glycoprotein.[14] This efflux transporter reduces the bioavailability of drugs by transporting absorbed drugs back into the gastro intestinal tract. A

good number of surfactants were proved to be having the capacity to inhibit this efflux transporter.[15] Soft gels improve the stability of loaded drugs as they do not allow oxygen permeability.[16] The present liquid fill formulations for soft gels contain a vehicle, solubility enhancer, recrystallization retardant, viscosifying agent, P-gp efflux inhibitor and a preservative as fill excipients along with the selected drug NLF.

From the literature review, it is clearly evident that most (or) all of the works were published with cyclodextrin based drug delivery system,[17] polymeric nanocarriers,[18] β-cyclodextrin inclusion complexes, [19] micro capsules of NLF using cellulose acetate,[20] solid dispersions using modified starch,[21] for improving the solubility, dissolution, bioavailability of NLF. However, no reports were published on the liquid fill formulations for soft gel dosage forms in order to improve the *in vitro* dissolution and oral bioavailability of NLF.

MATERIALS AND METHODS

Materials

NLF was supplied by Lantec pharmaceuticals, Hyderabad, as a gift sample. PVP K-30 (gift sample from ISP Pharmaceuticals), PEG-400 (gift sample from S.D Fine chemicals Ltd, Mumbai), Propylene glycol (gift sample from Central drug house, Bombay), Tween 80 (gift sample from Central drug house, Bombay), Ethyl alcohol of HPLC grade (gift sample from Changshu Yang chemicals, China), Butylated Hydroxy toulene (gift sample from Merck specialities Ltd, Mumbai), Empty soft gelatine capsules (gift sample from Kahira pharm.chem.co, Cairo, Egypt), Distilled de-ionized water. All the materials used were of pharamcopoeial and analytical grades.

Methods

Analytical methods

A UV-VIS spectrophotometric method based on the measurement of absorbance at 252 nm in methanol stock solution was used in the present research work for the estimation of NLF *in vitro* studies.

HPLC method for estimation of NLF in rabbit plasma

A new reverse phase HPLC method with UV detection was developed for the estimation of NLF in plasma samples. For this purpose a calibration curve was constructed by analyzing plasma samples containing different amounts of NLF. The experiment was conducted to develop a liquid chromatographic method for the determination of NLF using Waters Alliance 2695, HPLC system with Auto Sampler and 2487 UV-Visible detector. The chromatographic studies were performed using Hypersil ODS C_{18} column (4.6 ID X 150 mm, 5µm) at ambient temperature. Data acquisition was done by using Empower 2 software.

Nelfinavir mesylate equivalent to 10 mg of nelfinavir base (i.e.11.92mg) was weighed accurately and transferred into a 10 ml volumetric flask containing 5 ml of methanol. The contents were sonicated for 5 minutes and then the volume was made up with a further quantity of methanol to get a free base concentration of 1 mg/ml. This stock solution of the drug was stored in a refrigerator at a temperature below 10^0C. One ml of the drug stock solution was diluted up to 10 ml in a volumetric flask using mobile phase [Phosphate buffer (pH 3.0) and ACN in a ratio of 40:60 v/v] as a diluent to get a concentration of 100µg/ml. The secondary stock solution of the drug was stored in a refrigerator at a temperature below 10^0C.

Preparation of liquid fill formulations

Liquid fill formulations were prepared as per the formulae given to a batch size of 6g. Initially propylene glycol, Tween 80 and PEG-400 were taken into a small beaker and stirred to dissolve well. PVP K-30 was then added and dissolved. Accurate amount of NLF was then weighed and transferred into this beaker and mixed thoroughly. It was followed by the addition of ethyl alcohol to dissolve the drug completely (evaporation of ethyl alchohol is avoided by covering the beaker during stirring). Nelfinavir base, 10 mg is equivalent to 11.69 mg of nelfinavir mesylate salt. Therefore for 250 mg of nelfinavir base that is required in the formulation, salt equivalent taken was 292.3 mg. BHT was then added and the system was mixed thoroughly. The prepared formulation was sonicated for 3 minutes in order to remove any entrapped air. The weight of liquid ingredients like ethyl alchohol, propylene glycol (PG), polyethylene glycol – 400, Tween 80 was converted to volume from density values and taken accordingly. The volume of the above liquid ingredients was derived from the available values of density reported in standard literature (density of ethyl alchohol is 1 gm/cm^3, propylene glycol is 1.038 gm/cm^3, PEG -400 is 1.12 gm/cm^3, Tween 80 is 1.02 gm/cm^3). Empty soft gelatine capsules were incubated at 40° C for 10 minutes with an objective of removing moisture taken up by the capsules during storage.[16] Each oval shaped soft gelatin capsule of size 20 equivalent to 1.232 ml was taken for filling. Each capsule was filled by injection with 1.0 ml of each of the formulation. Each capsule should be filled up to 75 percent of its total volume.[14] Using a glass syringe the liquid fill was injected into the capsule, which was then sealed by heat.

The soft gelatin capsules filled with liquid fill formulations of NLF were then subjected to different tests to evaluate for various parameters.[22]

Table 1: Formulation of nelfinavir mesylate liquid fill formulations for soft gels

Ingredients (mg)/capsule	F1	F2	F3	F4	F5	F6
NLF	250	250	250	250	250	250
PVP k-30	100	100	100	-	50	150
Ethyl alcohol + water (9:1 ratio)	100	100	100	100	100	100
PG	275	275	250	275	275	275
Tween 80	-	100	50	-	-	-
PEG-400	275	174	249	374	324	224
BHT	-	1	1	1	1	1
Total wt	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**

Evaluation parameters for liquid filling formulations[23]

Appearance

Appearance is one of the most important parameter of liquid filling formulations. All the formulations were evaluated for clarity by visual observation against a black background. Clarity is the most important characteristic feature of liquid fill formulations.

pH

The developed NLF liuid fill formulations were evaluated for pH by using Elico LI 120 pH meter and estimations were carried out in triplicate. Soft gel formulations should have a pH range between 2.5 and 7.5.

Drug content uniformity

Drug content was estimated in the liquid fill formulations by weighing approximately 25mg of the fill formulation into a 5 ml volumetric flask. A small volume of methanol was then added, the flask was stirred well, and the volume was made up to 5 ml with remaining methanol. Samples were suitably diluted with 0.1N HCl and the samples were analysed for NLF content by measuring absorbance at 252 nm. The estimations were carried out in triplicate.

Moisture absorption studies

In order to study the effect of liquid fill composition on the water sorption behavior of the softgels, they were subjected to water migration studies. Three capsules from each formula were weighed, transferred to a small dry pre-weighed beaker and kept in a sealed glass humidity chamber containing 100 ml of a saturated aqueous solution of sodium chloride (to provide an atmosphere of 75% relative humidity). The weight of the beaker, with its contents, was recorded every day until it became constant indicating that equilibrated moisture absorption had been achieved. The water content of the prepared softgels was determined at the beginning of the water migration study and after equilibrium to calculate the weight of water gained by each formula at equilibrium, and this was expressed as a percentage of the initial capsule weight. A Karl Fischer titrator (Veego, Matic-MD, Veego Instruments Corporation, India) was used to determine the moisture content of the softgels and to do this the capsules were cut, inserted into the titration vessel containing dried methanol (Karl-Fisher grade) and titrated with Hydranal Composite 5 reagent (Riedel-de-Haën, Seelze, Germany) after stirring for 2 minutes. Three capsules were analyzed from each formula and the results were presented as a mean value ± SD. For comparision, the water sorption behaviour of empty capsule shells and filled soft gelatin capsules was studied.

Rheological studies

Viscosity of all the formulations was measured using a Brookfield DV-II + PRO viscometer. The formulations were taken in the cup of the Brookfield DV-II + PRO viscometer and it was rotated with CP52 spindle. The angular velocity was fixed at 10-100 rpm. The viscosity measurements were made in triplicate using fresh samples each time at room temperature.

FTIR studies

Samples were analyzed using an ATR-FTIR spectrometer (Bruker, Germany). ATR spectra were measured over the wave number range of 4000–500 cm−1 at a resolution of 1.0 cm^{-1}. The powder or film sample is simply placed onto the ATR crystal and the sample spectrum is collected. The sample is then cleaned from the crystal surface and the accessory is ready to collect additional spectra. ATR analysis is less complicated than using KBr pellets. It is a fast process and a very small amount of the sample is needed.

***In vitro* drug release studies**

In vitro dissolution studies were conducted using 900mL of 0.1N HCl, as dissolution medium using USP XXI type I/II (paddle method) dissolution apparatus (DISSO 8000, LAB INDIA). A temperature of 37 ± 0.5°C and a rotation speed of 100/50rpm were maintained. Dissolution studies were performed. As the capsule tends to float in the dissolution medium, sinkers were used. Samples of 5ml were withdrawn at predetermined time intervals over a period 1hr, passed through a 0.45µm nylon membrane. Then the sample removed was replaced with the same volume of fresh dissolution medium in order to maintain constant dissolution medium. The filtered samples were suitably diluted and analyzed at 252nm using UV-Visible Elico SL150 spectrophotometer. Dissolution experiments were conducted in triplicate.

Drug release kinetics & mechanisms

There are a number of kinetic models, to describe the overall release of drug from the dosage form. Because

qualitative and quantitative changes in a formulation may alter drug release and *in vivo* performance, developing tools that facilitate product development by reducing the necessity of bio-studies is always desirable. The rate of release of NLF from prepared dosage form was analyzed by fitting drug release data into first order release kinetics equation:

$\text{Log } C = \log C_O - k\ t/2.303.$

Where, C_O is the initial concentration of the drug and k is the first order constant. By plotting log cumulative of % drug unreleased against time a line was obtained and from its slope, k was calculated.

Stability studies on NLF liquid fill formulations for soft gels

Liquid fill formulations, apart from their other requirements, should be stable with regard to their properties, especially their dissolution characteristics. The stability of nelfinavir liquid fill formulations developed in the present study was evaluated as per ICH guidelines. The capsules were packed in amber coloured bottles and stored at 40^0 C and 75% RH for 6 months. During storage, the products were monitored for viscosity, pH of the formulation, drug content, appearance, precipitation, and dissolution profile studies. These studies were carried out at 3rd and 6th months.

***In vivo* studies**

In vivo pharmacokinetic studies were carried out on the reference formulation, NLF suspension-code-N (Each 1 ml of suspension made with 0.9% sodium carboxy methyl cellulose contained 250 mg of nelfinavir, (M/s Lantec Pharmaceuticals pvt. Ltd. Hyderabad) and test formulation code- SGN (Liquid fill formulation for soft gel F2 containing 250 mg of nelfinavir mesylate).

In vivo experiments were carried out on healthy rabbits as per the following experimental design and protocol.

Experimental design

Dose regimen and administration

The reference compound N was suspended in 0.9 % sodium carboxy methyl cellulose to yield a concentration of 250mg of nelfinavir free base (292.3mg of nelfinavir mesylate) per 1gm of suspension. The formulations N and SGN were prepared as suspensions 30 minutes before administration at room temperature. The pure drug suspension was prepared and kept overnight. Suspension was prepared with a magnetic stirrer. The dose administered was 9mg per kg body weight of the rabbit.

In the present study, *in vivo* animal studies of test formulation SGN and pure drug N were performed in healthy rabbits of either sex weighing (1.0 ± 0.2 kg) and were divided into two groups, each consisting of six animals. First group received pure drug suspension (N), and the second group received the test formulation (SGN). Food was withdrawn from the rabbits 12 hrs before drug administration and until 24 hrs post dosing. All rabbits had free access to water throughout the study. Animal housing and handling were in accordance with the CPCSEA guide lines. This study on animals was carried out in the pharmacology laboratories of the University College of Pharmaceutical Sciences, Andhra university, is approved by the institutional ethics committee, and has a registration CPCSEA(Regd.No.516 – 01/ A/ CPCSEA) for experimentation on animals.

***In vivo* study protocol**

After collecting the zero hour blood sample (blank), the products involved in the study were administered orally at a dose equivalent to 9 mg of nelfinavir/kg of body weight of the rabbit. Blood samples of 0.5 ml (500 µl) volume were collected from the marginal ear vein at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 8.0, 10 and 12.0 hrs after administration of the product. The blood samples were centrifuged at 4,000 rpm and the separated plasma samples were stored at -20°C until analysis.

Extraction procedure of nelfinavir from rabbit plasma

To 0.5 ml of plasma, 1 ml of ACN was added in 1.5 ml of poly propylene tubes. The tubes were vortex-mixed for 5 minutes in cyclomixer and then centrifuged for 10 minutes at 4000 rpm. The upper organic layers of the tubes were collected, filtered and injected into the column and then analyzed by using a sensitive high performance liquid chromatography (HPLC) assay method.

Nelfinavir concentrations in plasma following the administration of pure drug, and experimental products are given in Tables and are shown in Figures. From time versus plasma concentration data, various pharmacokinetic parameters such as peak concentration (C_{max}), time at which peak concentration occurred (T_{max}), area under the curve (AUC), elimination rate constant (K_{el}), biological half - life ($t_{1/2}$) and mean residence time (MRT) were calculated in each case.

Statistical Analysis of the data

All the data was given as mean ± SD. Statistical analysis of the data was carried out with a one-way ANOVA analysis (using Fisher's Least-Significant-Difference post hoc test) using SYSTAT 13 software (Systat Software Inc., CA, USA). Statistical significance was checked at a threshold of $p < 0.05$.

RESULTS AND DISCUSSION

The HPLC method was developed, validated and the total run time was set at 08 minutes. Nelfinavir was appeared on the chromatogram at 5.916 minutes. The retention time of the drug was the same for all the six injection samples. The regression of nelfinavir concentration (250 - 1250 ng/ml) over its peak area of drug was found to be Y= 32.094X- 0.2 with a high correlation coefficient (r =0.999), where Y is peak area and X is concentration of nelfinavir mesylate. This

regression equation was used to estimate the amount of nelfinavir in plasma.

The developed HPLC method was validated for intra-day and inter-day variation. When the solutions containing 500, 750 and 1000 ng/ml of nelfinavir were injected repeatedly on the same day and on other day, the coefficient of variation in amounts estimated was less than 0.1709%. The results indicated that the HPLC method is highly reproducible. In the accuracy assessment, the recovery was found to be 98.93 - 99.62%. Thus, the developed HPLC method is simple, sensitive, precise and highly accurate and required only a small quantity of plasma sample. This method is applicable to the estimation of nelfinavir mesylate in rabbit plasma obtained in pharmacokinetic evaluation of liquid fill formulations for soft gels.

The formulations prepared were evaluated for various parameters like viscosity, pH, appearance, drug content, water absorption, drug-excipient compatibility and *in vitro* drug release studies and stability studies.

Appearance

All liquid filling formulations of NLF were visually tested for clarity, colour and precipitation of drug if any. The formulations were clear, homogenous and free of precipitation.

pH

pH is another important parameter for liquid filling formulations. The two areas of critical importance are the effect of pH on solubility and stability. Liquid fill formulations for soft gels should have their pH in the range of 2.5 to 7.5.[24] At pH values below 2.5, gelatin is hydrolyzed causing leakage of the soft gel, whereas at pH values above 7.5, gelatin may be either hydrolyzed or tanned (i.e., cross-linked) resulting in decreased solubility of the gelatin shell. [24] The pH of all the formulations was close to 7.2. The pH of the soft gelatin fill formulation without drug was found to be 5.4 (placebo). Therefore, all the batches of the formulations are suitable for capsule filling.

Drug content estimation

The drug content was found to be in acceptable range for all formulations indicating uniform distribution of drug. Percent dug content was found to be in the range of 98.89 ± 0.05 - 99.68 ± 0.31.

Moisture absorption studies

The moisture absorption was found to be in the acceptable range for all formulations indicating their stability. Percent moisture absorption was found to be in the range of 1.17 ± 0.95 – 4.83 ± 0.64.

Rheological studies

Viscosity is one of the important parameters, which provides vital information during the optimization of the liquid filling formulation for soft gels. In general, the viscosity of liquid filling formulations for soft gels is in the range of 0.222-3000 cps.[25]

Rheological studies were carried out for all the liquid filling formulations in Brookfield DV-II PRO viscometer. The consistency of F1, F2, F3, and F5 formulations, in which 10 percent PVP K-30 was used, was like that of a fluid. Formulation, F6 with 15 percent PVP K-30 had thicker consistency. Formulation F4 with zero percent PVP K-30 showed very less viscosity. The viscosity was measured by plotting the shear stress on x-axis, and shear rate on y-axis. The resultant rheograms were straight lines. From the slope values viscosity was calculated for each formulation. The consistency and viscosity of the liquid fill formulations were related to each other because both are dependent on the concentration of PVP K-30.

All the formulations showed Newtonian type of fluid behaviour. A significant increase in viscosity was observed with increase in PVP K-30 concentration, as, because of PVP K-30, the system offers more resistance to flow. The decrease in shear viscosity with increasing shear rate is due to tendency of PVP K-30 molecules to orient more in the direction of shear. It is clearly evident that changes in the viscosity and consistency of liquid fill formulations for soft gels were because of change in concentration of PVP K-30. All the liquid ingredients in the formulations are co- solvents to ethanol. Therefore, solution form of dosage form, that shows Newtonian type of fluid behaviour, results in the liquid fill formulations for soft gels of nelfinavir mesylate, in the present investigation Dispersion/suspension/emulsion forms show pseudoplastic type of fluid behaviour when PVP K-30, PEG 400, Tween 80 are used. The viscosity of all the formulations was studied.

FTIR spectra for liquid fill formulations

FTIR spectrum of pure nelfinavir mesylate showed OH-group bending(alcohols) at 1659.48 cm^{-1},C=N stretching at 1642.46 cm^{-1}, N-H stretching at 3361.88 cm^{-1}, C-H bending at 836.56 cm^{-1}, S=O stretching at 1375.44 cm^{-1}, C-O stretching at 1358.14 cm^{-1} N-H bending at 1540.59 cm^{-1}. It indicates that there is no interaction between drug and excipients.

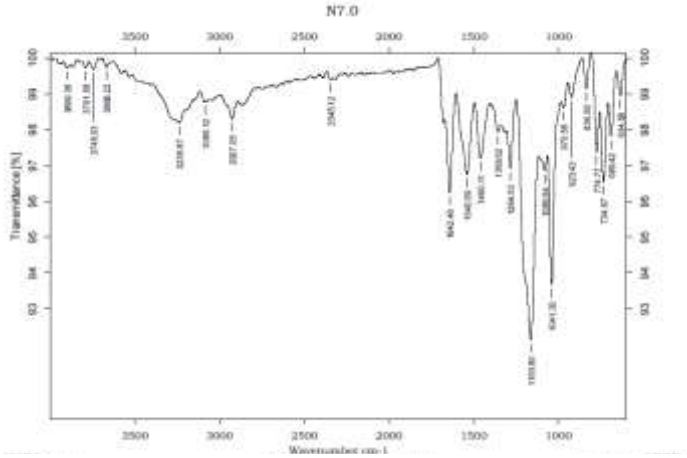

Figure 1: FTIR spectrum of pure Nelfinavir mesylate

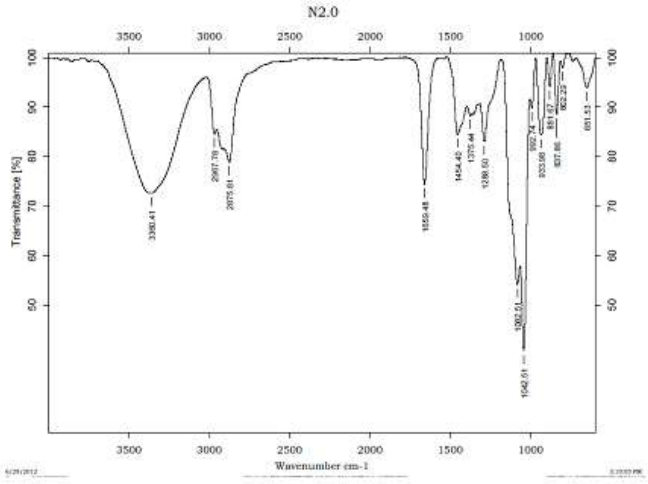

Figure 2: FTIR spectrum of F2 formulation of Nelfinavir mesylate

***In vitro* drug release studies**

In vitro drug release studies were carried out for both nelfinavir mesylate pure drug and liquid fill formulations for soft gels. The dissolution test helps to study the dissolution characteristics of drug and also measures the rate of release of drug from dosage form. For effective absorption of oral solid dosage forms, the dissolution of drug into surrounding medium plays an important role. It can be looked upon as a tool which provides valuable information about the bioavailability of a drug. *In vitro* dissolution studies were carried out in USP Type II (paddle method) dissolution apparatus (DISSO 8000, LAB INDIA). Dissolution medium was 900mL of 0.1N HCl and it was maintained at a temperature of 37 ± 0.5ºC, with an agitation speed of 50/100rpm for a period of 60 minutes. *In vitro* dissolution studies were performed to assess the dissolution parameters like drug percent released at 2 minutes (DP_2), at 4 minutes (DP_4), and first order release kinetic data for pure drug and its liquid fill formulations (F1 –F6).Initially, dissolution studies were carried out with NLF in powder form at a dosage strength of 250 mg. The DP_2 and DP_4 values for pure NLF were 3.6 ± 0.563 and 6.14 ± 0.461. The cumulative percent of NLF dissolved was found to be 33.97± 1.407 at the end of 120 minutes. All the dissolution studies were carried out in triplicate and in each case mean values and standard deviation values were calculated.

Selection of Apparatus, paddle/basket

In the present study, the effect of apparatus (Basket and paddle) on drug release from F1 was studied by maintaining the speed at 50 rpm. The cumulative percent NLF released was 99.52 ± 0.672 and 99.71 ± 0.88 in the case of basket and paddle methods at the end of 20 minutes and 15 minutes, respectively. Hence paddle method was selected. When paddle method was used the initial burst release at the end of 10 minutes was 85.01 ± 1.54 %, whereas it was 69.18 ± 3.22 % for the basket method. From the above results it was clear that the initial burst release and cumulative % NLF released in the case of paddle with sinker method was higher when

compared to the basket method. This may be due to the fact that in the paddle apparatus, the drug released has more surface area to get distributed in the entire dissolution bowl. In the basket method, initially the drug released has less surface area in the basket to get uniformly distributed throughout the bowl. This is according to Fick's law of diffusion.

Therefore, keeping in view of the dissolution profile obtained with paddle method, further studies were carried out using paddle method to obtain ideal and reproducible dissolution rates.

Selection of rpm

Agitation speed plays a vital role in drug release characteristics. Agitation speed affects the release of drug from the dosage form and thereby its dissolution in the dissolution medium. Therefore agitation speed is one of the crucial variables that affect the dissolution rate of the drug.

In the present investigation, the effect of agitation speed on drug release profiles of F1 and F2 was evaluated. F1 and F2 were evaluated with agitation speeds of 50 and 100 rpm. The cumulative percent NLF release from F1 was 99.71 ± 0.88 and 99.94 ± 0.64 with 50 and 100 rpm respectively. The cumulative % NLF released in the case of F2 was 99.91 ± 0.32 and 99.94 ± 0.64 with 50 and 100 rpm respectively.

When 50 rpm was applied, the initial burst release at the end of 5 minutes from F1 was 44.3 ± 1.94%. Whereas, the release from F1, was 78.54 ± 1.47 %, at the end of 4 minutes, when 100 rpm was applied. For formulation F2, when 50 rpm was applied, the initial burst release at the end of 4 minutes was 36.19 ± 1.430, whereas it was 100.79 ± 0.28 %, when 100 rpm was applied. Hence, from the above results it was clear that the initial burst release and cumulative % NLF release from F1 and F2 with 100 rpm was higher and faster when compared to values at 50 rpm. This may be due to faster drug release at higher agitation. From the above dissolution profiles obtained with 100 rpm, further studies were carried out with 100 rpm as agitation speed, to obtain ideal and reproducible dissolution rates.

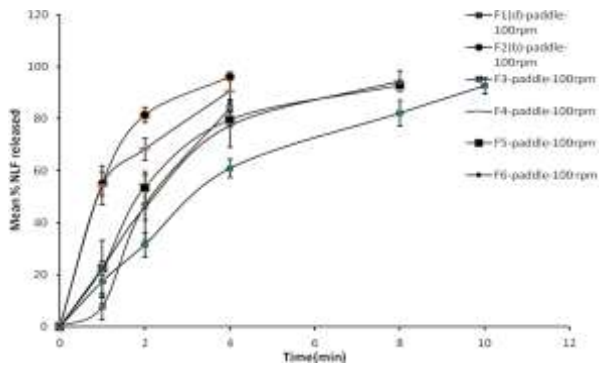

Figure 3: Comparative *in vitro* dissolution profiles of F1,F2,F3,F4,F5,F6 of Nelfinavir mesylate

Effect of PVP K-30 and Tween 80 on dissolution of NLF

PVP K-30, which inhibits the precipitation of NLF, if any, and affects the dissolution rate of NLF was used in all the formulations at different concentrations. In all the liquid fill formulations it was majorly used as viscosity modifier to maintain the gel consistency.

In this investigation, the NLF release profiles of formulations with and without PVP K 30 were evaluated at 100 rpm agitation speed. In F1, F2 and F3 formulations 10% PVP K-30 was used. In F1 formulation antioxidant was not used. In F2,F3,F4,F5,F6 antioxidant (0.1% BHT) was used. F2 was formulated with 10 percent Tween 80 and F3 with 5 percent Tween 80. Formulation F3 had more percent of propylene glycol compared to F2. The DP_2 and DP_4 values for these liquid fill formulations F1, F2 and F3 were 53.16 ± 3.547, 81.74 ± 2.782, 41.78 ± 1.979 and 78.54 ± 1.477, 100.79 ± 0.287, 71.62 ± 1.755 respectively. Formulation F2 showed better results. F3, even though PG concentration was more, showed prolonged dissolution upto 8 minutes, because of less percent of Tween 80 than F2. Tween 80 is a non-ionic surfactant and is used as a solubility enhancer.

A 12.78, 16.40, 11.65 folds increase in DP_4 values was observed with formulations F1, F2 and F3 respectively, when compared to pure NLF data. Formulation F2 contains ten percent Tween 80. Tween 80 is a non-ionic surfactant used as a solubility enhancer through micellar solubilisation technique. The critical micellar concentration of Tween 80 is 0.03 percent. Above critical micellar concentration, Tween 80 shows solubility enhancing action. The solubilizing action of Tween 80 increases with increase in its concentration. Surfactants are amphiphiles having both lipophilic and hydrophilic portions within the same molecule. At low concentration surfactant molecules exist independently and at high concentration they form aggregates called micelle. Critical micellar concentration (CMC), is the concentration at which micelle are formed. At CMC, physical properties of a surfactant such as surface tension, interfacial tension, osmotic pressure, and electrical conductance change sharply. HLB value of Tween 80 is 15. Tweens are hydrophilic in nature and have high HLB values. Formulation F2 with ten percent Tween 80 got better dissolution release than F3 with five percent Tween 80.

In F4, F5 and F6 formulations, further studies were carried out to study the effect of concentration of PVP, in the range of 0%, 5%, 15% w/w respectively. The DP_2 and DP_4 values for these liquid filling formulations F4, F5, F6 were 70.86 ± 3.363, 89.02 ± 2.365, 40.02 ± 2.618 and 99.04 ± 0.810, 100.18 ± 0.380, 69.79 ± 0.964 respectively. When PVP K-30 concentration was increased in F6 to 15%, the dissolution was prolonged to 10 minutes even with 100 rpm agitation speed which may be due to high viscosity of the formulation that

inhibits drug release. A 16.11,16.30,11.35 folds increase in DP_4 values was observed with formulations F4, F5 and F6 respectively, when compared to pure NLF data.
From the comparison of the above six formulations, DP_4 values for F2, F4, and F5 have 100 % release. F4 with zero percent PVP K-30 due to low viscosity was eliminated from the study. Formulation F2 (10% PVP K-30) was selected because it showed 100 % drug release within 4 minute interval along with good gel consistency. F5 with only five percent PVP K-30 was also discarded from the study because of lack of optimum gel consistency. Finally F2 was regarded as the optimized formulation. F2 and F5 were compared for release profiles and release kinetics.

First order release kinetics
The dissolution data were analyzed as per zero order and first order kinetics in each case. The r^2 values were higher in the first order model than in the zero order models indicating that the release of NLF from these liquid fill formulations followed first order kinetics. The first order rate constant 'k' (min^{-1}) values for liquid fill formulation were calculated from dissolution data by fitting the data into first order equation. First order kinetic values were significantly higher for NLF liquid filling formulations compared to NLF. The 'k' (min^{-1}) values for pure NLF and its formulations F1, F2 were 0.018, 0.109, 0.103 at 50 rpm and F1, F2, F3, F4, F5, F6 were 0.375, 0.818, 0.269, 0.614, 0.877, 0.371 at100 rpm. The effect of agitation speed on 'k' values was also studied. The 'k' values for formulations F1 and F2 were 0.109, 0.103 at 50rpm and whereas at 100rpm the values were 0.375, 0.818. A 7.9 folds increase in 'k' value for F2 at 100rpm when compared to F2 at 50rpm. A 1.32, 2.2 folds increase in 'k' value was observed for formulation F2 (10 % PVP) when compared to formulations F4 (0% PVP) and F6 (15% PVP) respectively. The 'k' values are significantly higher for liquid fill formulations containing PVP K-30 when compared to formulations without PVP K-30.

The order of coefficient of determination (R^2) values for all the formulations was F2>F4>F6>F3>F1>F5. In F2 formulation the R^2 values are higher and it indicates that F2 has superior release characteristics at 100 rpm speed among all the liquid fill formulations and also comapared with 50 rpm. This may be due to more uniform release of the drug from the F2 formulation that followed first order because of optimized concentration of excipients.
Finally, the release kinetics showed that F2 best fits the first order release kinetics among all the formulations. The viscosity modifier, PVP k-30 was added in this formulation as per the specified limits and better release of NLF was observed compared to remaining formulations.

Stability studies
The optimised liquid fill formulation F2 was charged in triplicate on accelerated stability studies and monitored for appearance, pH, percent drug content, viscosity, *in vitro* dissolution profile studies, at 40 ± 2°C / 75 ± 5 % RH for 6 months. Physical observation and drug release studies were conducted after 3 months of storage and after 6 months of storage. The stability study reveals no significant variation in appearance, pH, percent drug content, viscosity, *in vitro* dissolution profile studies up to three and six months. Statistical analysis was done by paired t-test, to verify whether the differences observed between the physical parameters before and after storage at 40 ± 2°C and at 75±5% relative humidity (RH), for 6 months period, were significant or not. No significant difference was observed in any case (P>0.05). The results thus indicated that the formulation was stable under accelerated conditions of temperature and humidity.

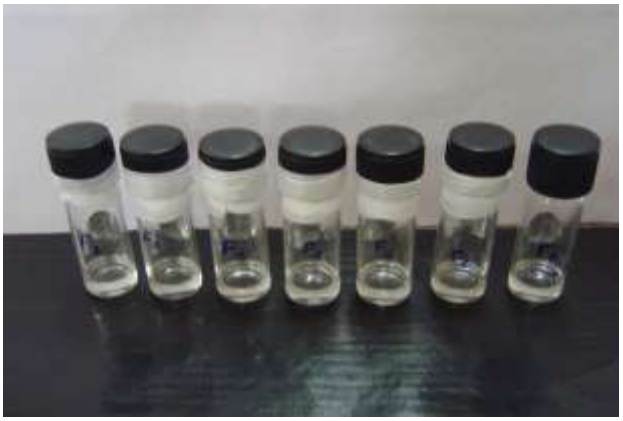

Figure 4: Appearance at zero months

Figure 5: Appearance at 3 months

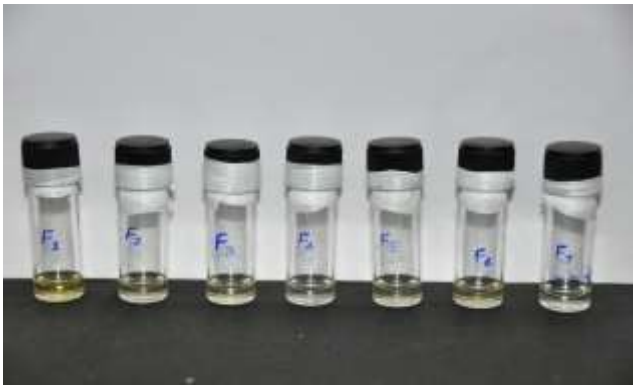

Figure 6: Appearance at 6 months

***In vivo* studies**

As per *in vivo* study protocol, nelfinavir mesylate products were administered per orally to healthy rabbits at a dose equivalent to 9 mg of nelfinavir/kg of body weight of rabbit and the plasma concentrations were determined by HPLC method.

Pharmacokinetic parameters were determined, after the oral administration of pure drug (N) suspension, elimination rate constant k_{el} was found to be 0.203 hr^{-1} and the corresponding biological half-life ($t_{1/2}$) was found to be 3.412 hours. The MRT was found to be 5.39 hours. A peak plasma concentration of 290 ng/ml was observed at 1.5 hours after administration of pure drug suspension (N).When the experimental SGN formulation was administered orally, peak concentration of 790 ng/ml was observed at 2 hours. The elimination rate constant k_{el} for nelfinavir was found to be 0.1098 hr^{-1}, and the corresponding biological half life ($t_{1/2}$) was found to be 6.31 hours. The MRT was found to be 10.205 hr. Very high C_{max}, AUC0-∞ (extent of absorption) and $AUMC_{0-\infty}$ were observed with SGN, when compared to N. So, it may be concluded, that the test formulation SGN of nelfinavir is having higher absorption and bioavailability in comparison with the pure drug N.

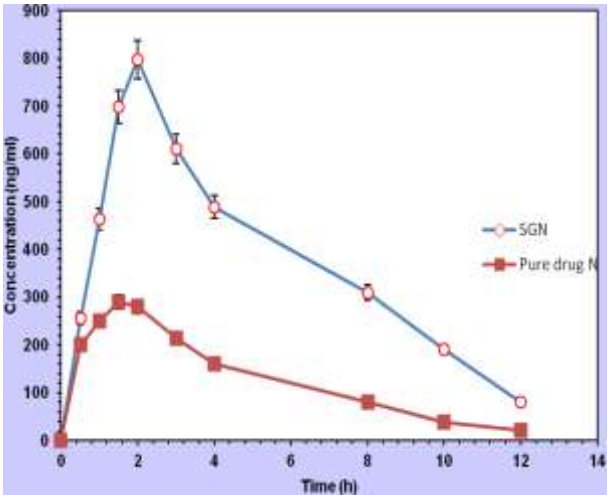

Figure 7: Comparative *in vivo* drug release profiles of pure Nelfinavir mesylate(N) and liquid fill formulation for soft gels of Nelfinavir mwsylate(SGN)

CONCLUSION

Development and evaluation of a liquid fill for soft gel of nelfinavir mesylate was successfully achieved which can be used as an immediate release dosage form that can give greater bioavailability than a tablet of the same strength.

Conflict of interest

The authors declare that they have no conflict of interests.

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