ENHANCEMENT OF SOLUBILITY OF ATORVASTATIN CALCIUM BY NANOSUSPENSION TECHNIQUE

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ABSTRACT
The current research provides that more than 60% of active pharmaceutical ingredients shows poor solubility in biological fluids i.e., lipophilic or poorly water-soluble compounds. Many formulation approaches like micronization, use of fatty solutions and penetration enhancer, use of co-solvents, surfactant dispersion method, salt formation, precipitation, etc., are available to solve the problems of low solubility and low bioavailability of drugs. All these techniques are not suitable for some of the drugs, so some advanced techniques like Nanosuspension, nanosuspension, liposomes, Nanoliposome, solid dispersion and nanocrystals are used to enhance the solubility and bioavailability of BCS class II, III and IV type of drugs. Additional to this approaches emulsion and microemulsion methods, inclusion complexes with complex forming agent like EDTA, are also as drug delivery for enhancing the solubility of drugs. In this study Atorvastatin calcium was high lipophilic drug with very less aqueous solubility. To enhance the solubility of Atorvastatin calcium, it was modulated into Nanosuspension to make a desired soluble enhanced dosage form.

Key words: Nano suspension, Atorvastatin calcium, Solubility, Bioavailability, Nanosuspension.

INTRODUCTION
Solubility problems of many newly developed high-potential drugs are a severe obstacle in formulation development, especially when they show poor solubility simultaneously in aqueous and organic media. Various attempts to increase the saturation solubility, and thus solving the problem, have been tried. One of the most advanced and easy technique to enhance both bioavailability and solubility of drug was by Nanosuspension (Rajesh Dubey et al., 2006).

Nanosuspensions are submicron colloidal dispersions of nanosized drug particles stabilized by surfactants. Nanosuspensions consist of the poorly water-soluble drug without any matrix material suspended in dispersion. These can be used to enhance the solubility of drugs that are poorly soluble in water as well as lipid media. As a result of increased solubility, the rate of flooding of the active compound increases and the maximum plasma level is reached faster. This approach is useful for molecules with poor solubility, poor permeability, or both, which poses a significant challenge for the formulators. The reduced particle size renders the possibility of intravenous administration of poorly soluble drugs without any blockade of the blood capillaries. The suspensions can also be lyophilized and into a solid matrix. Apart from these advantages, it also has the advantages of liquid formulations over others. In the present review, we are mainly focusing on the different methods of preparation associated merits, demerits, and its pharmaceutical application as drug delivery system (Patravale VB et al., 2004).

The advantages of Nanosuspension was as follows: Enhance the solubility and bioavailability of drugs, Suitable for hydrophilic drugs, Higher drug
loading can be achieved. Dose reduction is possible, Enhance the physical and chemical stability of drugs, Provides a passive drug targeting (Lakshmi P et al., 2010).

MATERIALS AND METHODS
Atorvastatin calcium was acquired as a gift sample from Sashun Pvt. Ltd. Instrument used for the formulation and evaluation of Nanosuspension like UV spectrophotometer, FTIR, High speed homogenizer. And all solvents used in the formulation are selected as analytical grade solvents.

Micro precipitation – High-pressure homogenization (Nanoedge)
This technique involve the following steps: Method includes precipitation of friable materials followed by fragmentation under high shear and/or thermal energy. First, drug powders are dispersed in a stabilizer solution to form presuspension; after that, presuspension is homogenized by high pressure homogenizer at a low pressure sometimes for premilling; and finally homogenized at a high pressure for 10 to 25 cycles until the nanosuspensions are formed with desired size (Nagaraju P et al., 2010; Keck CM et al., 2006).

Evaluation of Nanosuspension: FTIR Studies (Compatibility of drug and polymer)
Infrared Spectrometry (IR) is a widely used technique in both research and industry. It is a simple and reliable technique for the identification of unknown materials, determination of the amount of components in a mixture as well as for quality control of materials. Infrared (IR) spectroscopy gives molecular structural information using the frequencies of the vibrational modes for a compound. IR is a type of energy absorption spectroscopy that uses the infrared region of the electromagnetic spectrum.

Fourier Transform Infra Red (FTIR) spectroscopy is a measurement technique whereby IR spectra are collected by using a time domain measurement. The original IR spectrometers are of the dispersive or filter types. They measure the amount of energy at each frequency of the IR spectra with the aid of a prism or grating. FTIR uses an interferometer which measures the signal and performs a Fourier transform on the data to provide an IR spectrum. From these data it can be concluded that whether the excipients and polymers used in the formulation are interacting or otherwise compatible with the drug or not (Pu X et al., 2009).

Scanning Electron Microscopy (SEM)
The scanning electron microscope (SEM) is one of the most versatile instruments widely applied to surface microstructure imaging. SEM is a type of electron microscopy that images the sample surface of a solid specimen by using a focused beam of high-energy electrons. The signal contains information about surface topography, external morphology, chemical composition, crystallographic information and electrical conductivity. (Kumar AN et al., 2009).

Transmission Electron Microscopy (TEM)
Transmission Electron Microscope (TEM) is a type of microscopy technique which operates on the same basic principle as the light microscope except TEM uses a beam of electrons, instead of light. The image is formed by the interaction of the sample specimen when electron beams are transmitted through it. Due to the small de Broglie wavelength of electrons, it is possible to get significantly higher resolution down to 0.1 nm in TEM over light microscopy. Visualization of small details in the cell or materials down to near atomic levels makes it a viable tool in medical, biological, material science, and environmental geochemistry research (Chen Y et al., 2005).

Mean Particle Size and Particle Size Distribution
The mean particle size and particle size distribution affects saturation solubility, dissolution rate, physical stability, and in vivo performance of nanosuspensions. The particle size distribution and its range named polydispersity index (PDI) can be determined by laser diffraction (LD), photon correlation spectroscopy, microscope, and coulter counter. PDI gives the physical stability of nanosuspensions and should be as lower as possible for the long-time stability of nanosuspensions. A PI value of 0.1 to 0.25 shows a fairly narrow size distribution and PI value more than 0.5 indicates a very broad distribution. LD can detect and quantify the drug microparticles during the production process. It also gives a volume size distribution and can be used to measure particles ranging from 0.05 up to 2000 μm. The coulter counter gives the absolute number of particles per volume for the different size classes. It is more efficient and suitable than LD to quantify the contamination of nanosuspensions (Chen Y et al., 2005).

Surface Charge (Zeta Potential)
Surface charge properties of the nanosuspensions are studied through zeta potential. The value of particle surface charge indicates the stability of nanosuspensions at the macroscopic level. A minimum zeta potential of ±20 mV to ±30 mV is required for electro statically stabilized. The zeta potential values are commonly calculated by determining the particle’s electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential. Electro acoustic technique is also used for the determination of
the zeta potential in the areas of material sciences (Chen Y et al., 2005).

**Saturation Solubility and Dissolution Velocity:**
Nanosuspensions have an important advantage over other techniques, that it can increase the dissolution velocity as well as the saturation solubility. The saturation solubility of the drug in different physiological buffers as well as at different temperatures should be assessed using methods described in the literature. The investigation of the dissolution velocity of nanosuspensions reflects the advantages that can be achieved over conventional formulations, especially when designing the sustained-release dosage forms based on Nanoparticulate drugs. The assessment of saturation solubility and dissolution velocity helps in determining the in vitro behavior of the formulation (Peters et al., 1996).

**Invitro drug release studies:**
An in vitro drug release study is an integral part of characterization for any drug delivery system. It is considered an indicator of batch to batch variability associated with quality control. It can discriminate between various batches for the same formulation consisting of the same ingredients at various levels. Most interestingly, it can be used as an indicator for the in vivo performance of the formulation. There are several factors which can govern drug release from a polymeric nanoparticulate carrier such as: solubility; diffusion; desorption; matrix erosion; degradation; and particle size. No standardized methods are available to study the drug release from nanosized carries. The methods available for in vitro drug release from a nanosuspension can be classified into four categories: dialysis method; sample and separate method; flow-through cell; and in situ techniques.

The Static Franz diffusion cell was used for studying the *in vitro* release of the nanosuspension. A cellulose acetate membrane was adapted to the terminal portion of the cylindrical donor compartment. A 10 mL portion of the nanosuspension containing drug, sufficient for establishing sink conditions for the assay was placed into the donor compartment. The receptor compartment contained 90 mL of 0.2M Phosphate buffer solution of pH 7.4 maintained at 37°C under mild agitation using a magnetic stirrer. At specific time intervals, aliquots of 1mL were withdrawn and immediately restored with the same volume of fresh phosphate buffer. The amount of drug released was assessed by measuring the absorbance at 246 nm using a single beam UV spectrophotometer (Chen Y et al., 2005).

**RESULT AND DISCUSSION**
SEM studies shown that nanosuspension appeared dark and with bright surroundings and a positive image. The droplet sizes are ranged between 298.6 and 452.8 nm, which was within the acceptable nanometric range. The droplet size decreases with the increase in surfactant concentration in the formulations. The droplet size of formulation F4, containing 1.0 % of surfactant tween 80 was 298.67 nm, which was lower as compared to other formulations. This may due to the effect of concentration of the surfactant and homogenizer speed i.e., 4000 RPM /12 hrs. This result is in accordance with the report that the addition of surfactant to nanosuspension systems causes the interfacial film to condense and stabilize the suspended nanoparticle. All the formulations had droplets in the nano range which is well evident from the low values of polydispersity in the range of 0.234-0.324. This signifies the uniformity of droplet size dispersion in the external medium within the formulation. If the polydispersity value is high mean, uniformity of dispersion will be low in the formulation. The polydispersity values of nanosuspension formulations are very low in all the formulation which indicates a good uniformity of droplet size within the formulation.

The solubility of Atorvastatin in Optimized formulation F4 was determined and compared with aqueous solubility of pure Atorvastatin calcium. The reports shows an significant increase in solubility of Atorvastatin calcium in nanosuspension F4 when compared to water solubility of pure drug in the presence of surfactant (Tween-80) , thus it was indirectly conclude that there may be an enhanced permeability of Atorvastatin calcium through biological membrane.

The *invitro* drug release studies shows that F4 formulation shows maximum release of drug at 10 minutes time interval itself i.e., 98.54% of drug release at 10 minutes itself, when compared to all the other formulation.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Atorvastatin calcium</th>
<th>Acetic acid</th>
<th>Tween 80</th>
<th>Homogenization stirring speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>50 mg</td>
<td>20 ml</td>
<td>0.25 %</td>
<td>1000 rpm/12 hrs</td>
</tr>
<tr>
<td>F2</td>
<td>50 mg</td>
<td>20 ml</td>
<td>0.5 %</td>
<td>2000 rpm/12 hrs</td>
</tr>
<tr>
<td>F3</td>
<td>50 mg</td>
<td>20 ml</td>
<td>0.75 %</td>
<td>3000 rpm/12 hrs</td>
</tr>
<tr>
<td>F4</td>
<td>50 mg</td>
<td>20 ml</td>
<td>1.0 %</td>
<td>4000 rpm/12 hrs</td>
</tr>
</tbody>
</table>
### Table 2. Evaluation of Nanosuspensions F1-F4

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Particle size (nm)</th>
<th>Zeta Potential (mV)</th>
<th>Poly Dispersibility Index (PDI)</th>
<th>Drug content in (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>452.8 nm</td>
<td>-3.42</td>
<td>0.234</td>
<td>82.5</td>
</tr>
<tr>
<td>F2</td>
<td>402.6 nm</td>
<td>-6.34</td>
<td>0.324</td>
<td>79.8</td>
</tr>
<tr>
<td>F3</td>
<td>344.9 nm</td>
<td>-11.24</td>
<td>0.272</td>
<td>70.42</td>
</tr>
<tr>
<td>F4</td>
<td>298.6 nm</td>
<td>-14.44</td>
<td>0.266</td>
<td>80.80</td>
</tr>
</tbody>
</table>

### Figure 1. Phase solubility studies of Atorvastatin calcium and Atorvastatin calcium Nanosuspension

![Phase solubility studies](image)

### Figure 2. *In vitro* Drug Diffusion Studies of Atorvastatin Calcium Nanosuspension in 7.4 pH Buffer

![Drug diffusion studies](image)

### CONCLUSION

From the studies it was concluded that Nanosuspension plays a major role in enhancing the solubility and permeability of BCS class II, III and IV type of drugs. Thus Nanosuspension which was an simple and easy technology having a higher chance to launch products which having low solubility on the market.

### REFERENCES


