FORM-2

THE PATENTS ACT, 1970
&
THE PATENTS RULES, 2003

COMPLETE SPECIFICATION

Nanocrystals of poorly water soluble drugs and their Preparation Process

ORIGINAL

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The following specification particularly describes and ascertains the nature of this invention and the manner in which it is to be performed

FIELD OF THE INVENTION

The invention relates to preparation of nanocrystals of poorly water soluble drugs to increase their solubility and dissolution rate; and thereby bioavailability. It specifically relates to the preparation of statin Nanocrystals. More particularly it relates to the process for preparation lovastatin nanocrystals by precipitation method and its formulations.

BACKGROUND OF THE INVENTION

The solubility of a solute is the maximum quantity of solute that can dissolve in a certain quantity of solvent or quantity of solution at specified temperature. Aqueous solubility is one of the key determinants in development of new chemical entities as successful drugs. However, new drug development technologies, such as combinational chemistry and high throughput screening are based on the basic principles of medicinal chemistry, teaching that the most reliable method to increase *in vitro* potency is to add lipophilic moiety at appropriate position of the lead structure. This has led to an increase in the number of lipophilic and poorly soluble molecules being investigated for their therapeutic activity. Various formulation techniques are applied to compensate for their insolubility and consequent slow dissolution rate. These include formulation of the amorphous solid form, nanoparticles, microemulsions, solid dispersion; melt extrusion, salt formation and formation of water soluble complexes. Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules. Solubility is one of the parameter to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown. Currently only 8% of new drug candidates have both high solubility and permeability. It has been estimated that roughly 40% of all investigational compounds fail development because of poor bioavailability that is often associated with aqueous insolubility (Prentis et al, 1998).

The Biopharmaceutics classification system (BCS) is guidance for predicting the intestinal drug absorption provided by the U.S.FDA. The BCS has been developed to provide a scientific approach to allow for the prediction if *in vivo* pharmacokinetic of oral immediate release (IR) drug products. The importance of drug dissolution in the gastrointestinal tract and permeability across the gut wall barrier in the oral absorption

process has been well known since 1960s. It provides very clear and easily applied rules in determining the rate limiting factor in the gastrointestinal drug absorption process. BCS classifies drugs into four classes based on the solubility and permeability: Class I: High solubility, high permeability; Class II: Low solubility, high permeability; Class III: High solubility, low permeability; & Class IV: Low solubility, low permeability. BCS identifies the problems associated with oral delivery of the drug and gives the pharmaceutical scientist a channel to work in drug delivery.

Bioavailability is the degree to which a drug becomes available to the target tissue after administration. Many factors can affect bioavailability including the dosage form and various properties, e.g., dissolution rate of the drug. Poor bioavailability is a significant problem encountered in the development of pharmaceutical compositions, particularly those containing an active ingredient that is poorly soluble in water. Poorly water soluble drugs, i.e., those having a solubility less than about 10 mg/ml, tend to be eliminated from the gastrointestinal tract before being absorbed into the circulation. Moreover, poorly water soluble drugs tend to be unsafe for intravenous administration techniques, which are used primarily in conjunction with fully soluble drug substances. The oral route is the most convenient method of drug administration, but is subject to the bioavailability complications imposed by GI tract physiology and the effects of first pass metabolism and biotransformation. While current methods may continue to offer fast and cost effective solutions without compromising product quality, the future appears brighter for new technologies being developed to obtain ultrafine particles in the micro and nano-scale range.

It is known that the rate of dissolution of a particular drug can increase with increasing surface area, i.e., decreasing particle size. Consequently, methods of making finely divided drugs have been studied and efforts have been made to control the size and size range of drug particles in pharmaceutical compositions. Oral delivery of poorly soluble drugs has become one of the most challenging problems for advanced pharmaceutical research. Usually, drugs with low water solubility show poor bioavailability and a high variability of plasma levels among subjects. This in turn leads to formulations with high drug content which often must be delivered repeatedly to obtain and maintain therapeutic plasma levels. Several studies have been made with the purpose of improving the solubility of these drugs by physical means, without resorting e.g. to

chemical derivatisation or the use of additional chemicals. The unfavourable biopharmaceutical behaviour of poorly soluble drugs is strictly correlated with well defined physical-chemical characteristics. Drug uptake can occur in different ways but, for small synthetic molecules, absorption via a non-saturable passive process (diffusion through the GI barrier) plays a primary role. The ability of poorly water soluble drugs to be passively absorbed is strictly dependent on their physical properties, such as steric hindrance, crystal form, solubility, lipophilicity, wettability and surface area.

In case of substances of poor solubility and strong lipophilic character, this crosses the membrane only by passive diffusion; dissolution rate of a substance from a particular pharmaceutical formulation is one of the key factors which define the rate and extent of absorption. The faster a substance dissolves, the higher is the local concentrations of the substance, resulting in more rapid passage across the membrane. The number of drugs coming from synthesis and being poorly soluble is steadily increasing. At present about 40% of the drugs in the development pipelines and approximately 60% of the drugs coming directly from synthesis are poorly soluble. The increasing number of poorly soluble drugs requires innovative formulation approaches to reach a sufficiently high bioavailability after oral administration or at least to make available intravenously injectable forms.

Poorly soluble drugs present big challenges for the formulation scientist. The range of technologies currently available for improving bioavailability (i.e. increasing the solubility and/or the permeability or absorption) of poorly water soluble chemical compounds and high molecular weight drugs can be split broadly into conventional methods and nanotechnology.

"Nanoparticles" are defined as particulate dispersion or solid particles with size in the range 10-100 nm. Nanoparticles are solid, submicron sized drug particles that may or may not be biodegradable. The term nanoparticle is a collective name for both nanospheres which have a matrix type of structure or encapsulated within the particle. Drugs delivered as nanoparticles are either intended to dissolve in the GIT or in most cases, to be taken as such through the mucosa and the BCS per se, does not apply in the latter case, but in this non-applicability of BCS, lies the biggest utility of nanoparticulate delivery systems. Most of the applications of NP are spread around delivery of drugs

that have compromised solubility or poor bioavailability (Lennernas & Abrahamson, J Pharm Pharmacol. 2005; 57: 273-285).

Advantages of nanoparticles:

- Increased bioavailability
- Dose proportionality
- Decreased toxicity
- Smaller dosage form (i.e., smaller tablet)
- Stable dosage forms of drugs which are either unstable or have unacceptably low bioavailability in non-nanoparticulate dosage forms.
- Increased active agent surface area results in a faster dissolution of the active agent in an aqueous environment, such as the human body. Faster dissolution generally equates with greater bioavailability.
- Reduction in fed/fasted variability.

"**Nanocrystals**" are the nanoparticles being composed of 100% drug without any matrix material. When the size of the material is reduced to less than 100 nanometers, the realm of quantum physics takes over and materials begin to demonstrate entirely new properties. Hence nano-design of drugs by various techniques like milling, high pressure homogenization, controlled precipitation etc., are explored to produce drug nanocrystals, nanoparticles, nanoprecipitates, nanosuspensions. As decreased size will increase the solubility of drugs hence, this technology is explored to increase oral bioavailability of sparingly water soluble drugs. On the other hand engineering of nanocrystals will avoid the use of toxic solvents and surfactants to develop injectable solutions of sparingly water soluble drugs. It is also possible to develop formulations for various routes of administration where size is the critical factor (injectables, ophthalmics and topical preparation).

Advantages of Nanocrystals:

i. In contrast to micronized powders the drug nanocrystals can be administered using very different administration routes.
ii. Oral administration is possible as a suspension.

iii. More patient convenient dosage forms can be produced by transferring the liquid nanosuspensions to solid dosage forms, i.e. tablets or pellets or granulate containing capsules.

iv. In addition, because of their small size the nanosuspensions can be injected parenterally, especially intravenously. Intravenous injection leads 'per definition' to a 100% bioavailability.

The rational of producing micronized drugs for oral administration is the enhancement in bioavailability for BCS class II drugs. The limiting step of oral absorption is the dissolution velocity. The dissolution velocity of micronised drug powders is enhanced by their enlarged surface area. The same effect, but much more pronounced, is valid for nanosized drug powders. As the surface is enlarged, the dissolution velocity is also enhanced.

Another important aspect is the increase in saturation solubility. The increase in saturation solubility has two effects:

i. Based on the Noyes–Whitney equation an increase in saturation solubility leads to an increase in dissolution velocity.

ii. An increase in saturation solubility in the lumen of the gut increases the concentration gradient between lumen and the blood, thus accelerating drug-diffusion and promoting absorption.

There is a third special feature of drug nanocrystals, the general adhesiveness of nanoparticles. Due to their large surface area, the nanoparticles tend to stick to surfaces.

To sum up the special features of drug nanocrystals are those, the further enlargement in surface area by one dimension compared to micronized powders, the increase in saturation solubility, both leading to a distinctly increased dissolution velocity. Consequently, nanonization is the ultimate universal formulation approach for drugs of BCS class II. In addition, due to their ultrafine character and adhesiveness, they further enhance oral bioavailability of drugs and reduce variability in bioavailability due to the reproducibility of there adhesion process in the gut wall.

There are reports available for the preparation of nanoparticles for drugs and interested ones include Suresh et al.(AAPS Pharm. Sci. Tech., 2007; 8 (1); Article 24: E1-E9)

reports the lovastatin solid lipid nanoparticle (SLN) using triglycerides by hot homogenization followed by ultrasonication to improve the bioavailability of Lovastatin. Zhang et al. (Int. J. Pharm. 2006; 323: 153-160) reported the preparation of amorphous nanoparticles of cefuroxime axetil (CFA), a poorly water-soluble drug, by the controlled nanoprecipitation method without any surfactants at room temperature. Waard et al. (J. Cont. Rel. 2008; 128: 179-183) has developed a novel bottom –up process based upon freeze drying which allows for the production of nanocrystalline particles and concluded that this novel process could strongly increase the dissolution behavior of Fenofibrate. D. Douroumis, A. Fahr. (Eur. J. Pharm. Biopharm. 2006; 63: 173-175) reported a novel technique for the production of nano- and micro-particulate formulations of poorly water-soluble drugs. This technique involves the use of static mixer elements to provide fast precipitation by continuous turbulent mixing of two liquid flows, an aqueous phase and an organic phase.

There are patents of interest available for preparing nanosized particles of drugs, the relevant ones include U.S patent no. 4826689 which discloses a method for making uniformly sized particles from water-insoluble drugs or other organic compounds. First, a suitable solid organic compound is dissolved in an organic solvent, and the solution can be diluted with a non-solvent. Then, an aqueous precipitating liquid is infused, precipitating non-aggregated particles with substantially uniform mean diameter. The particles are then separated from the organic solvent. U.S patent no. 6696086 discloses solid pharmaceutical formulation containing lovastatin and simvastatin with a particle size D(0.9) between 15 and 100 μm and a specific particle surface area between 1 and 4 m^2/g, and to the process for its preparation. U.S patent application no. 20050255164 discloses a method of preparing low water-soluble medicine into solid nanometer pharmaceutical formulation. U.S patent application no. 20080213378 discloses nanoparticulate compositions comprising statin, preferably lovastatin or simvastatin, and novel statin combinations. The nanoparticulate statin particles preferably have an effective average particle size of less than about 2000 nm. International patent application no.WO2008048205 discloses nanocrystals of hydrophobic drug molecules such as tetra- pyrrole compounds which have been synthesized and remain stably dispersed in an aqueous system without the necessity of stabilizers like surfactants. The nanocrystals of a hydrophobic photosensitizing

anticancer drug 2-devinyl-2-(l-hexyloxyethyl) pyropheophorbide (HPPH), were synthesized using reprecipitation method. International patent application no. WO2008065502 discloses the compositions comprising nanoparticles comprising a low-solubility drug and an enteric polymer, and casein or a pharmaceutically acceptable form thereof.

Accordingly, there is a real and continuing need for development of cost-effective and easy method for preparation of stable lovastatin nanocrystals to increase the solubility and dissolution rate and thereby bioavailability of lovastatin which is taken as a model drug. Hence the present inventors aim is to develop the convenient process to prepare nanocrystals of poorly soluble drugs such as lovastatin as a model drug and its formulations.

OBJECTS OF THE INVENTION

The primary object of the present invention is the development of an easy process for preparation of nanocrystals of poorly soluble drug to increase the solubility and dissolution rate and thereby bioavailability.

Another object of the present invention is to develop an improved process for preparation of nanocrystals of lovastatin (LVS) to increase the solubility and dissolution rate and thereby bioavailability of lovastatin which is taken as a model drug.

It is yet another object of the present invention for preparation of stable nanocrystals of lovastatin of particle size below 1000 nm.

It is the further object of this invention to develop formulations of lovastatin nanocrystals for the improved drug delivery system and bioavailability.

STATEMENT OF THE INVENTION

The invention is a process for preparation of nanocrystals of poorly water soluble drugs to increase the solubility and dissolution rate and thereby bioavailability. The process mainly comprises the steps: i) preparation of drug solution; ii) addition of drug solution to water; iii) removal of solvent; iv) centrifugation; and v) solidification of supernatant to obtain the nanocrystals. The various concentrations of drug were dissolved in suitable organic solvents such as acetone, methanol and acetonitrile to prepare the drug solution. Then the drug solution of step (i) is to be added into required quantity of water with continuous stirring on magnetic stirrer at 1000 rpm. The organic solvent was removed by overnight stirring at 500 rpm. After removal of organic solvent the solution was centrifuged at 5000 rpm. Then the precipitate obtained after step (iv) was subjected to solidification by freeze drying to obtain the Nanocrystals. The poorly water soluble drug lovastatin is used as a model drug and by the process produced lovastatin nanocrystals of having particle size less than 1000nm. The formulations of nanocrystals comprising lovastatin nanocrystals of having particle size less than 1000nm and suitable excipients.

DETAILED DESCRIPTION OF THE INVENTION

LOVASTATIN

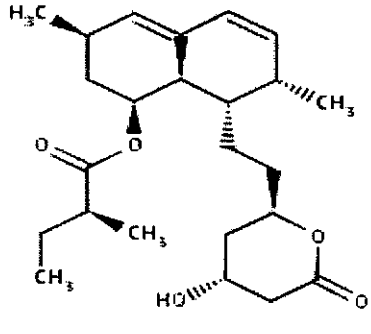
Chemical IUPAC Name: [8-[2—(4-hydroxy-6-oxo-oxan-2-yl) ethyl]-3,7-dimethyl-1,23,7,8,8a-hexahydronapthalen-1-yl] 2—methylbutanoate

CAS Registry Number: 75330-75-5

Molecular Weight: 404.54

Molecular formula: $C_{24}H_{36}O_5$

Structure:



Appearance: White crystalline powder

Melting point: 174.5^{0}C

Solubility: Freely soluble in chloroform, soluble in acetone, acetonitrile and in methanol, sparingly soluble in alcohol, practically insoluble in hexane and water.

Lovastatin is one of the most important known cholesterol lowering agents. Lovastatin as used herein (CAS Registry No. 75330-75-5) is also known as mevinolin or monacolin K and is chemically known as beta, beta-dihydroxy-7-[1,2,6,7,8,8a-hexahydro-2,6-dimethyl-8-(2-methyl-bu- tyryloxy)-1-napthalen-1-yl]-heptanoic acid beta-lactone. Lovastatin is one member of a class of compounds which are referred to generally as statins and which are known to exist in open ring hydroxy acid and in lactone form. Lovastatin and its analogs inhibit HMG-CoA reductase. Lovastatin is specifically advantageous because, as a result of its application, biosynthetic intermediates that have a toxic steroid skeleton formed at a later stage of biosynthesis fail to accumulate. Lovastatin also increases the number of LDL-receptors at the surface of the cell membrane, which remove the LDL cholesterol circulating in the blood, thereby inducing the lowering of blood plasma cholesterol level.

Lovastatin tablets are commercially supplied as 10 mg, 20 mg, and 40 mg tablets for oral administration. In addition to the active ingredient lovastatin, each tablet contains cellulose, lactose, magnesium stearate, and starch. Butylated hydroxyanisole (BHA) is added as a preservative. Lovastatin is well known in the art and is readily recognized by one of ordinary skill. High LDL cholesterol is usually first treated with exercise, weight loss in obese individuals, and a diet low in cholesterol and saturated fats. When these measures fail, cholesterol-lowering medications such as lovastatin can be added. The National Cholesterol Education Program (NCEP) has published treatment guidelines for use of statins such as lovastatin. These treatment guidelines take into account the level of LDL cholesterol as well as the presence of other risk factors such as diabetes, hypertension, cigarette smoking, low HDL cholesterol level, and family history of early coronary heart disease. The effectiveness of the statin medications in lowering cholesterol is dose-related. Blood cholesterol determinations are performed in regular intervals during treatment so that dosage adjustments can be made. A reduction in LDL cholesterol level can be seen two weeks after starting therapy with a statin.

Process for preparation of Nanocrystals:

The Nanocrystals of poorly water soluble drugs were prepared by Precipitation method. The preparation process involves following steps:

i) Preparation of drug solution;

ii) Addition of drug solution to water;

iii) Removal of solvent;

iv) Centrifugation;

v) Solidification of supernatant to obtain the Nanocrystals.

i) Preparation of drug solution:

The different concentrations (ranging from 1 mM to 10 mM) of drug were added to suitable organic solvents to prepare the drug solution.

ii) Addition of drug solution to water:

The above prepared drug solution is to be added into required quantity of water with continuous stirring on magnetic stirrer at 1000 rpm. The volume of drug solution added to water ranges from 30 to 60 times less than the volume of water and preferably 50 times less than the volume of water.

iii) Removal of solvent:

The organic solvent was removed by overnight stirring at 500 rpm.

iv) Centrifugation:

After removal of solvent the solution was centrifuged at 5000 rpm.

v) Solidification of supernatant to obtain the Nanocrystals:

The supernatant obtained at above step was subjected to solidification by freeze drying to obtain the nanocrystals.

EXAMPLES:

The following specific examples presented to illustrate the best mode of carrying out the process and formulation of the present invention but do not limit the scope of the invention.

The present study was carried out to develop nanocrystals of lovastatin in order to enhance solubility, dissolution and bioavailability by decreasing the particle size of the drug. In concern to this approach, the primary necessity is to reduce the particle size by precipitation process and dissolution profiles of obtained nanocrystals were compared with pure drug in a capsule. In this case six different nanocrystals of LVS were prepared. In these the effect of different solvents were observed on particle size.

Lovastatin nanocrystals are prepared by following process:

Two different concentrations of solution of lovastatin (3 mM/4mM) were prepared by dissolving it in organic solvent such as acetone or methanol or acetonitrile as shown in **Table 1**). Then the prepared lovastatin solution of 12.3 ml/10.8 ml was added to water (615 ml/540 ml) with continuous stirring on magnetic stirrer at 1000 rpm and the organic solvent was removed by overnight stirring at 500 rpm. After removal of solvent the solution was centrifuged at 5000 rpm. Then the supernatant solution obtained was subjected to solidification by freeze drying at -40°C for 24 hours to obtain the lovastatin nanocrystals.

Table 1. Lovastatin Nanocrystals and their evaluation

Code	Organic solvent	LVS concn (mM)	Vol. of LVS soln. (ml)	Vol. of water (ml)	Mean Particle Size (nm)	Yield (%)	Solubility in Distilled water (mg/ml)	Solubility in Acid buffer (pH 1.2) (mg/ml)	Solubility in Phosphate buffer (pH7.4) (mg/ml)
F_1A	Acetone	3	12.3	615	579.33	66.75	0.092	0.148	0.176
F_1B	Acetone	4	10.8	540	620.00	60.63	0.089	0.136	0.161
F_2A	Methanol	3	12.3	615	584.58	66.70	0.090	0.131	0.173
F_2B	Methanol	4	10.8	540	711.85	66.31	0.084	0.129	0.154
F_3A	Acetonitrile	3	12.3	615	803.71	59.75	0.081	0.097	0.134
F_3B	Acetonitrile	4	10.8	540	848.06	65.32	0.076	0.089	0.105
Pure LVS	--------	------	--------	-------	--------	-------	0.005	0.007	0.008

The prepared Nanocrystals were evaluated for following parameters:

1. Particle Morphology
2. Particle size analysis
3. Crystalline state evaluation
4. Solubility of formulation
5. *In- vitro* release studies
6. *In- vivo* Evaluation.
7. Stability Study

1. Particle morphology by scanning electron microscopy (SEM):

Nanocrystals were examined using Jeol JSM-840A scanning electron microscope (Japan). The surface morphology of nanocrystal was examined at pH 7.4. The samples were mounted on aluminum mount and then were critical point dried, sputter-coated with 9 nm of gold/palladium and imaged using scanning electron

microscope. The SEM images of pure drug and F_1A and F_2A were shown in Plate no. 1 to 3 of **Fig. 2.**

2. Particle size analysis:

Particle size analysis of the nanocrystals was done by using particle size analyzer. Size and size distribution of the particles in dried state following water redispersion were determined through particle size analyzer with a wet sampling system (Nanotrac150) and the diameters reported were calculated using mean particle size distribution and the results are given in **Table. 1**. The smallest particle size was given by nanocrystals F_1A and F_2A as compared to other nanocrystals.

3. Crystalline state evaluation:

Crystalline state evaluation of nanocrystal formulation was done by using Powder X-Ray diffraction (PXRD) and Differential Scanning Colorimeter (DSC).

a) ***Powder X-ray diffraction (PXRD):***

PXRD diffractograms of each nanocrystal and pure drug were recorded using Philips analytical XRD PW3710 with a Cr line as source of radiation. Standard runs using a 40 Kv voltage, a 25 mA current and a scanning rate of 1^0 min^{-1} over 2θ range of $10\text{-}70^0$ were used. The PXRD patterns of pure LVS and nanocrystals (F_1A, F_2A to F_3A) are presented in **Figure 3.** The PXRD patterns of pure LVS showed numerous sharp peaks, which are the characteristic of a crystalline compound and are compared with the PXRD patterns of nanocrystals. The PXRD patterns of pure drug lovastatin has highest peak (253) at 2θ range of 13.995, other peaks were (207) at 2θ range of 24.910, (135) at 2θ range of 26.740 and (193) at 2θ range of 28.270. While F_1A prepared by the addition of acetone has highest peak (67) at 2θ range of 26.240, other peaks were (55) at 2θ range of 25.710, (41) at 2θ range of 33.625 indicating the semi crystalline nature of the drug. F_2A also has highest peak (76) at 2θ range of 25.625, other peaks were (64) at 2θ range of 26.185, (59) at 2θ range of 24.385 indicating the semi crystalline nature of the drug. F_3A also has highest peak (154) at 2θ range of 24.440, other peaks were (125) at 2θ range of 25.735, (83) at 2θ range of 26.305 indicating the slight change in crystallinity of the drug, and indicating the semi

crystalline nature of the drug due to addition of acetonitrile as a solvent. Moreover, no other peaks than those that could be assigned to the pure LVS were detected with nanocrystals, thus indicating the absence of chemical instability in the solid state. These results confirm that LVS nanocrystals are present in crystalline state.

b) ***Differential scanning calorimetry (DSC):***

Thermal properties of the powder samples were investigated with a Perkin-Elmer DSC-7 differential scanning calorimeter. The amount of product to be analyzed shall range from 4 to 7 mg and be placed in crimped aluminum sealed 50 µl pans. Heat runs for each sample has been set from 50 to 300°C at a scanning rate of 10^0C/min, under dry nitrogen flow (100 ml/min). The results for pure LVS and nanocrystals are depicted in **Figure 4.** The pure LVS showed a large and sharp characteristic endothermic peak at 175.19°C due to its phase transition. The onset and endset of phase transition of lovastatin were observed at 171.44 °C and 180.59°C respectively. DSC thermogram of nanocrystals F_1A showed an endothermic peak at 173.92°C corresponding to the slight change in the crystalline nature. DSC thermograms of other two nanocrystals (F_2A and F_3A) showed a small and sharp characteristic endothermic peak at 174.87°C and 174.57°C respectively. The DSC thermograms of all formulations showed characteristic endothermic peaks corresponding to those of the pure drug and there is no appearance of one or more new peak or disappearance of one or more peak corresponding to those of the pure drug. This indicates that crystalline nature remains, with slight change in crystallinity due to some change in melting point. Besides this, no additional peaks to demonstrate the significant changes in the melting characteristics of lovastatin in the formulation indicating no polymorphic changes in the lovastatin. The peaks were found to be nearly identical, with a calculated ΔH of pure drug, F_1A, F_2A and F_3A were around -96.68 J/g, -70.45 J/g, -81.14 J/g and -86.65 J/g respectively.

4. Solubility determination:

Solubility of Lovastatin nanocrystals were tested in different solvents such as distilled water, acid buffer (P^H 1.2) and phosphate buffer (P^H 7.4). An excess amount of lovastatin nanocrystals were added in 150 ml of the pertinent solvents. The mixtures

were stirred in a mechanical shaker for 24 hours. Visual inspection was carefully made to ensure there were excess lovastatin solids in the mixture, indicating saturation had been reached. The mixtures were then filtered using 0.45 μm millipore filter and filtrates were diluted suitably to determine the solubility of lovastatin in each solvent. The results for pure LVS and nanocrystals are depicted in **Table 1.** F_1A and F_2A showed highest solubility in water (0.092 and 0.090 respectively), as compared with plain LVS (0.005) and other formulation of LVS are also shown to enhance the solubility of LVS in water significantly.

5. *In- vitro* release studies:

The release rate of lovastatin nanocrystal was determined using USP Dissolution testing apparatus II (Basket type). The dissolution test was performed using 900 ml of P^H 1.2 Buffer, at 37 ± 0.5^{0}C and 50 rpm. All drug formulations and pure drug were filled in capsule individually and subjected to dissolution study. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus every 15 minutes interval for first 1 hour and with Phosphate buffer P^H 7.4 containing 0.25% w/v of sodium lauryl sulphate as a dissolution medium at every 30 minutes interval for next 2 hours. The samples were replaced with fresh dissolution medium. The samples were filtered. This solution was then transfer to 50 ml volumetric flask. Absorbance values of these solutions were measured against respective buffer solutions at 238 nm using UV Spectrophotometer. The percentage drug release was calculated.

Details of Dissolution Test:

1. Apparatus : USPXXIII Basket Type
2. Volume of medium : 900 ml
3. Temperature : 37 ^{0}C ± 0.5 ^{0}C
4. Paddle speed : 50 rpm
5. Dissolution medium used : P^H 1.2 buffer, Phosphate buffer P^H 7.4
6. Aliquot taken at each time interval : 5 ml

Aliquots were withdrawn from a zone midway between the surface of dissolution medium and the top of rotating paddle not less than 1 cm apart from the vessel wall. The results for pure LVS and nanocrystals are depicted in **Table 2.** The *in vitro* release profiles of all nanocrystals (F_1A to F_3B) were compared with pure drug of lovastatin.

The release studies were carried out for 3 hours and the results obtained are tabulated in table 5.7 to 5.13. The drug release were found to be 95.69%, 78.97% and 92.80%, 75.09%, 69.50%, 63.60% and 40.46% for formulations F_1A, F_1B, F_2A, F_2B, F_3A and F_3B and pure drug respectively after 3 hours. Results showed that, using the nanocrystals F_1A to F_3B significantly increase the LVS release compared to pure LVS release.

TABLE 2: *In- vitro* LVS release profile

Time (Min)	Cumulative % of LVS Released						
	F_1A	**F_1B**	**F_2A**	**F_2B**	**F_3A**	**F_3B**	**Pure LVS**
15	47.60	37.10	52.90	29.10	37.10	29.10	13.20
30	61.14	45.20	61.17	34.64	42.55	34.57	18.66
45	72.07	50.75	66.80	40.13	48.08	40.05	21.41
60	77.76	58.97	72.47	48.29	51.00	45.57	26.82
90	85.80	65.74	82.95	57.16	57.23	51.44	31.43
120	89.08	70.24	86.22	65.91	63.17	54.54	37.23
150	92.37	75.91	89.50	71.89	66.32	60.46	40.24
180	95.69	78.97	92.80	75.09	69.50	63.60	40.46

6. *In- vivo* Evaluation:

In vivo studies were performed on groups of four male wistar rats weighing 200 ± 20 gm with no signs of disease. All animals were maintained according to CPCSEA guidelines.

Groups of animals: Group 1: IV pure lovastatin

Group 2: oral pure lovastatin

Group 3: oral lovastatin nanocrystal (F_1A)

Group 4: oral lovastatin nanocrystal (F_2A)

Dose for animal study:

Dose (mg/200 gram of rat) = Human dose (mg) x Body surface area

Dose (mg/200 gram of rat) = 10 x 0.018

Dose (mg/200 gram of rat) = 0.18 mg/200 gram rat

$$\text{For 220 gram rat: Dose} = \frac{0.18 \times 220}{200} = 0.198 \text{ mg/220 gm rat}$$

All animals were kept under fasting over night prior to experiment. For each animal, the formulation was suspended in methyl cellulose (0.5%w/v) to obtain 1 mg/ml lovastatin and this suspension was ultrasonicated for 2 minutes, just before oral dosing in each experiment. Pure drug was also treated same as above. Each formulation and pure drug suspension was administered orally to three rats by oral feeding needle as shown in figure 4.1. The blood samples were withdrawn by retro orbital venous plexus puncture at 5, 30, 60, 90, 120, 150, 180, 210 and 240 minutes for oral and iv control group and 5, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420 and 480 minutes. All the samples were collected in heparinized Eppendorf tubes and centrifuged (5000 rpm, 15 minutes), and plasma was collected and stored at -20^{0}C until analysis. Owing to the instability of lovastatin in rat plasma at ambient temperature, all sample preparations were performed on an ice water bath. The drug plasma concentration was analyzed by a modification of HPLC technique.

HPLC analysis:

Quantitative estimation of lovastatin was done by Mayura 3100 HPLC. The chromatographic system consisted of a solvent delivery pump equipped with a 20 µl loop and a UV visible detector. A Sepralyte C-18 column (50 x 4.63 µm) was used. An aqueous buffer (ammonium phosphate [0.05M] and phosphoric acid buffer [0.01M] and

acetonitrile) (50:50) was used as the mobile phase. The mobile phase was delivered at a flow rate of 1.5 ml/min, single injection volume was 20 µl and the effluent was monitored at 238 nm.

The absolute and relative bioavailability of oral nanocrystals was determined by taking the area under curve of pure drug i.v. administration and oral administration of pure drug and nanocrystals F_1A and F_2A as shown in **Table 3**. The results revealed that absolute and relative bioavailability was slightly increased as compared to oral control group; it was found that relative bioavailability was 1.015 µg/ml and 1.010 µg/ml respectively.

TABLE 3: Comparison of Bioavailability of LVS nanocrystals

Formulation code	Absolute bioavailability (µg/ml)	Relative bioavailability (µg/ml)	Area under curve(0-8) (µg/ml.hrs)	Cmax (µg/ml)	Tmax (hrs.)
Oral control group	----------	----------	802.8	5.849± 0.245	2
IV control group	----------	----------	986.7	9.546± 0.094	5*
F_1A	0.826	1.015	815.3	6.325± 0.324	2
F_2A	0.821	1.010	810.9	5.590± 0.432	2

* Time in minute
Values of Cmax are mean ± standard deviation

7. Stability studies:

Stability studies of the prepared nanocrystals were carried out, by storing F_1A at 4^0C in refrigerator room temperature at $30^0C \pm 2^0C$, 65%± 5% RH and at $40^0C \pm 2^0C$/ 65%± 5% RH in humidity control oven for thirty days. Two parameters namely residual percent drug content and *in vitro* release studies were carried out. The results of LVS content after 30 days of stability testing at different storage conditions are shown in **Table 4**. *In vitro* release profile after 30 days of stability testing is shown in **Table 5**. On comparing

this data with the previous release data of F_1A, it was observed that there is slight decrease in the drug release. These results may be due to oxidation of lovastatin formulation to some extent during storage. From the stability studies it was confirmed that nanocrystals of lovastatin remained more stable at 4°C. The maximum instability of nanocrystals was observed at 40±2°C.

Table 4: LVS content after 30 days storage of F_1A

Code	% LVS content at 4^0C	% LVS content at $30^0C±2^0C$ / 65%± 5% RH	% LVS content at $40^0C±2^0C$/ 65%± 5% RH
F_1A	66.46	66.32	60.54

Table 5: *In vitro* release profile after stability study of F_1A

Time (Min.)	% Cumulative LVS release stored at 4^0C	% Cumulative LVS release stored at $30^0C±2^0C$ / 65%± 5% RH	% Cumulative LVS release stored at $40^0C±2^0C$/ 65%± 5% RH
15	44.96	45.23	41.03
30	59.16	59.63	54.24
45	70.20	70.86	65.03
60	75.03	75.53	71.26
90	82.97	83.18	79.06
120	87.06	87.45	82.36
150	90.31	90.35	88.65
180	93.76	93.06	89.30

Formulation of Nanocrystals:

The formulations of LVS nanocrystals was prepared by mixing 10-80 mg of LVS nanocrystals having particle size less than 1000 nm with 50-400 mg of directly compressible lactose, 1-8 mg of magnesium stearate and 1-8 mg of colloidal silicon dioxide along with other suitable excipients as shown in **Table.6**. The blend was compressed on an eight-station, single-rotary machine (Cadmach, India) using round-shaped, flat punches to obtain tablets of 4–6 kg/cm^2 hardness and 3.4–3.6 mm thickness.

Table 6: LVS Nanocrystal Formulations

Ingredients	Formulations (mg)		
	1	2	3
LVS Nanocrystals below 1000 nm	10	20	40
Directly Compressible Lactose	50	100	200
Magnesium Stearate	1	2	4
Colloidal Silicon dioxide	1	2	4
Other excipients	38	76	152

Brief Description of Figures:

Figure 1 show the photograph of nanocrystals of LVS which are prepared by the above method.

Figure 2 shows the SEM micrograph of pure LVS (plate no.1), F_1A (plate no.2) and F_2A (plate no. 3)

Figure 3 shows the Powder X-ray Diffraction (PXRD) Pattern of pure LVS, F_1A, F_2A and F_3A.

Figure 4 shows the Differential Scanning Calorimetry (DSC) thermogram of pure LVS, F_1A, F_2A and F_3A.

We Claim,

1. Process for preparation of nanocrystals of poorly water soluble drugs to increase the solubility and dissolution rate and thereby bioavailability; wherein said process comprises:

   i. preparation of drug solution by dissolving in suitable organic solvent;

   ii. addition of drug solution to water;

   iii. removal of solvent;

   iv. centrifugation;

   v. solidification of supernatant to obtain the nanocrystals.

2. The process as claimed in claim1 wherein the organic solvent is selected from the group of acetone, methanol and acetonitrile.

3. The process as claimed in claim 1 wherein the drug solution of step (i) is to be added into required quantity of water with continuous stirring on magnetic stirrer at 1000 rpm.

4. The process as claimed in claim 1 wherein the organic solvent was removed by overnight stirring at 500 rpm.

5. The process as claimed in claim 1 wherein after removal of solvent the solution was centrifuged at 5000 rpm.

6. The process as claimed in claim 1 wherein the supernatant obtained after step (iv) was subjected to solidification by freeze drying to obtain the nanocrystals.

7. The process as claimed in claim1 wherein the drug is lovastatin.

8. Lovastatin nanocrystals of having particle size less than 1000 nm.

9. Formulation of nanocrystals comprising lovastatin Nanocrystals of having particle size less than 1000 nm and suitable pharmaceutical excipients.

10. Lovastatin nanocrystals and their formulations as claimed in any of the preceding claims and as substantially described with reference to the foregoing examples.

Dated: 11th May 2009

Signature

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