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Preparation and Evaluation of Sustained Release Nelfinavir Microspheres by Emulsification-Solvent Evaporation Method

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ABSTRACT:

The present study aimed to formulate and evaluate sustained release microspheres of nelfinavir using HPMCK15M, HPMCK100M, ethyl cellulose, and carbopol as encapsulating polymers through a W/O/O double emulsification–solvent evaporation method. Nelfinavir, an antiretroviral drug with a short half-life (3.5 h), requires frequent dosing; sustained release formulations can reduce dosing frequency and maintain therapeutic plasma levels. To address poor encapsulation efficiency, Tween-80 was used as stabilizer under constant stirring (750–1000 rpm) for 7–10 hours, followed by centrifugation. Prepared microspheres were characterized for drug–excipient compatibility (FTIR), yield, drug entrapment, loading, particle size, flow properties, surface morphology (SEM), solid-state properties (DSC), in-vitro dissolution, and release kinetics. The percentage yield ranged from 75.4–80.8%. Angle of repose (25º–30º) and Carr's index (9.37–19.81%) indicated excellent flowability. Particle size varied from 96.3–253.3 µm, increasing with polymer concentration. Drug content and encapsulation efficiency ranged between 64.4–79.08%, with maximum entrapment observed in formulation F7 (79.08%). SEM revealed spherical, smooth microspheres with uniform polymer coating. FTIR showed no drug–polymer interaction, while DSC confirmed transformation from crystalline to amorphous form. In vitro studies showed slower drug release in acidic medium (pH 1.2) compared to alkaline buffer (pH 7.4). The optimized formulation (F7) achieved 98.4% drug release, sustained up to 12 hours, following Higuchi diffusion kinetics. Stability studies indicated consistent performance. Overall, nelfinavir-loaded microspheres demonstrated good yield, flow properties, satisfactory encapsulation, and sustained release potential. The developed system can minimize dosing frequency and maintain therapeutic drug levels, offering an effective controlled drug delivery approach for long-term management of HIV infection.

Keywords*:* Nelfinavir, ethyl cellulose, carbopol, hydroxyl propyl methyl cellulose- HPMCK100M, HPMCK15M and solvent evaporation method.

INTRODUCTION:

The population of patient with chronic diseases has recently been increasing. These situations necessitate taking drug for a long period and/or multiple medicines simultaneously, which can lead to increase in non-compliance. The problem would be worse for drugs with short biological half-life. One method to solve such problems is to find a dosage form capable of releasing the drug gradually. With many drugs, the basic goal of therapy is to achieve a steady-state blood or tissue level that is therapeutically effective and nontoxic for an extended period. The design of proper dosage regimen is an important element in accomplishing this goal. A basic objective in dosage form design is to optimize the delivery of medication to achieve a measure of control of therapeutic effect in the face of uncertain fluctuation in the *in-vivo* environment in which drug release takes place. This is usually accomplished by maximizing drug availability i.e. by attempting to attain a maximum rate and extent of drug absorption; however control of drug action through formulation also implies controlling bioavailability to reduce drug absorption rates.[1] For any drug therapy to be successful, the drug must reach the target tissue or systemic circulation in optimum concentration which should be maintained for desired time. Therapeutic response of the drug also depends on the pharmacokinetics of the drug in an individual patient and frequency of dosing. Drug with short half-life also require frequent dosing, some problem may be arises like patient noncompliance to the prescribed drug regimen, particularly in case of chronic treatment or in the treatment of a silent disease such as arthritis.[2] Most of the Antiviral Drugs are easily absorbed from the GIT and are eliminated quickly from the blood circulation due to their short half-life. Administration of such drugs in the form of conventional drug delivery may cause several problems to the patient. Because

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these drugs must be taken 3 to 5 times daily this causes problems to the patients. To avoid these problems controlled drug delivery systems have been developed as they releases the drug slowly into the GIT and maintain a constant drug concentration in the serum for longer period of time for the treatment of chronic infectious disease associated with the frequent medication. As the drug needs to be given repeatedly, there may be accumulation which leads to toxicity. In such circumstances, the problem can be solved by developing new drugs or dosage forms, like prolonged release dosage form with similar therapeutics response as that of conventional dosage forms and longer duration of action**.** Sustained release systems include any drug delivery system that achieves slow release of drug over an extended period. More precisely, sustained drug delivery can be defined as "Sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects.[3,4] In sustained release dosage forms, a sufficient amount of drug is initially made available to the body to cause a desired pharmacological response. The remaining fraction is released periodically and is required to maintain the maximum initial pharmacological activity for some desirable period of time in excess of time expected from usual single dose. A sustained release is facilitated through the consistent rejuvenation of drug molecules. For last so many decades conventional dosage forms like tablets, capsules, pills, powders, parenteral preparations, solutions, emulsions, suspensions, creams, ointments and aerosols are used in the treatment of acute or chronic diseases. Even today, these formulations can be considered as primary pharmaceutical products commonly seen in the market[5].

Long acting dosage forms can be broadly classified into the following classes as 1. Sustained release a) Prolonged release, b) Controlled release. 2. Delayed release. 3. Repeat action.

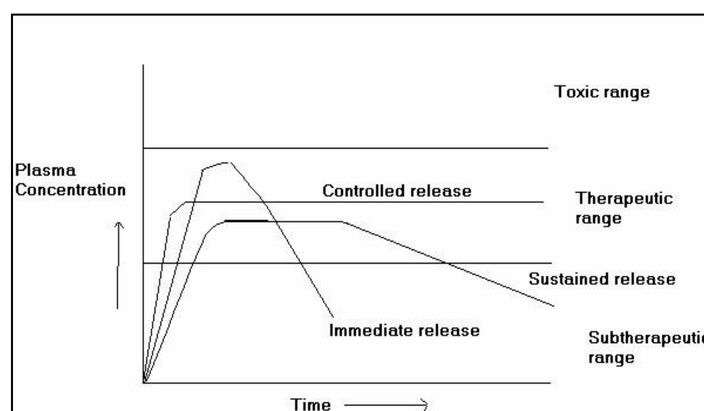

Figure No.1: Plasma concentration Vs Time profile showing difference between controlled release, sustained release and immediate release

Sustained drug delivery can be defined as "Sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects. Prolonged release system can be defined as "system that provides the duration of action over than that achieved by conventional delivery". Controlled release System can be defined as "system that delivers an agent at a controlled rate for an extended time". Delayed release system can be defined as "system that provides at least a twofold reduction in dosing frequency as compared to conventional fast release dosage form". Repeat action preparation can be defined as the one which provides a usual single dose of drug and is so designed to provide another single dose at some later time i.e. it sequentially release two full dose of drug. "Repeat action" preparation is the multiple dosing would give rise to a number of problems, which can be overcome by sustained release drug formulations. Wide ranges of probable method are available for the development of controlled release dosage form, micro-encapsulation being one of them[6, 7]. The objectives of oral sustained release formulations are to attain blood concentration of the drug rapidly, this will elicit the desired therapeutics effects, to maintain the concentration at constant level for a desired period of time, to reduce the frequency of doses administrated as compared to conventional dosage form, to give a uniform biological response and reduce the incidence of side effects. The advantages of sustained release formulations are **e**mploy less total drug that minimize or eliminates local side effects, minimize or eliminates systemic side effects, minimize drug accumulation with chronic dosing, obtains less potentiation or reduction in drug activity on chronic use, enhanced patient compliance and convenience, reduction in dosing frequency, improve efficiency in treatment by cure or control of condition, improve or control condition, make use of specific effect (E.g. SR Aspirin for morning relief of Arthritis), improve bioavailability of some drugs, reduced fluctuations in circulating drug levels, more uniform effect, safety margin of potent drug is increased by technically excellent designing of formulation, patients care time is reduced, night time dosing can be avoided for patient convenience and product life time is increased[8]. The ideal characteristics of drug for sustained release formulations are a number of drug characteristics were determine whether the drug is suitable for SR formulation or not, needed to be considered such as biological half-life, therapeutics index, dose of drug, absorption kinetics, solubility, first pass effect and stability. The different mechanisms of drug release in controlled release systems are dissolution-controlled release, osmotically-

controlled release, diffusion-controlled release, erosion-controlled release, and miscellaneous controlled release such as ion-exchange resins, drug-coated micropellets (formulated by altering the densities), barrier coating and embedment in slowly eroding matrix, embedment in plastic matrix repeat action[9]. Microspheres are small spherical particles, with diameters in the micrometer range (typically1 µm to 1000 µm). Microspheres are sometimes referred to as microparticles. Microspheres are characteristically flowing powders consisting of proteins or synthetic polymers. The range of techniques for the preparation of microspheres offers a variety of opportunities to control aspects of drug administration and enhance the therapeutic efficacy of a given drug. There are various in delivering a drug or therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs also known as micro particles. It is the reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest. Microspheres can be manufactured from various natural and synthetic materials such as glass microspheres, polymer microspheres and ceramic microspheres commercially available. Solid and hollow microspheres vary widely in density and, therefore, are used for different applications. Hollow microspheres are typically used as additives to lower the density of a material[10, 11]. Solid microspheres have numerous applications depending on what material they are constructed of and what size they are. Polyethylene and polystyrene microspheres are two most common types of polymer microspheres. Polystyrene microspheres are typically used in biomedical applications due to their ability to facilitate procedures such as cell sorting and immune precipitation. Polystyrene microspheres are suitable for medical research and biological laboratory experiments and commonly used as permanent or temporary filler. Lower melting temperature enables polyethylene microspheres to create porous structures in ceramics and other materials. High sphericity of polyethylene microspheres, as well as availability of coloured and fluorescent microspheres, makes them highly desirable for flow visualization and fluid flow analysis, microscopy techniques, health sciences, process troubleshooting and numerous research applications. Charged polyethylene microspheres are also used in electronic paper digital displays[12]. Glass microspheres are primarily used as filler for weight reduction, retro-reflector for highway safety, additive for cosmetics and adhesives, with limited applications in medical technology. Ceramic microspheres are used primarily as grinding media. Microspheres vary widely in quality, sphericity, uniformity of particle and particle size distribution. The appropriate microsphere needs to be chosen for each unique application. The advantages of microspheres are delivers the drug to the, target site with specificity, solid biodegradable microspheres have the potential throughout the particle matrix for the controlled release of drug, Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumor, The size, surface charge and surface hydrophilicity of microspheres have been found to be important in determining the fate of particles *in-vivo,* Studies on the macrophage uptake of microspheres have demonstrated their potential in targeting drugs to pathogens residing intracellularly. The ideal characteristics of microspheres are they ability to incorporate reasonably high concentrations of the drug, stability of the preparation after synthesis with a clinically acceptable shelf life, controlled particle size and dispersability in aqueous vehicles for injection, release of active reagent with a good control over a wide timescale and biocompatibility with a controllable biodegradability. The types of microspheres are bioadhesive microspheres, magnetic microspheres, floating microspheres, radioactive microspheres and polymeric microspheres[13, 14]. The various methods of preparation of microspheres are 1. Solvent evaporation method a) Single emulsion technique, b) Double emulsion technique. 2. Coacervation phase separation method. 3. Spray drying and Spray congealing method. 4. Polymerization method.

Solvent Evaporation Method: a) Single emulsion technique: The micro particulate carriers of natural polymers, i.e. those of proteins & carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved/ dispersed in aqueous medium followed by dispersion in the non-aqueous medium (E.g.: oil) as shown in figure 2. In the 2nd step, cross-linking of the dispersed globule is carried out either by means of heat or by using chemical cross linkers. The chemical crosslinking agents used gluteraldehyde, formaldehyde, terephthalate chloride, diacidchloride and calcium chloride. Cross-linking by heat is effected by adding the dispersion to previously heated oil. Heat denaturation is not suitable for the thermo labile drugs while the chemical cross-linking suffers disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation[15].

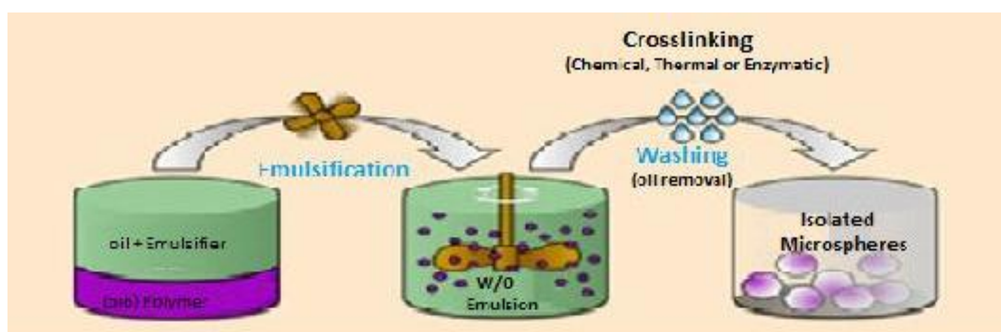

Figure No.2: Processing scheme for microspheres-preparation by single emulsion technique

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b) Double emulsion technique: Involves the formation of the multiple emulsions or the double emulsion of type w/o/w & is best suited to the water-soluble drugs, peptides, proteins and vaccines. The aqueous protein solution is dispersed in a lipophilic organic continuous phase which is generally consisted of polymer solution that eventually encapsulates protein contained in dispersed aqueous phase as shown in figure 3. The primary emulsion is then subjected to the homogenization before addition to aqueous solution of PVA. This results in formation of double emulsion which is then subjected to solvent removal by solvent evaporation maintaining the emulsion at reduced pressure or by stirring so that organic phase evaporates out. Examples: hydrophilic drugs like LHRH agonist and vaccines[16].

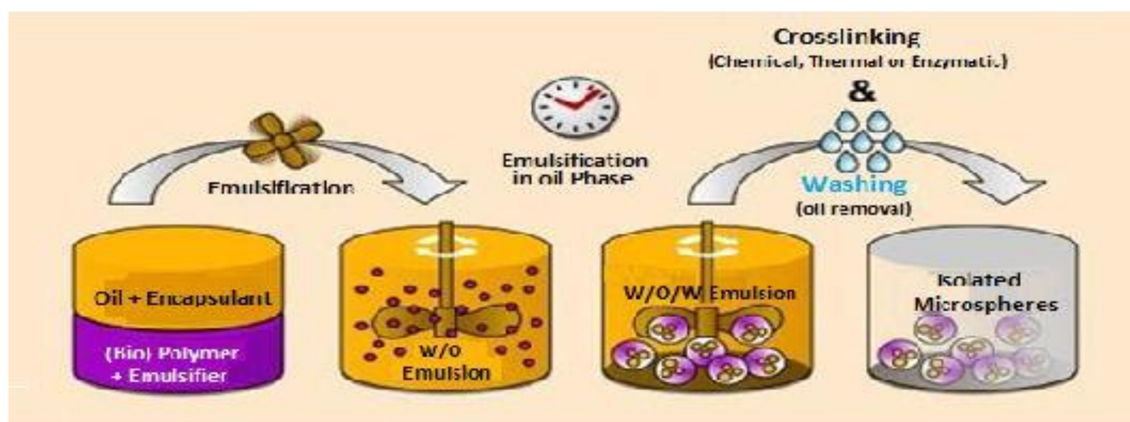

Figure No.3: Processing scheme for microspheres preparation by double emulsion technique

Double emulsion method of microspheres preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w and w/o/o are best suited to water soluble drugs, peptides, proteins, vaccines and water insoluble drugs.

New applications for microspheres are discovered day by day, below are just a few medical application such as release of proteins, hormones and peptides over extended period of time. Gene therapy with DNA plasmids, also delivery of insulin, vaccine delivery for treatment of diseases like hepatitis, influenza, pertusis, ricin toxoid, diphtheria, birth control, passive targeting of leaky tumour vessels, active targeting of tumour cells, antigens, by intra-arterial/intravenous application and tumour targeting with doxorubicin and also treatment of leishmaniasis. Magnetic microspheres can be used for stem cell extraction and bone marrow purging. Radioactive microspheres application can be used for radioembolisation of liver and spleen tumors, imaging of liver, spleen, bone marrow, lung and even imaging of thrombus in deep vein thrombosis can be done. Fluorescent microspheres can be used for membrane based technologies for flow cytomettry, cell biology, microbiology, fluorescent linked immuno-sorbent assay. Yttrium-90 can be used for primary treatment of hepatocellular carcinoma and also used for pre-transplant management of HCC with promising results. Some microsphere products in the market are tretinoin gel microspheres for the topical treatment of acne vulgaris. Dexamethasone microspheres are used for its anti-inflammatory action[17, 18]. Azithromycin extended release (Zmax, Pfizer Inc) microspheres is a novel single-dose administration formulation of azithromycin approved by FDA in June 2005. It is currently being used for the treatment of community acquired pneumonia and acute bacterial sinusitis (Zmax package insert). Acetazolamide microspheres are widely used in the treatment of glaucoma and also used as diuretic which are prepared by solvent evaporation technique. Degradable starch microspheres (DSM) are also known as spherex are most frequently used microsphere system for nasal drug delivery and the other applications and drugs available in the market are metformin HCL, amoxicillin trihydrate, ibuprofen, trimetazidine HCL, furosemide, acyclovir, atenolol, propranolol, rantidine HCL and glipizide etc. The applications of microspheres are to mask the bitter taste of drugs like paracetamol, nitrofurantoin etc and to reduce gastric and other G.I. tract irritations. Sustained release aspirin preparations have been reported to cause significantly less G.I. bleeding than conventional preparations. A liquid can be converted to a pseudo-solid for easy handling and storage (eg. Eprazinone). Hygroscopic properties of core materials may be reduced by microencapsulation (eg. sodium chloride). Carbon tetrachloride and a number of other substances have been microencapsulated to reduce their odour[19, 20].

MATERIALS AND METHODS:

Nelfinavir is a gift sample from hetero drugs pvt ltd, HPMCK100M, HPMCK15M, ethyl cellulose and carbopol are from lobachem pvt ltd, light liquid paraffin, tween-80 and n-hexane are from SD fine chemicals pvt ltd, dichloromethane and methanol are from spectrochem pvt ltd Mumbai, all the ingredients are AR grade.

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METHODS:

The preformulation studies with nelfinavir obtained were performed using conventional and reported techniques. In the present work, preformulation parameters such as U.V-Visible spectroscopy, solubility, flow properties, drug crystallinity, drug excipient compatibility studies (FTIR), melting point and pH were performed and determined. Preformulation testing is the first step in the rationale development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients[21]. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass-produced. The pH of nelfinavir was carried out by digital pH meter and melting point of nelfinavir was determined by open capillary method. The solubility of nelfinavir was determined in different solvents like water, methanol, chloroform, pH 1.2 and pH 7.4 Phosphate buffer. Excess amount of drug was added to the 5 mL of the buffers in a 25mL stoppered conical flasks and the mixtures were shaken for 24 hours at room temperature (28±1oC) on rotary shaker. After 24 hours of shaking 1mL aliquots were withdrawn at different time intervals and filtered immediately using a 0.45µ nylon disc filter. The filtered samples were diluted suitably and assayed for nelfinavir by measuring the absorbance at 254nm. Shaking was continued until three consecutive estimations were same[22].

Determination of λ_{max} of Nelfinavir (pH 1.2 Buffer): Nelfinavir 10µg/ml solution was prepared by using p^H 1.2 hydrochloric acid buffer. This prepared solution was scanned from 200-400 nm in UV-Visible Spectrophotometer against pH 1.2 hydrochloric acid buffer as blank solution. The maximum absorbance of the drug solution was found to be 254nm and this was determined as λ_{max} of the nelfinavir.

Determination of λ_{max} of Nelfinavir (pH 7.4 Buffer): Nelfinavir 10µg/ml solution was prepared by using pH 7.4 phosphate buffer. This solution was scanned from 200-400nm in UV-Visible spectrophotometer against pH 7.4 phosphate buffer as blank solution. The maximum absorbance of the drug solution was found to be 254nm and this was determined as λ_{max} of the nelfinavir.

Standard graph of Nelfinavir:

Preparation of Primary stock solution: Weigh accurately 10mg of nelfinavir and transferred into a clean and dried 10ml volumetric flask. The drug was then dissolved and diluted up to the mark with buffer solution.

Preparation of Secondary stock solution: From Primary stock solution 1ml was pipetted out and transferred into a clean and dried 10ml volumetric flask and diluted up to the mark with buffer solution.

Preparation of Sample solution: From this solution aliquots of 0.5, 1, 1.5, 2, 2.5, 3ml were transferred to 10ml volumetric flask and diluted up to the mark with buffer solution to get 5, 10, 15, 20, 25 and 30µg/ml of nelfinavir respectively. The absorbance was measured in the UV-Visible spectrophotometer at 254nm using buffer solutions as blank and graph of concentration versus absorbance was plotted[23].

Preparation of microspheres of nelfinavir (Emulsification-solvent evaporation method): The nelfinavir loaded microspheres were prepared by Emulsification-solvent evaporation technique. Require quantity of polymer was dissolved in a mixture of equal volumes methanol and dichloromethane (1:1) at room temperature and stirred until a homogenous solution was formed. Core material nelfinavir was added to the polymer solution and mixed thoroughly. The resulting mixture was then added to the beaker containing 100 ml of liquid Paraffin and 0.01% of tween-80 was added and maintained at room temperature while stirring at 600rpm as shown in table 1. The resultant mixture was stirred for 4 hours until the solvent evaporated completely. The liquid paraffin was decanted, the microspheres were collected, washed twice in n-hexane to remove any adhering oily phase and was air dried for at least 12 hours to obtain discrete microspheres[24,25,26].

Table No.1: Formulation design of nelfinavir microspheres:

S.No	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
1	**NELFINAVIR (mg)**	500	500	500	500	500	500	500	500
2	**HPMCK100M (mg)**	500	-	-	-	1000	-	-	-
3	**HPMCK15M (mg)**	-	500	-	-	-	1000	-	-
4	**ETHYL CELLULOSE (mg)**	-	-	500	-	-	-	1000	-
5	**CARBOPOL 934 (mg)**	-	-	-	500	-	-	-	1000
6	**TWEEN-80 (ml)**	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
7	**METHANOL (ml)**	10	10	10	10	10	10	10	10
8	**DICLOROMETHANE (ml)**	10	10	10	10	10	10	10	10
9	**LIQUID PARAFFIN (ml)**	100	100	100	100	100	100	100	100
10	**n-HEXANE (ml)**	10	10	10	10	10	10	10	10

CHARACTERIZATION AND EVALUATION OF MICROSPHERES:

Tapped Density: The microspheres were tapped gently on surface till the powder occupies maximum volume and noted the volume as tapped volume. The mechanical tapping of cylinder was carried out manually 50 times. The tapped density was calculated in g/cm^2 by the following formula.

Tapped density = Weight of microspheres/Tapped volume.

Carr's compressibility index: The percentage compressibility index was calculated according to following formula.

$$\% \text{ Compressibility index} = [1 - V/V_0] \times 100$$

Where, V and V_O are the volume of the sample after and before the standard tapping respectively. Each determination was made in triplicate.

Angle of repose (θ): The frictional forces in floating microspheres can be measured by the angle of repose θ. This is the maximum angle possible between the surface of a pile of microspheres and the horizontal plane. A funnel is fixed at a particular height 'h' on a burette stand. A white paper is placed bellow the funnel. The sample is passed slowly through the funnel until it forms a pile further addition of drug stopped as soon as the drug pile touches the tube of the funnel. Circle of the pile of drug is drawn without disturbing the pile of radius of the pile is noted as shown in table 2. Angle of repose is calculated from the following formula:

$$\tan\theta = h/r$$

Θ= angle of repose degrees, h= height of pile, r= radius of the pile in cm

Table No.2: Angle of repose

S.N0	Angle of repose (θ)	Carr's index	Type of flow
1	<25	5-15	Excellent
2	25-30	12-16	Good
3	30-40	18-21	Passable
4	-	23-35	Poor
5	-	33-38	Very poor
6	>40	>40	Extremely poor

Particle size analysis: Determination of average particle size of nelfinavir microspheres was carried out by optical microscopy in which stage micrometer was employed. Minute quantity of microsphere was spread on a clean glass slide. The particle size of the

microparticles was measured, along the longest axis and the shortest axis (cross shaped measurement). Average of these two readings was given as mean diameter of particles and average size of 25 microspheres was determined in each batch[27].

Percentage yield: Percentage yield is calculated to know about percentage yield or efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of nelfinavir microspheres recovered from each batch in relation to the sum of starting material. The percentage yield of prepared microspheres was determined by using the following formula:

Percentage yield = (Practical yield/theoretical yield) x 100.

Theoretical drug content: It was determined by calculation assuming that the entire nelfinavir present in the polymer solution gets entrapped in microspheres, and no loss occurs at any stage of preparation of microspheres.

Practical drug content: A weighed quantity of microspheres (equivalent to 100 mg of a drug) was crushed into powder and added to 100 ml of phosphate buffer of pH 7.4. The resulting mixture was kept stirring at 1000 rpm for 2 hrs and kept it overnight for 24 hrs. Then the solution was filtered through membrane filter (0.45µm pore size) and 1 ml of this solution was diluted using phosphate buffer of pH 7.4 and analysed spectrophotometrically for nelfinavir content at 254 nm using a regression equation derived from the standard graph (R^2=0.999) to get practical drug content.

Determination of percentage drug entrapment (PDE): A weighed quantity of microspheres (equivalent to 100 mg of a drug) was crushed into powder and added to 100 ml of phosphate buffer of pH 7.4. The resulting mixture was kept stirring at 1000 rpm for 2 hrs and kept it overnight for 24 hrs. Then the solution was filtered through membrane filter (0.45 µm pore size) and 1 ml of this solution was diluted using phosphate buffer of pH 7.4 and analysed spectrophotometrically for nelfinavir content at 254 nm using a regression equation derived from the standard graph (R2=0.999) to get practical drug content.

The percentage drug entrapment was calculated as follows.

$$\textbf{Drug entrapment efficiency (\%)} = \frac{\textbf{Amount of drug actually present}}{\textbf{Theoretical drug load expected}} \times 100$$

Surface morphology (SEM): Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. SEM studies were carried out by using JEOL 840A scanning electron microscope (Japan). Dry nelfinavir microspheres were placed on an electron microscope brass stub and coated with in an ion sputter. Pictograms of nelfinavir microspheres were taken by random scanning of the stub[28].

Drug-excipient compatibility studies (FTIR studies): The FT-IR analysis was conducted for the analysis of drug-polymer interaction and stability of the drug during microencapsulation process. The FT-IR spectrum of pure nelfinavir, HPMCK15M, HPMC K100M, ethyl cellulose, carbopol was studied by FTIR spectrometer (Shimadzu 8400S, Tokyo, Japan) and the spectrum was scanned over the wave number range of 4000-400 cm-1 in a scan time of 12 minutes. 2% (w/w) of the sample, with respect to a potassium bromide was mixed with dry KBr and the mixture was grinded into a fine powder using mortar and then compressed into a KBr discs in a hydraulic press at a pressure of 10000 PSI. Each KBr disc was scanned 10 times at a resolution of 2 cm^{-1}. The characteristic peaks were recorded. IR helps to confirm the identity of the drug and to detect the interaction of the drug with the carriers.

Differential scanning calorimetry (DSC): DSC studies were performed to understand the nature of the encapsulated drug in the polymer and the physical state of drug in the polymer matrix would also influence its release characteristics. To probe this effect, DSC analysis was performed. Thermal analysis and properties of the powder samples (Nelfinavir and optimized nelifinavir microspheres) were investigated with a DSC (Shimadzu DSC 60, Tokyo, Japan). Approximately 10mg of sample was analyzed in an open aluminum pan, and heated at scanning rate of 10°C/min between 0°C and 400°C under nitrogen atmosphere. Magnesia was used as the standard reference material to identify physical changes of drug in the formulation. Hence, it indicates the physical nature of drug is changed in the formulation from crystalline to amorphous.

***In vitro* dissolution studies:** The *in-vitro* release rate of nelfinavir was carried out for 12 hours using basket type dissolution apparatus containing 900 ml of dissolution medium maintained at 37±0.5ºC and speed of agitation at 100 rpm. An accurately

weighed quantity of microspheres were suspended in dissolution medium consisting 900 ml of simulated gastric (buffer solution pH 1.2) fluid, and dissolution was carried out for 2 hours. Then the dissolution medium was changed to simulated intestinal (phosphate buffer pH 7.4) fluid, and the process was further continued for up to 12 hours. At present time intervals 5 ml aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. After suitable dilutions, the samples were analysed spectrophotometrically at 254 nm. The release data obtained were fitted into various mathematical models like zero order, first order, higuchi, korsmeyer-peppas to know the mathematical model which is best fitting the obtained release profile[29].

Drug release kinetics (Harris shoaib et al., 2006): The obtained dissolution data was fitted into mathematical equation for zero order, first order, highuchi model and korsemeyer equation/peppa's model in order to describe and analyse the *in-vitro* release data and mechanism of drug release from the microspheres formulations. The results of *in-vitro* release profiles obtained for all the best formulations were fitted into four models of data treatment as follows such as cumulative percent drug released versus time (zero-order kinetic model), log cumulative percent drug remaining versus time (First-order kinetic model), cumulative percent drug released versus square root of time (Higuchi's model) and log cumulative percent drug released versus log time (Korsmeyer-Peppas equation). The zero order describes the systems where the drug release rate is independent of its concentration. The first order describes the release from system where release rate is concentration dependent. Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on fiction diffusion.

Stability studies: The purpose of stability study is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature and humidity and to establish a retest period for the drug substance or a shelf-life for the drug product and recommended storage conditions. The stability study of the selected formulations of nelfinavir loaded microspheres was carried out by storing 1 gm of micro particles in an amber colored screw capped bottle for a period of 3 months at 5-8ºC, 27ºC, 42ºC temperature and 75±5% RH using stability chamber. Sampling was carried out at 30 days interval and examined for percent entrapment[30].

RESULTS AND DISCUSSION:

Nelfinavir, an anti-retroviral drug was selected as novel drug for the experiment. Nelfinavir microspheres were prepared by solvent evaporation technique using HPMCK15M, HPMCK100M, carbopol, ethyl cellulose as a rate-controlling polymer and the microspheres were evaluated. From the scanning of drug in simulated gastric fluid (pH 1.2) and phosphate buffer (pH 7.4), it was concluded that the drug had λmax of 254 nm. The linear equations were obtained for simulated gastric fluid is $y = 0.0174x$ $R^2 = 0.998$ and for saline phosphate buffer is $y = 0.01736x$ $R^2 = 0.999$. In the present work, preformulation parameters such as FTIR, solubility study, melting point, U.V-Visible spectroscopy were performed. The prepared microspheres were then subjected to various evaluation studies such as tapped density, angle of repose, compressibility index, percentage yield (%), particle size analysis, drug content, drug entrapment efficiency, SEM, *In-vitro* dissolution studies, release order kinetics, DSC and stability studies. Solubility study reveals that the drug is soluble in methanol, ethanol and slightly soluble in water. The melting point of nelfinavir was found to be 349.84 °C. FT-IR spectras were obtained for nelfinavir, polymers, physical mixture of nelfinavir and optimized formulation, and spectras were represented in figure 4, 5 and 6. The characteristic peaks of the nelfinavir were compared with the peaks obtained for physical mixture of nelfinavir and polymers. Drug polymer interaction was studied by FTIR analysis, NH stretching, CH stretching, C=O stretching, C=C stretching, O-H stretching of pure nelfinavir and the nelfinavir formulations containing polymer were in the standard wave number ranges. There was no change or shifting of characteristic peaks of nelfinavir in drug loaded microspheres and suggested that there was no significant drug polymer interaction as shown in and indicates the stable nature of drug in formulations.

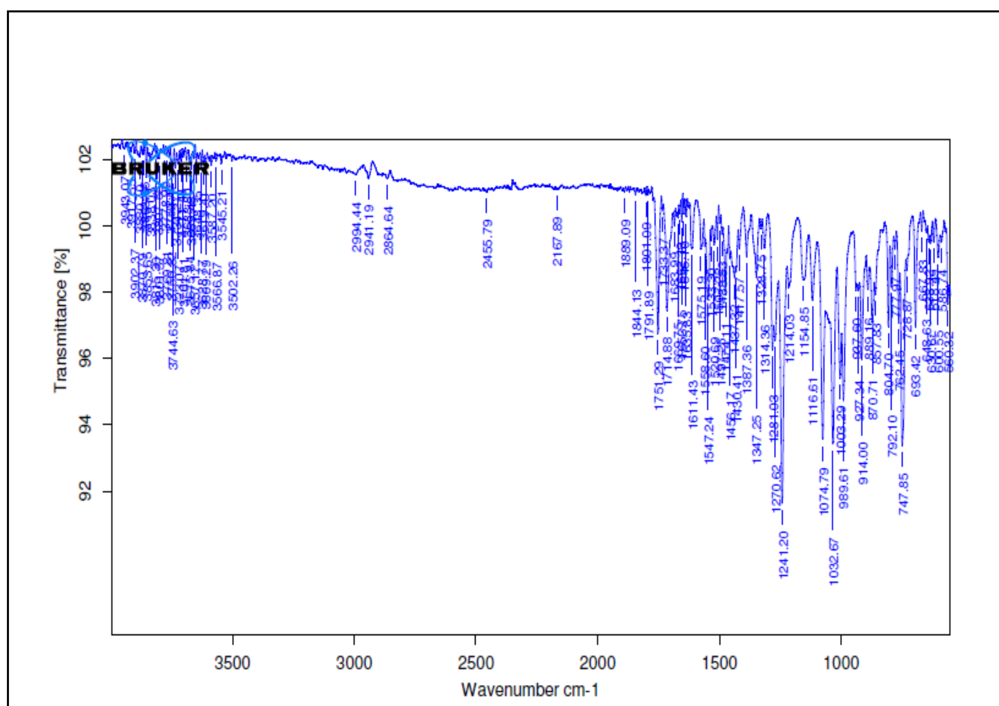

Figure No.4: FTIR Spectrum of nelfinavir pure

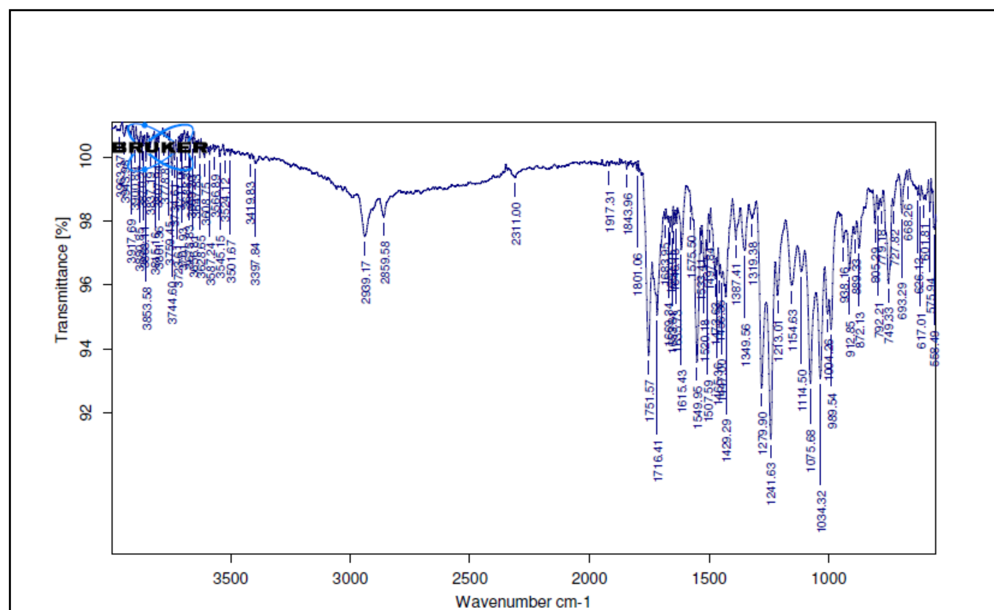

Figure No.5: FTIR Spectrum of Nelfinavir Physical Mixture (Nelfinavir+HPMCK15M+Ethyl cellulose+Carbopal)

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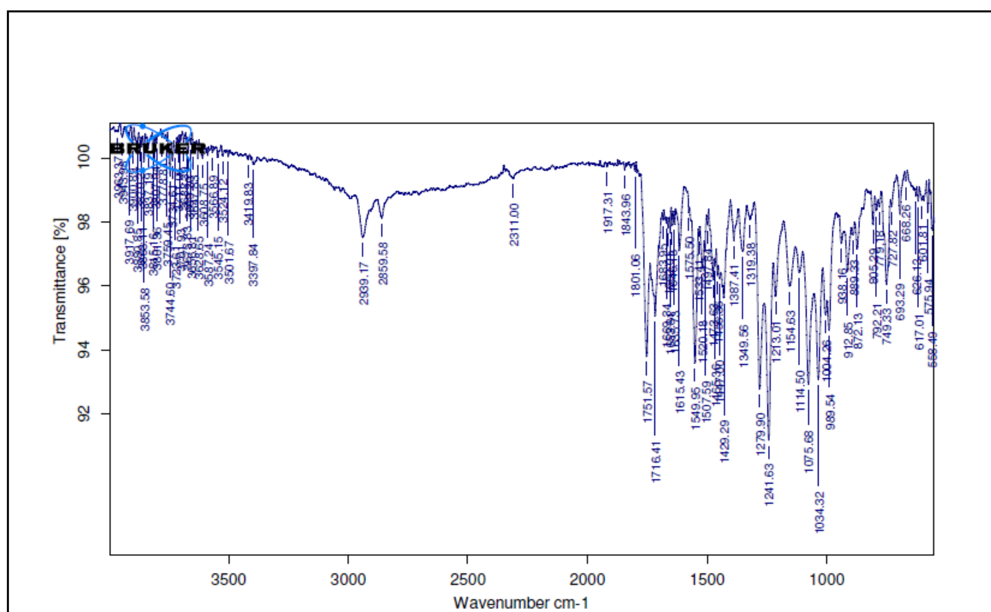

Figure No.6: FTIR Spectrum of nelfinavir microspheres optimized formulation (F7)

Characterization and evaluation of nelfinavir microspheres: The percentage yield for nelfinavir loaded microspheres were 75.4, 77.6%, 78.3%, 76.8%, 76.1%, 82.4%, 79.3% and 80.8% formulations respectively. Angle repose of microspheres was in the range of 25º31'- 30º38'. All formulation showed excellent flow ability as represented in term of angle of repose. The tapped density value of different microspheres ranges from 0.303–0.402 g/cc. Compressibility index range was found to be 9.37-19.81%. The Carr's index for all formulation was good which indicates good flow property. The mean particle size of microspheres was in range 96.3-253.3 µm and particle size analysis of different formulations of nelfinavir microspheres was carried out using stage micrometer. By increasing the concentration of polymer, the mean particle size of microspheres increased. The major parameters that affect the particle size were concentration of polymer. The percentage of drug content in the formulations was found to be in the range of 64.4% to 79.08%. A low to moderate encapsulation efficiency was observed in different formulations of nelfinavir. As it was evident from the results that the, drug loading increases as the polymer concentration increases. Microencapsulation was found to be in the range of 64.4%to 79.08% and the formulation F7 showed maximum drug entrapment of 79.08. All the results are shown in table 3. Encapsulation efficiency is largely depending upon the concentration of polymer and the reason of low encapsulation was found that drug tend to go in external medium before it could be encapsulate in to spheres and due to the decreased concentration of polymer.

Table No.3: Evaluation Parameters of nelfinavir microspheres

Parameters	F1	F2	F3	F4	F5	F6	F7	F8
Angle of Repose (ϴ)	29º31	27º59	28º47	26º51	30º38	29º54	28⁰30	26⁰53
Tapped Density (g/cc)	0.321	0.303	0.402	0.383	0.364	0.326	0.371	0.363
Compressibility Index (%)	17.59	19.81	9.37	12.46	14.23	16.78	12.54	15.31
Mean Particle Size (µm)	96.3	151.4	201.1	110.6	151.5	198.5	253.5	109.7
Percentage Yield (%)	75.4	77.6	78.3	76.8	76.1	82.4	79.3	80.8
Drug Entrapment Efficiency(%)	65.6	68.4	71.2	64.4	73.2	72.08	79.08	71.6

From SEM, it was observed that the size of the optimized formulation was found to be 292.5µm and the particles were spherical spherical in nature with smooth surface with wavy and free flowing nature and completely covered with polymer coat shown in figures 7 & 8. The surface of the drug-loaded microspheres was rough and dentations manifested the presence of drug particles.

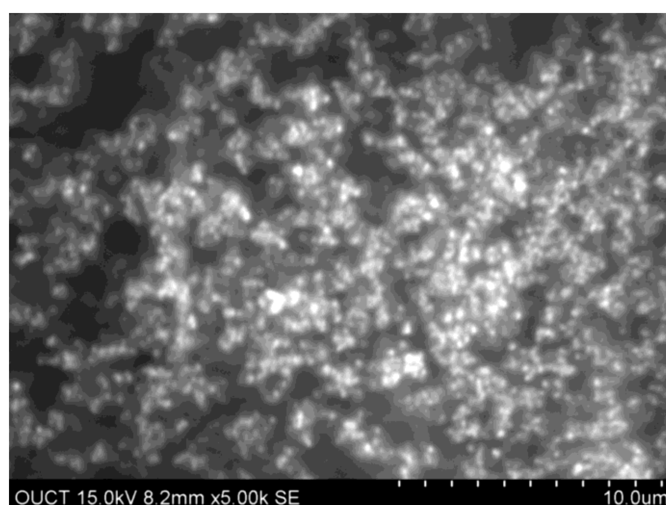

Figure No.7: SEM photograph of optimized nelfinavir microspheres (F7) showing surface morphology

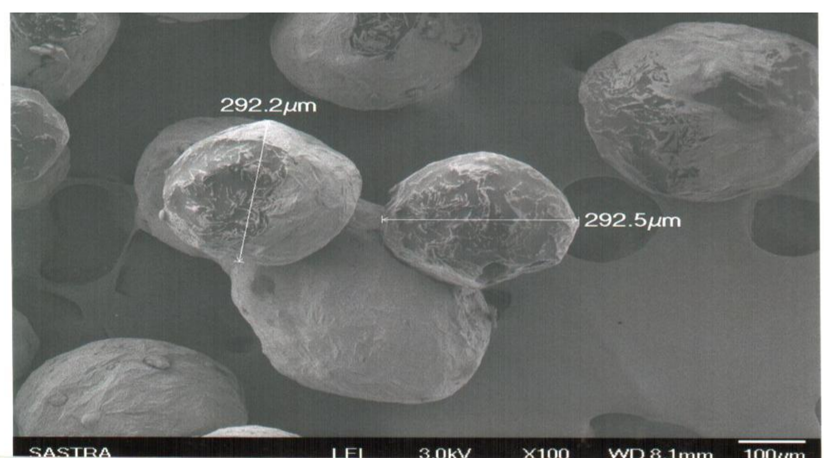

Figure No.8: SEM photograph of optimized Nelfinavir microspheres (F7) showing particle size

In-vitro release studies of Nelfinavir Microspheres: The cumulative *in-vitro* release studies of different formulations were performed in pH 1.2 buffer for first 2 hrs. Then buffer was replaced with pH 7.4 after 2 hrs and release was studied for further 12 hrs. The cumulative release of drug significantly decreased with increasing polymer concentration. The release data of microspheres are shown in table 4 and figure 9. All the formulations exhibited initial burst effect, which was due to the presence of drug particles on the surface of the microspheres. The initial burst effect may be attributed as a desired effect to ensure initial therapeutic plasma concentrations of drug. Drug releases from mixture of polymer containing microspheres were good compare to other formulations. The Formulation F1, F2, F3, F4, F5, F6, F7 and F8 were prepared by HPMCK15M, HPMCK100M, carbopol, ethyl cellulose and their percentage drug releases were 97.06(8hrs), 96.84(8hrs), 97.27(10hrs), 96.73(8hrs), 97.94(9hrs), 96.11(9hrs), 98.74(12hrs) and 95.08(9hrs) respectively. The F7 showed highest drug release of 98.74(12hrs) with extended and sustained release. This may be due to increase in the concentration of the polymer.

Table No. 4: In-vitro drug release data of nelfinavir microspheres/Percentage drug released data (pH 7.4 Phosphate buffer)

Time in hrs	F1	F2	F3	F4	F5	F6	F7	F8
1	18.24	18.87	15.84	15.74	15.32	11.88	9.69	11.46
2	27.21	33.25	27.62	26.27	22.52	21.99	18.87	19.18
3	39.09	54.84	38.99	37.53	30.65	41.60	25.33	28.67
4	51.40	65.68	47.64	56.82	41.39	53.79	35.65	42.43
5	69.96	76.84	55.67	68.91	53.38	65.68	41.80	51.71
6	86.85	87.68	63.80	72.35	71.41	74.65	54.32	63.28
7	91.43	92.37	70.89	83.30	85.59	87.89	60.68	79.65
8	97.06	96.84	81.95	96.73	91.33	92.68	72.78	87.68
9	-	-	90.91	-	97.94	96.11	80.67	95.08
10	-	-	97.27	-	-	-	85.53	-
11	-	-	-	-	-	-	96.09	-
12	-	-	-	-	-	-	98.73	-

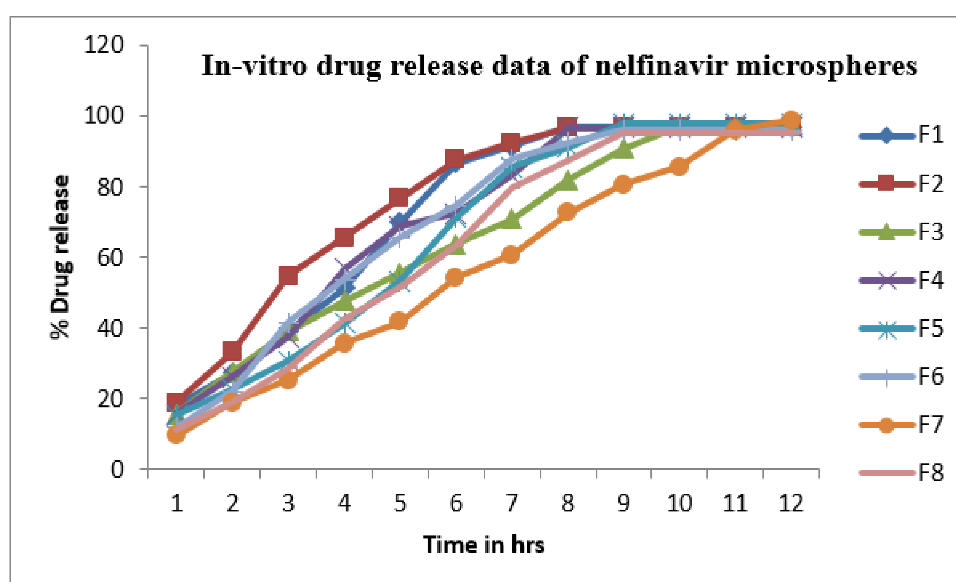

Figure No.9: Comparative graph of in-vitro drug release data of nelfinavir microspheres

Drug release kinetics data obtained that, among the formulations F1 to F8, F7 formulation containing ethyl cellulose showed more sustained effect and it may be due to the hydrophobicity of the polymer. All the release data was fitted into various kinetic models like zero order, first order, higuchi, and korsmeyer-peppas in order to find out the mechanism of drug release from polymeric spheres. The correlation coefficient, rate constant and diffusion coefficient were calculated and is given in table 5. Analysis of the release data as per zero order kinetic model is best suited to describe release rate of drug from the microspheres. Plots of percent release Vs square root of time (Higuchi's plots) were found to be linear and indicating that the dug release from the microspheres was by Higuchi diffusion mechanism. The mechanism of release for the optimized formulations was determined by finding the R^2 value for each kinetic model viz. zero-order, first-order, higuchi, and korsmeyer-peppas corresponding to the release data of formulations as shown in and figures 10, 11, 12 and 13.

For most of the formulations, the R^2 value of zero-order model is very near to 1 than the R^2 values of other kinetic models. Thus, it can be said that the drug release follows zero-order model mechanism. Plots of percent release Vs square root of time (Higuchi's plots) were found to be linear indicating that the dug release from the microspheres was by higuchi diffusion mechanism.

Table No.5: Drug release kinetics data of nelfinavir microspheres formulations F1 to F8

FORMULATION	ZERO ORDER		FIRST ORDER		HIGUCHI		PEPPAS		
	R^2	K0	R^2	K1	R^2	K	R^2	K	n
F1	0.9843	0.375	0.9218	0.001	0.9241	34.321	0.7064	0.1288	1.524
F2	0.9623	0.250	0.8713	0.0006	0.9665	34.950	0.6769	0.1225	1.516
F3	0.9901	0.273	0.8665	0.0013	0.9610	30.78	0.7147	0.0970	1.332
F4	0.9884	0.158	0.8965	0.006	0.9318	35.01	0.7223	0.1225	1.532
F5	0.9894	0.117	0.8143	0.005	0.9002	32.98	0.7523	0.1099	1.464
F6	0.9939	0.209	0.8567	0.009	0.9899	32.70	0.7781	0.109	1.535
F7	0.9931	0.112	0.8342	0.001	0.9261	27.93	0.8279	0.0805	1.346
F8	0.9943	0.545	0.8761	0.002	0.9949	31.69	0.7834	0.1056	1.560

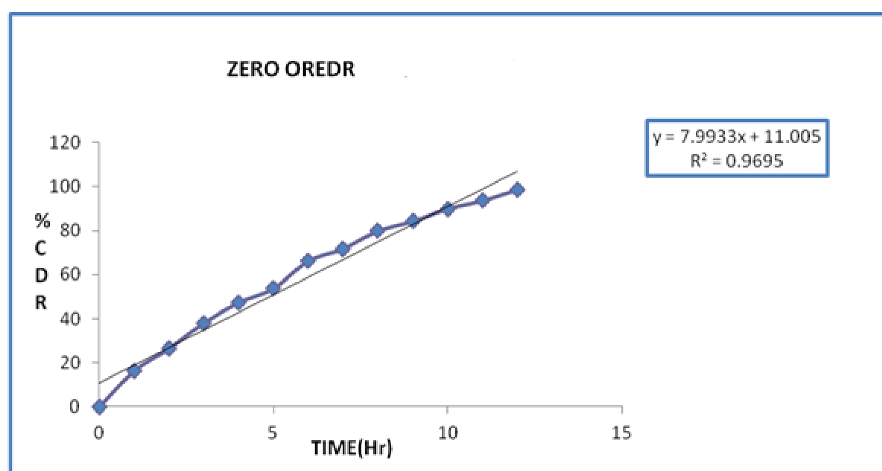

Figure No.10: Zero-order release kinetics graph of Nelfinavir microspheres (F7)

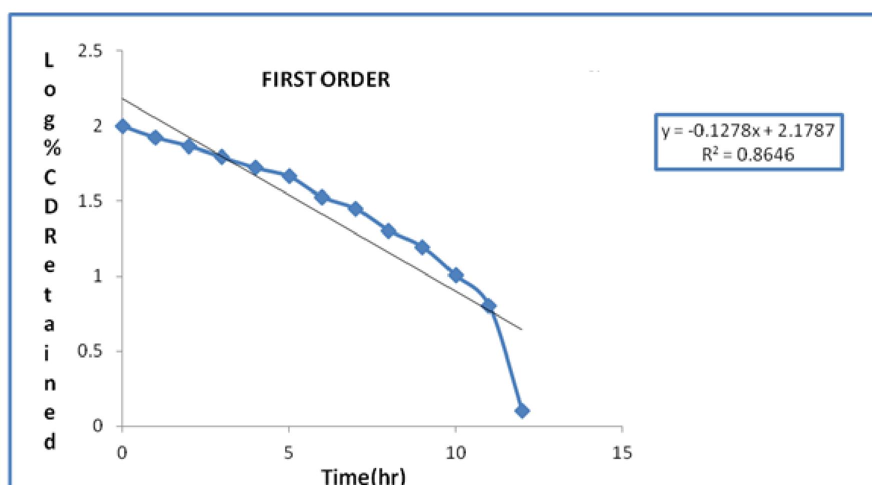

Figure No.11: First order release kinetics graph of Nelfinavir microspheres (F7)

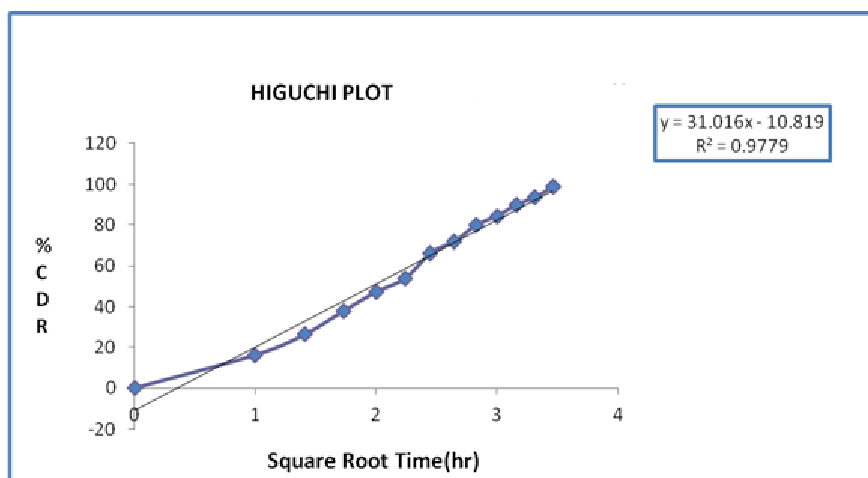

Figure No.12: Higuchi release kinetic graph of Nelfinavir microspheres (F7)

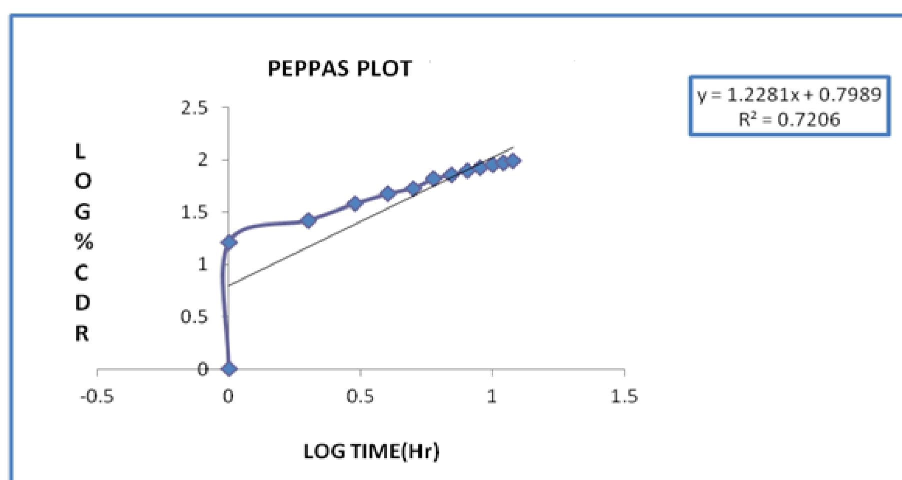

Figure No.13: Peppas release kinetic graph of Nelfinavir microspheres (F7)

The thermogram of the drug has been shifted or changed, so the intensity and peaks are changed as shown in the figures 14 & 15. Hence, by this we can confirm that physical change of drug happened, thus crystalline state of drug has been changed to amorphous form.

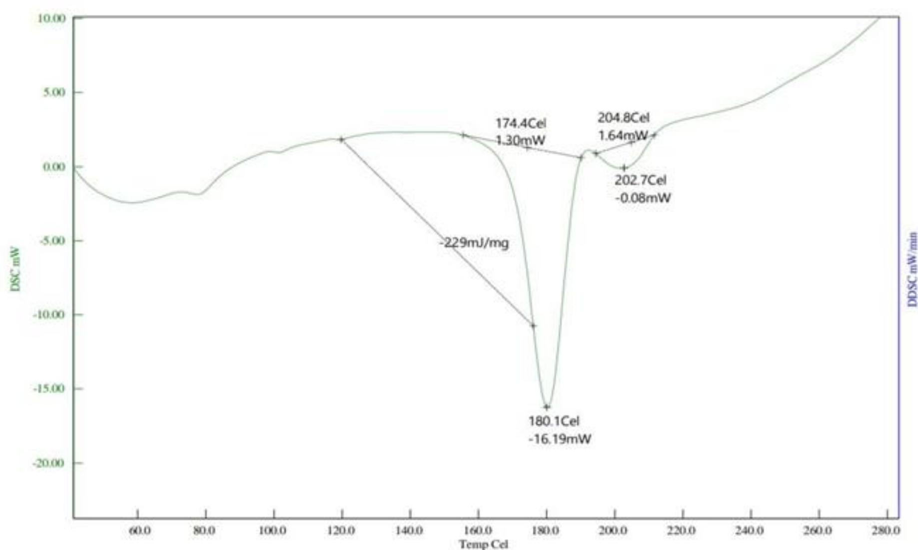

Figure No.14: DSC of Pure drug Nelfinavir

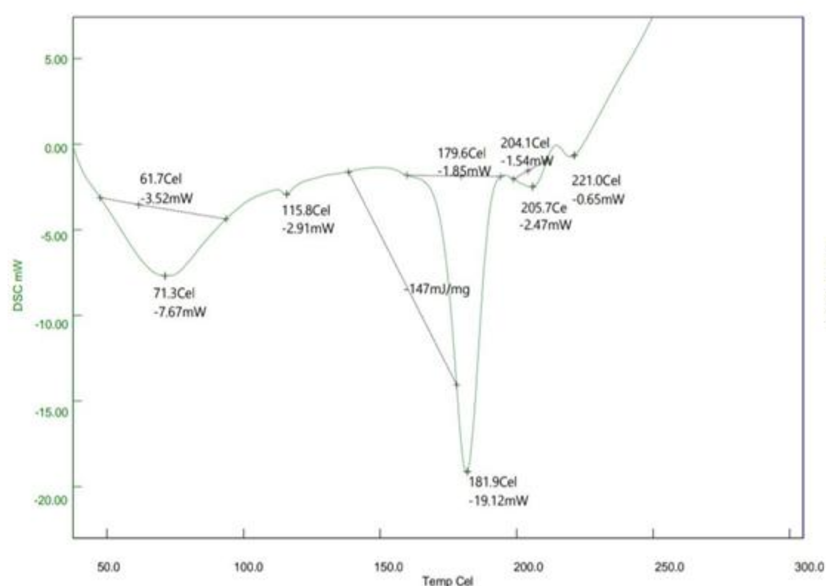

Figure No.15: DSC of Nelfinavir microspheres (F7)

The stability study was performed on the prepared formulation as per the ICH guidelines for 90 days and it showed that the formulations were stable, with no physical change and also there was no significant reduction in drug content and the % Drug retained was found to be 97.1% and the results were shown in table 6.

Table No.6: Stability Studies of optimized Formulation (F7)

S. No	Study Period (in days)	Temperature Conditions		
		% Drug retained at 5-8ºC	% Drug retained at 27ºC	% Drug retained at 42ºC
1	0	100±00	100±00	100±00
2	30	98.7±0.015	98.4±0.003	98.1±0.041
3	60	97.8±0.013	97.5±0.027	97.2±0.036
4	90	97.5±0.15	97.2±0.012	97.1±0.02

CONCLUSION:

The nelfinavir microspheres prolonged drug release for and sustained release drug for 12 hours or longer would be capable of reducing the frequency of administration and the dose-dependent side effects associated with the repeated administration of conventional nelfinavir. The investigation of optimum formulation showed controlled drug release and could therefore, produce some benefits such as reduction in total dose, frequency of administration, and dose related systemic side effects in HIV/AIDS patients.

In the present study, nelfinavir microspheres were attempted and showed encouraging results with increased patient compliance. Therefore, further refinement in the formulations can be attempted, such an attempt will be useful to put in market for the near future.

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