Fabrication and Evaluation of Atorvastatin Calcium Loaded Sustained Release Microspheres using O/W/O Double Emulsion Solvent Evaporation Technique

Neelam Singh*, Tania Munjal, Sukhbir Singh and Sandeep Arora
Chitkara College of Pharmacy, Chitkara University, Chandigarh Patiala National Highway (NH-64), Tehsil-Rajpura, Distt-Patiala-140401, Punjab, India.

ABSTRACT
Microspheres are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability, reducing side effects and improving patient compliance. A double emulsion solvent evaporation method was employed for the preparation of Atorvastatin Calcium Microspheres using Eudragit RL 100 and Eudragit RS 100 polymers. The influence of formulation factors (polymer: drug ratio, emulsifier concentration, viscosity of aqueous phase, stirring speed and stirring time) on particle size, morphology, encapsulation efficiency, drug loading, process yield and in vitro release behavior was studied. The in-vitro performances of microspheres were evaluated by recovery efficiency, particle size analysis, surface topography (using scanning electron microscopy), drug-polymer compatibility (FTIR) and drug release studies. The o/w/o emulsion solvent evaporation method was suitable for the preparation of microspheres in the size range of 99.52 ± 1.06 μm, the encapsulation efficiency was 71.48 ± 1.21% (w/w) and the process yield was 82.43 ± 1.24% (w/w). SEM revealed that microparticles were smooth, spherical in shape. FTIR, XRD studies showed no potential chemical interaction between the drug and polymer used. In vitro release studies revealed a controlled release of microspheres suitable for peroral administration. Drug release from microspheres followed Higuchi kinetics.

Keywords: Solvent evaporation method, Entrapment efficiency, Process yield, Surface topography.

INTRODUCTION
Conventional oral drug administration does not usually provide rate-controlled release or target specificity. In many cases, conventional drug delivery provides sharp increase of drug concentration at potentially toxic levels. Following a relatively short period at the therapeutic level, drug concentration eventually drops off until re-administration. Today new methods of drug delivery are possible: desired drug release can be provided by rate-controlling membranes. The relatively high GI concentration and plasma peaks associated with conventional formulations result in an increased incidence of side effects; therefore multiple daily administrations were needed.

The purpose of this research was to formulate and evaluate sustained release microspheres of atorvastatin calcium. As the bioavailability of atorvastatin Calcium is 13% to 14% owing to a major hepatic first pass metabolism. It has an elimination half life of 13h and has an absorption zone from the upper intestinal tract. Efficacy of the administered dose may get diminished due to the incomplete drug release from the device above the absorption zone. Atorvastatin Calcium requires twice a day dosage in order to maintain adequate plasma concentration. Because of poor bioavailability and rather high first pass metabolism, it is necessary to develop sustained release preparation with extended clinical effect.

MATERIALS AND METHODS
MATERIALS
Atorvastatin Calcium was supplied as a gift by Panacea Biotech Ltd, Punjab, India. Eudragit RS100 and Eudragit RL100 was supplied by Evonik Industries AG Mumbai, India. Various chemicals including Light Liquid Paraffin, n-hexane, acetone (Merck Specialties Private Limited, Mumbai), and Methanol was obtained from Loba Chemicals Pvt. Ltd., Mumbai, India. All other chemical reagents were of analytical grade and were used without any further purification. Distilled water was used for all of the experiments.

METHODS
Preparation of microspheres
A solution of ATC (10 ml containing 80 mg Drug and 50 mg stearate) in dichloromethane (oil phase) was homogenized in eudragit RS100 and eudragit RL100 (240 mg) in 10 ml acetone and methanol mixture (3:2, aqueous
phase) using sonicator with approximately 2 minutes of sonication at room temperature. The resulting oil-in-water (o/w) emulsion was then emulsified at room temperature into (100 ml) of light liquid paraffin solution using 0.1% v/v span 60 emulsifier and 10% v/v n-hexane as a hardening agent and stirred with a mechanical stirrer at a stirring rate of 1000 rpm for 2 h to allow the evaporation of the organic solvent. The hardened microspheres were separated from the oil phase by filtration, rinsed with 40 ml of petroleum ether. The washings were checked for the absence of organic solvents spectrophotometrically and successive washings continued till this was achieved, and vacuum dried overnight at room temperature 8-12.

In these studies the effect of the following formulation variables on the microspheres size, surface morphology, drug loading and encapsulation efficiency, process yield were investigated: All microspheres formulations were prepared in triplicate. Following are the Polymer:drug ratio: This was investigated by variation in the polymer:drug ratio (1:1, 2:1, 3:1, 4:1 and 5:1, w/w).

1. Nature and concentration of emulsion stabilizer in the external aqueous phase: While maintaining a constant volume for the external oil phase, microspheres were produced using stabilizer span 60 at various concentration (0.05, 0.1, 0.2% w/v).
2. Volume of n-hexane.
4. Stirring rate (500, 1000 and 1500 rpm).
5. Duration of agitation during emulsification (1, 2, 3 h).

RESULTS AND DISCUSSION 12-17

The hydrophobic drugs require the use of o/w/o emulsion solvent evaporation method, first the drug is dissolved in oil phase before dissolving in aqueous polymer solution. The fast evaporation rate of the solvent permits a reduction in the processing time; moreover the evaporation rate may be used to control the microspheres size as compared with other methods where evaporation follows the microspheres formation.

Polymer:drug ratio

Atorvastatin Calcium loaded microspheres were prepared using using different polymer:drug ratio (from 1:1, 2:1, 3:1, 4:1, 5:1, w/w) by variation in the weight of polymer dissolved in Acetone and methanol solution (3:2) to investigate the eventual modification of the particle size, drug loading, efficiency of entrapment and process yield. Increasing the weight of polymer in a fixed volume of organic solvent resulted in an increase in mean particle size (from 75.43 ± 2.14 μm to 118.42 ± 2.07 μm for 1:1 to 5:1, i.e. a increase of 64.95%). suggesting that the higher weight of polymer in the sample may have led to an increased frequency of collision, resulting in fusion of semi-formed particles and producing finally an overall increase in the size of the microspheres. For 1:1 polymer:drug ratio the particles obtained were spherical in shape but with a rough surface and a mean diameter of 75.43 ± 2.14 μm, an encapsulation efficiency of 41.48 ± 2.52% (w/w) and a drug loading of 20.74 ± 1.14 % (w/w). Further increase in polymer:drug ratio from 2:1 to 4:1 led to the mean diameters of 86.83 ± 1.92 μm, 99.52 ± 1.06 μm and 110.34 ± 1.12 μm, the encapsulation efficiencies of 58.16 ± 1.33% (w/w), 70.48 ± 1.67% (w/w) and 64.92 ± 2.52% (w/w) and the drug loadings of 19.75 ± 1.17% (w/w), 18.42 ± 1.04% (w/w) and 13.98 ± 1.06% (w/w), respectively. A further increase in polymer:drug ratio, i.e., 5:1 led to production of spherical particles in aggregates with a mean diameters of 118.42 ± 2.07 μm, an encapsulation efficiency of 62.35 ± 1.09% (w/w) and a drug loading of 11.13 ± 2.53% (w/w).

Table 1: Effect of polymer:drug ratio on microspheres characteristics

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Polymer: Drug Ratio (w/w)</th>
<th>Mean Diameter* (µm) ± S.D.</th>
<th>Drug Loading* (% w/w) ± S.D.</th>
<th>Entrapment Efficiency** (%, w/w) ± S.D.</th>
<th>Process Yield### (% w/w) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE-1</td>
<td>1:1</td>
<td>75.43 ± 2.14</td>
<td>20.74 ± 1.01</td>
<td>61.37 ± 1.67</td>
<td>59.08 ± 1.38</td>
</tr>
<tr>
<td>AE-2</td>
<td>2:1</td>
<td>86.83 ± 1.92</td>
<td>19.75 ± 1.17</td>
<td>66.53 ± 2.14</td>
<td>63.46 ± 2.21</td>
</tr>
<tr>
<td>AE-3</td>
<td>3:1</td>
<td>99.52 ± 1.06</td>
<td>18.42 ± 1.04</td>
<td>71.48 ± 1.21</td>
<td>82.41 ± 1.24</td>
</tr>
<tr>
<td>AE-4</td>
<td>4:1</td>
<td>110.34 ± 1.12</td>
<td>13.98 ± 1.06</td>
<td>66.53 ± 2.14</td>
<td>63.55 ± 1.98</td>
</tr>
<tr>
<td>AE-5</td>
<td>5:1</td>
<td>118.42 ± 2.07</td>
<td>11.13 ± 2.53</td>
<td>62.35 ± 1.09</td>
<td>61.37 ± 1.67</td>
</tr>
</tbody>
</table>

* Data represent the mean of three independent experiments.

** Percentage of weight of microparticles recovered with respect to weight of polymer utilized.

### Percentage of encapsulated drug with respect to the total amount used
Microspheres were produced by o/w/o double emulsion solvent evaporation method using light liquid paraffin (100 ml) as dispersion medium, emulsifier concentration 0.1% (w/v), n-hexane concentration (10% v/v) stirring speed: 1000 rpm and stirring time: 2 h.

**Concentration of n-Hexane**

n-Hexane was selected as a hardening agent of choice, it allowed the preparation of particles in the size range of 115.88 ± 1.03 μm with an interesting drug loading of 18.98 ± 1.14% (w/w). For all the n-hexane concentration in our experimental conditions, respective emulsion droplets formed during the agitation seemed to be stable enough to harden after solvent evaporation and form the microspheres.

**Table 2: Effect of n-Hexane concentration on microspheres characteristics**

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>n-Hexane Concentration (%)v/v</th>
<th>Mean Diameter (μm) ± S.D.</th>
<th>Drug Loading (%)w/w ± S.D.</th>
<th>Entrapment Efficiency (%)w/w ± S.D.</th>
<th>Process Yield (%)w/w ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1AE-3</td>
<td>5</td>
<td>115.88 ± 1.03</td>
<td>18.98 ± 1.14</td>
<td>70.94 ± 0.68</td>
<td>60.30 ± 1.23</td>
</tr>
<tr>
<td>H2AE-3</td>
<td>10</td>
<td>101.64 ± 1.93</td>
<td>19.37 ± 2.05</td>
<td>73.48 ± 0.89</td>
<td>79.30 ± 2.05</td>
</tr>
<tr>
<td>H3AE-3</td>
<td>15</td>
<td>92.54 ± 2.03</td>
<td>16.16 ± 1.06</td>
<td>68.68 ± 1.57</td>
<td>87.80 ± 1.89</td>
</tr>
</tbody>
</table>

* Data represent the mean of three independent experiments.

**Table 3: Effect of Emulsifier concentration on microspheres characteristics**

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Emulsifier Conc. (%)w/v</th>
<th>Mean Diameter (μm) ± S.D.</th>
<th>Drug Loading (%)w/w ± S.D.</th>
<th>Entrapment Efficiency (%)w/w ± S.D.</th>
<th>Process Yield (%)w/w ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ec1AE-3</td>
<td>0.05</td>
<td>110.98 ± 1.53</td>
<td>17.98 ± 2.14</td>
<td>71.94 ± 1.82</td>
<td>63.30 ± 1.83</td>
</tr>
<tr>
<td>Ec2AE-3</td>
<td>0.1</td>
<td>101.64 ± 1.93</td>
<td>18.37 ± 1.05</td>
<td>72.48 ± 1.01</td>
<td>83.30 ± 2.11</td>
</tr>
<tr>
<td>Ec3AE-3</td>
<td>0.2</td>
<td>93.54 ± 1.03</td>
<td>17.16 ± 1.73</td>
<td>69.66 ± 0.96</td>
<td>66.80 ± 1.58</td>
</tr>
</tbody>
</table>

* Data represent the mean of three independent experiments.

**Nature and concentration of emulsifier stabilizer in the external oil phase**

Of the stabilizer studied (tween 80, span 20, span 60), span 60 resulted in successful preparation of microspheres. Nevertheless 0.1% w/v span 60 was selected as a stabilizer of choice, since it allowed the preparation of particles in the size range of 110.98 ± 1.53 μm with an interesting drug loading of 17.98 ± 2.14% (w/w). Except when 0.1% span 60 is used, it could be shown that changes in span 60 concentration were devoided of the effect on drug entrapment, and particle size distribution. For all the span 60 concentration the respective emulsion droplets formed during the agitation seemed to be stable enough to harden after solvent evaporation and form the microspheres.

**Table 3: Effect of Emulsifier concentration on microspheres characteristics**

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Emulsifier Conc. (%)w/v</th>
<th>Mean Diameter (μm) ± S.D.</th>
<th>Drug Loading (%)w/w ± S.D.</th>
<th>Entrapment Efficiency (%)w/w ± S.D.</th>
<th>Process Yield (%)w/w ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ec1AE-3</td>
<td>0.05</td>
<td>110.98 ± 1.53</td>
<td>17.98 ± 2.14</td>
<td>71.94 ± 1.82</td>
<td>63.30 ± 1.83</td>
</tr>
<tr>
<td>Ec2AE-3</td>
<td>0.1</td>
<td>101.64 ± 1.93</td>
<td>18.37 ± 1.05</td>
<td>72.48 ± 1.01</td>
<td>83.30 ± 2.11</td>
</tr>
<tr>
<td>Ec3AE-3</td>
<td>0.2</td>
<td>93.54 ± 1.03</td>
<td>17.16 ± 1.73</td>
<td>69.66 ± 0.96</td>
<td>66.80 ± 1.58</td>
</tr>
</tbody>
</table>

* Data represent the mean of three independent experiments.

**Microspheres were produced by o/w/o double emulsion solvent evaporation method**

In phase ratio, different volumes of light liquid paraffin (150 ml) were employed as external dispersing phase, resulting in different ratios between aqueous external and oil internal phases (o/w ratio), namely 15:1, 20:1, 30:1. The polymer:drug ratio was 3:1. Using as dispersion medium light liquid paraffin (100 ml) emulsifier concentration 0.1% (w/v), n-hexane concentration (10% v/v) stirring speed: 1000 rpm and stirring time: 2 h.

**Oil:Aqueous phase ratio**

In phase ratio, different volumes of light liquid paraffin (150, 200, 300 ml) were employed as external dispersing phase, resulting in different ratios between aqueous external and oil internal phases (o/w ratio), namely 15:1, 20:1, 30:1. The polymer:drug ratio was 3:1. The use of the lower w/o ratio (15:1, i.e., 150 ml) led to formation of irregular microspheres with a mean diameter of 132.91 ± 1.64 μm, an encapsulation efficiency of 66.72 ± 1.12% (w/w) and drug loading of 16.68 ± 1.64% (w/w). The highest w/o ratio (30:1, i.e., 300 ml) led to aggregates of particles after isolation. Conversely particles produced by a 20:1 w/o ratio (200 ml) enabled the production of spherical microspheres with a mean diameter of 104.47 ± 2.03 μm, the encapsulation efficiency of 74.53 ± 1.72% (w/w) and drug loading of 18.63 ± 1.08% (w/w).
Microspheres were produced by o/w/o double emulsion solvent evaporation method using as dispersion medium light liquid paraffin (100 ml) emulsifier concentration 0.1% (w/v ),n-hexane concentration(10% v/v) stirring speed: 1000 rpm and stirring time: 2 h.

**Stirring speed**

Stirring speed plays an important role in the microspheres size distribution and drug loading. In fact using 3:1 polymer:drug ratio, 20:1 o/w ratio it was found that a 500 rpm stirring speed produced particles with rough and irregular surface. On the contrary, a triple stirring speed, namely 1500 rpm, led to the production of spherical microspheres, characterized by 71.46 ± 2.12 μm mean diameter, 80.37 ±1.18% (w/w), drug loading 16.79 ± 2.18% (w/w) and 66.18 ± 1.43% (w/w) encapsulation efficiency.

The best results in term of process yield were obtained by the use of 1000 rpm stirring speed (96.85 ± 1.43%, w/w), microspheres in this condition were spherical, with a 98.56 ± 1.83 μm mean diameter, drug loading 18.17 ± 1.92% (w/w) and 71.48 ± 1.09% (w/w) encapsulation efficiency.

### Table 4: Effect of Oil:aqueous phase ratio on microspheres characteristics

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Oil:aqueous ratio</th>
<th>Drug Loading(^a) (%W/W) ± S.D.</th>
<th>Drug Loading(^b) (%W/W) ± S.D.</th>
<th>Entrapment Efficiency(^c) (% W/W) ± S.D.</th>
<th>Process Yield(^d) (%W/W) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1AE-3</td>
<td>15:1</td>
<td>132.91±1.64</td>
<td>16.68±1.64</td>
<td>66.72±1.12</td>
<td>65.34±2.05</td>
</tr>
<tr>
<td>P2AE-3</td>
<td>20:1</td>
<td>104.47±2.03</td>
<td>18.63±1.08</td>
<td>74.53±1.72</td>
<td>85.34±1.97</td>
</tr>
<tr>
<td>P3AE-3</td>
<td>30:1</td>
<td>91.38±2.48</td>
<td>17.12±2.11</td>
<td>65.49±2.15</td>
<td>65.34±1.88</td>
</tr>
</tbody>
</table>

\(^a\) Data represent the mean of three independent experiments.  
\(^b\) Percentage of weight of microparticles recovered with respect to weight of polymer utilized.  
\(^c\) Percentage of encapsulated drug with respect to the total amount used.

### Table 5: Effect of stirring speed on microspheres characteristics

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Stirring Speed (rpm)</th>
<th>Mean Diameter(^a) (μm) ± S.D.</th>
<th>Drug Loading(^a) (%W/W) ± S.D.</th>
<th>Entrapment Efficiency(^b) (% W/W) ± S.D.</th>
<th>Process Yield(^c) (%W/W) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1AE-3</td>
<td>500</td>
<td>119.48±2.08</td>
<td>16.37±1.14</td>
<td>67.47±2.08</td>
<td>61.37±1.02</td>
</tr>
<tr>
<td>T2AE-3</td>
<td>1000</td>
<td>96.85±1.43</td>
<td>18.17±1.92</td>
<td>71.48±1.09</td>
<td>80.37±1.18</td>
</tr>
<tr>
<td>T3AE-3</td>
<td>1500</td>
<td>71.46±2.12</td>
<td>16.79±2.11</td>
<td>66.18±1.43</td>
<td>64.48±1.74</td>
</tr>
</tbody>
</table>

\(^a\) Data represent the mean of three independent experiments.  
\(^b\) Percentage of weight of microparticles recovered with respect to weight of polymer utilized.  
\(^c\) Percentage of encapsulated drug with respect to the total amount used.

Microspheres were produced by o/w/o double emulsion solvent evaporation method using as dispersion medium light liquid paraffin (100 ml) emulsifier concentration 0.1% (w/v ),n-hexane concentration(10% v/v) stirring speed: 1000 rpm and stirring time: 2 h.

**Stirring Time**

For a constant speed of 1000 rpm, a polymer:drug ratio of 3:1, a o/w ratio of 20:1 an increase of the stirring time from 1 to 3 h resulted in reduction in microspheres size (from 119.48 ± 2.13 to 71.46 ± 2.72 μm). These observations could be explained by the increased shear stress generated in the emulsions associated to the increase in the duration of agitation at high homogenization rates tending to divide the droplets of the emulsions and finally inducing a decrease in the mean particle size. A 2 h stirring time was chosen because the entrapment efficiency was higher (73.48 ± 1.09%, w/w) than after 2 h (67.18 ± 1.43%, w/w).

### Table 6: Effect of stirring time on microspheres characteristics

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Stirring Time (hrs)</th>
<th>Mean Diameter(^a) (μm) ± S.D.</th>
<th>Drug Loading(^a) (%W/W) ± S.D.</th>
<th>Entrapment Efficiency(^b) (% W/W) ± S.D.</th>
<th>Process Yield(^c) (%W/W) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1AT-3</td>
<td>1</td>
<td>119.48±2.13</td>
<td>17.37±2.14</td>
<td>69.47±2.08</td>
<td>63.37±1.09</td>
</tr>
<tr>
<td>T2AT-3</td>
<td>2</td>
<td>97.85±1.62</td>
<td>18.37±1.92</td>
<td>73.48±1.09</td>
<td>83.37±1.29</td>
</tr>
<tr>
<td>T3AT-3</td>
<td>3</td>
<td>71.46±2.72</td>
<td>16.79±2.11</td>
<td>67.18±1.43</td>
<td>65.48±1.82</td>
</tr>
</tbody>
</table>

\(^a\) Data represent the mean of three independent experiments.  
\(^b\) Percentage of weight of microparticles recovered with respect to weight of polymer utilized.  
\(^c\) Percentage of encapsulated drug with respect to the total amount used.
Microspheres were produced by **o/w/o double emulsion solvent evaporation method** using as dispersion medium light liquid paraffin (100 ml) emulsifier concentration 0.1% (w/v), n-hexane concentration(10% v/v) stirring speed: 1000 rpm and stirring time: 2 h.

**Particle size analysis**
The particle size and size distribution of the prepared microspheres were measured by laser diffraction in a particle size analyzer (Mastersizer, Malvern Instruments, UK). The dried powder samples were suspended in deionised water and sonicated for 1 min with an ultra-sound probe before measurement. The obtained homogeneous suspension was determined for the equivalent volume diameter and triplicate measurements were made for each batch of microspheres.

**FT-IR Spectroscopy studies**
Infrared (IR) spectroscopy was conducted using FTIR, ALPHA, Bruker and the spectrum was recorded in the wavelength region of 4000 to 500 cm\(^{-1}\). FTIR analysis revealed that there was no interaction between the drug and the polymer, thus these polymers can be conveniently used in further development of sustained release atorvastatin calcium microspheres. Further FTIR studies indicated that the drug crystallinity did not change upon fabrication. There was no formation of amorphous drug upon fabrication. Bands due to carbonyl bond stretches were studied to infer the physical state of the drug. FTIR data in the region (4000-400 cm\(^{-1}\)) were compared for pure atorvastatin calcium and atorvastatin calcium encapsulated in the microspheres. As expected, two main characteristic C=O bands were observed for crystalline drug at 1653 and 1590 cm\(^{-1}\), assigned to the aliphatic and aromatic carbonyl stretching bands, respectively. Microsphere formulation also yielded peaks at the same wavelengths. The FT-IR spectra of pure Atorvastatin Calcium and microspheres of the optimized batch (FOAT-3) are shown in **Figures 1 (a) & 1 (b)**. The FT-IR spectra of pure ATC showed characteristic peaks at 2955.15 cm\(^{-1}\) (C-H stretching), 1313.56 cm\(^{-1}\) (C-N stretching), 3059.15 cm\(^{-1}\) (C-H\(_2\) stretching alcoholic group), 1564.97 cm\(^{-1}\) (C=O stretching amide group), 3403.27 cm\(^{-1}\) (N-H stretching), 1656.97 cm\(^{-1}\) (C=C bending), 751.62 cm\(^{-1}\), 696.95 cm\(^{-1}\) (C-F stretching), 1104.39 cm\(^{-1}\) (O-H bending). It might be the possibility of intermolecular hydrogen bonding between adjunct ATC molecules. The spectrum of pure drug was equivalent to the spectra obtained by the optimized batch (FOAT-3). This indicated that no interaction occurred with microencapsulation of drug and polymers. The results revealed no considerable changes in the IR peaks of drug These observations indicated the compatibility of polymers and the drug.

![Fig. 1(a): FTIR Graph of Drug (Atorvastatin Calcium) Figure 1(b): FTIR Graph of Optimized Batch FOAT-3.](image-url)
Powder X-ray diffraction (pXRD) for assessment of crystallinity
In order to determine the physical state of the drug before and after microspheres preparations, the XRD patterns of the pure drug and the formulations were investigated using an Bruker’s D8 advance diffractometer (Germany). The samples were irradiated with monochromatized CuKα radiation, and the scanning range (2θ) was from 2–50°. The voltage and current were set to 30kv and 30mA, respectively. X-ray patterns were analyzed using an X-pert data collector and X-pert data viewer V-1.0 software.

The diffraction pattern of pure drug showed characteristic high-intensity diffraction peaks at 8.96, 9.35, 10.01, 10.37, 11.65, 12.00, 16.85, 19.26, 21.36, 22.50, 23.12, and 23.51, which indicates that the drug is present in the crystalline form that is also confirmed by DSC results, whereas FOAT-3 microspheres formulation showed reflections at 17.18, 18.66, 21.08, and 23.3. The pure drug exhibits reflections at 8.9, 9.3 and 10, these strong reflections of pure drug were masked in the microspheres formulation and exhibits weak reflections at 8.9, 9.3 and 10 (two theta) 2θ. This decrease in intensity of reflection is attributed to the presence of other excipients in the formulation, Figure 2 (b). It is clear that the reflections of the pure drug (Figure 2 (a) match satisfactorily with the reflections of the drug in the microsphere formulation. Thus, it can be concluded that the polymorph of pure drug was the same as that of Atorvastatin Calcium polymorph incorporated in microspheres, and no transformation took place during the manufacturing process and storage.

In vitro release studies
In vitro dissolution studies were carried out on the microspheres at 37°C (± 0.5°C) at 100 rpm with USP Dissolution Apparatus II (). For the acid stage, an accurately weighed sample of microparticles was suspended in the dissolution media consisting of 500 ml of 0.1 N (pH 1.2) hydrochloric acid without enzymes and dissolution was done for 2 h. At the end of the 2 h, 400 ml of 0.1M tribasic sodium phosphate was added to all dissolution vessels, the pH was adjusted to 7.2 (± 0.2) and the dissolution was continued until the microspheres were depleted of drug or for 24 h. Aliquots of dissolution fluid were withdrawn at specified time intervals to assay the released drug spectrophotometrically at 246 nm in both stages of dissolution. Each graphical data point was an average of dissolution data from three samples. Corrections were made for the removal of samples (Figure 3).
Drug release pattern from microspheres

In order to investigate the release mechanism of present drug delivery system, the release data of microparticles were fitted to classic drug-release kinetics models. The release rates were analysed by zero-order model, first order model, Higuchi model and Korsmeyer Peppas model, which have been suggested to describe drug-release kinetics from microparticles. Higuchi plot and Peppas plot of final optimized batch of microspheres (FOAT-3) are given in Figure 4(a) and 4(b) respectively.

Fig. 4(a): Higuchi Plot

Fig. 4(b): Korsemeyer Peppas Plot
Kinetics of drug release  
In order to investigate the release mechanism of present drug delivery system, the data obtained from in vitro release of final optimized batch were fitted into equations for the zero-order, first-order, and Higuchi release model and Peppas equation. The interpretation of data was based on the values of the resulting regression coefficients. The in vitro drug release showed the regression coefficient values for Higuchi’s model (Figure 4a) \( R^2 = 0.9782 \) and Peppas model \( R^2 = 0.9804 \) and a value of \( n = 0.587 \) (Figure 4b) indicating anomalous transport.

Statistical analysis  
Statistical data analysis were performed using the Student’s t-test and one-way analysis of variance (ANOVA) with \( p < 0.05 \) as the minimal level of significance.

Scanning electron microscopic analysis  
The prepared microparticles were coated with gold palladium under an air atmosphere for 150 seconds to achieve a 20 nm film (Coater Polaron, 18mA current at 1.4 kV). The coated sample were then examined using scanning electron microscope (Variable Pressure Scanning Electron Microscope (SEM) Hitachi S3400N). The morphology and appearance of microparticles were examined by scanning electron microscopy as shown in figure 5.

CONCLUSION  
O/W/O emulsion solvent evaporation method was used to prepare microspheres of Atorvastatin Calcium with Eudragit RS100 and Eudragit RL100. These investigations have also provided an understanding of the effects of some process parameters on particle size and shape, and encapsulation efficiency, drug loading and process yield. The investigated system has the potential to remain in treated site for a prolonged periods and capable of maintaining constant concentration of drug through a longer duration of time due to its sustained action. This can be expected to reduce the problem of low bioavailability and high first pass metabolism and also decrease the dose dependent side effects associated with repeated administration of conventional Atorvastatin Calcium loaded dosage forms, which ultimately improve patient compliance. O/W/O emulsion solvent evaporation method were suitable for the preparation of microspheres in the size range of 99.52 ±1.06 \( \mu m \), the encapsulation efficiency was 71.489 ± 1.21% (w/w), drug loading was 18.42 ± 1.04% (w/w) and the process yield was 82.43 ± 1.24% (w/w). It concluded that a sustained release Eudragit RS100 and Eudragit RL100 microsphere containing Atorvastatin Calcium were successfully prepared by using w/o/w emulsion solvent evaporation method with the selection of appropriate experimental conditions.

REFERENCES  
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