Title: A DRUG DELIVERY SOLID DOSAGE FORMULATION OF SIROLIMUS

Abstract: The present invention relates to a drug delivery solid dosage formulation of Sirolimus having improved in-vitro dissolution and in-vivo bioavailability, wherein Sirolimus is surface treated with a complexing agent and wetting agent in dissolved state, and to its method for preparation.
Title of the Invention:
A drug delivery solid dosage formulation of Sirolimus.

Field of the Invention:
The present invention relates to drug delivery formulation of Sirolimus.
Particularly it relates to drug delivery solid dosage formulation of Sirolimus.
More Particularly, it relates to drug delivery solid dosage formulation of Sirolimus having improved dissolution and bioavailability.

Background of the invention:
The drug Sirolimus, may also be referred to as rapamycin is an antibiotic macrolide produced by actinomycete bacterium - *Streptomyces hygroscopicus*. It was initially identified as antifungal agent capable of inhibiting growth of fungi such as *Candida albicans* and *Microsporum gypseum*. It is also reported to have antibiotic properties [US Patent No. 3,929,992 (US'992)]. It is also a potent immunosuppressant in preventing organ transplant rejection. Its immunosuppressant activities have been described in US Patent No. 5,100,899.

Although it is a structural analogue of tacrolimus, it acts somewhat differently and has different side-effects.

Additionally, Sirolimus is also effective against autoimmune diseases, solid tumors, and adult T-Cell Leukemia or Lymphoma, and is capable of acting as anti-inflammatory drug. It has been found to be capable of inhibiting T-lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (interleukin IL-2, IL-4 and IL-5). It stimulates distinctly different from other immunosuppressants.

Sirolimus is capable of preventing B cell differentiation into plasma cells, reducing production of IgM, IgG, and IgA antibodies.

It is also capable of being employed with or without other immunosuppressants, namely cyclosporine, corticosteroids.

It is also capable of inhibiting antibody production by cellularly binding to the immunophilin, FK Binding Protein 12 (FKBP-12) to generate an immunosuppressive complex, which binds to and inhibits the activities of the mammalian Target of Rapamycin (mTOR), a key regulatory Kinase and suppresses T-Cell proliferation. It is active against tumors that are PI3K/AKT/mTOR-dependent.

Due to above-described wide applicability of Sirolimus, it is center of attention of various research and development scientists.
The US patent 5,989,591 provides the drug delivery system having sugar coating with drug layering which is complicated skill oriented system and leaves room of variations and shows inconsistent *in-vitro* and *in-vivo* results.

The Sirolimus is insoluble in water, but freely soluble in organic solvents particularly, benzyl alcohol, chloroform, acetone and acetonitrile. Therefore, it has very poor dissolution and bioavailability. In case, Sirolimus is taken in the form of oral solution, its bioavailability is just 14% and in case it is taken in the form of a tablet its mean bioavailability is about 27%. The fat contents in food have been found to increase its bioavailability, but due to added disadvantages of fat contents these are not desirable in food contents.

**Need of the invention:**

Therefore, there is a need to provide a drug delivery solid dosage formulation of Sirolimus having improved dissolution and permeability, and improved uniform distribution of drug in the filler and easy to manufacture on mass scale, and improved bioavailability, drug dissolvability in biological media, and the formulation is independent of fat content in the physiological system.

**Objects of the Invention:**

Therefore, the present invention aims to provide an improved drug delivery system for a drug, particularly for an immunosuppressant drug, more particularly for Sirolimus which is capable of overcoming at least some of the above described disadvantages, limitations and drawbacks of prior art.

Accordingly, main object of the present invention is to provide a drug delivery solid dosage formulation of Sirolimus having improved dissolution.

This is also an object of the present invention to provide a drug delivery solid dosage formulation of Sirolimus having improved dissolution and bioavailability.

This is also an object of the present invention to provide a drug delivery solid dosage formulation of Sirolimus which is easy to be swallow with water.

This is also an object of the present invention to provide a drug delivery solid dosage formulation with film coating, which is easy to be produced on mass scale.

This is also an object of the present invention to provide a non dispersible drug delivery dosage formulation of Sirolimus which has improved dissolvability in biological media, and the formulation is independent of fat content in the physiological system.

**Description of the Invention:**
Accordingly, the present invention relates to a drug delivery solid dosage formulation of Sirolimus as immunosuppressant drug, wherein the formulation has improved dissolution, bioavailability, and is independent of fat content in the physiological system, and wherein the sirolimus is surface treated with a complexing agent and wetting agent in dissolved state, and wherein the complexing agent is hydroxypropyl β - cyclodextrin (Kleptose HPB) and the wetting agent is selected from a group comprising polysorbate 80, sodium lauryl sulphate [SLS], propylene glycol, polyoxyl 40 hydrogenated castor oil (cremophor RH 40) and mixture thereof.

In accordance with present invention, the Sirolimus is dissolved in an organic solvent, preferably in acetone.

In accordance with present invention, the solvent for complexing agent is selected from a group comprising polyethylene glycol, ethyl alcohol and propylene glycol.

In accordance with preferred embodiment of the present invention, the solvent for complexing agent is preferably propylene glycol, which in present invention has been found to act not only as solvent for complexing agent, but also act as wetting agent. This dual purpose of propylene glycol has been found to improve the dissolution of Sirolimus in the formulation of present invention.

In accordance with present invention, the wetting agent is preferably propylene glycol or polyoxyl 40 hydrogenated castor oil (Cremophor RH40), more preferably polyoxyl 40 hydrogenated castor oil (Cremophor RH40).

In accordance with present invention the solutions of Sirolimus in acetone and complexing agent in propylene glycol are mixed and to this solution a wetting agent is added which results in formation of a complex which has been surprisingly found to have enhanced dissolution inspite of Sirolimus being insoluble in water, and during the bioavailability studies it has been found to have improved bioavailability.

In accordance with present invention, the complex resulted by surface treatment of Sirolimus with complexing agent and wetting agent in dissolved state is adsorbed or spreaded on a filler selected from a group comprising microcrystalline cellulose, powdered cellulose, polyols, binders and mixture thereof. In accordance with present invention, the binder selected in accordance with present invention acts as filler as well as binder.

In accordance with present invention, the formulation may optionally comprise disintegrant.
In accordance with preferred embodiment of present invention, the binder is selected from a group comprising hydroxypropyl cellulose (Klucel LF Pharm), polyvinyl pyrrolidone, hydroxypropylmethyl cellulose, and mixture thereof.

In accordance with preferred embodiment of present invention, the binder is preferably hydroxypropyl cellulose (Klucel LF Pharm) having a viscosity of 75 to 150 cps at 25°C in a 5% aqueous solution, which has been found to further enhance the compressibility of the dosage form to tablet.

In accordance with preferred embodiment of present invention, the polyols is selected from group comprising pearlitol, sorbitol, mannitol, particularly the α-form of spray dried mannitol, and mixture thereof.

In accordance with preferred embodiment of present invention, the polyols is preferably pearlitol, which is more preferably pearlitol SD 200, which has been found to further enhance the flowability of powder to compress to a tablet as well as it adsorb complex solution for improved dissolution.

In accordance with preferred embodiment of present invention, the disintegrant is selected from a group comprising crosslinked polyvinyl pyrrolidone and Low-substituted Hydroxypropyl Cellulose (L-HPC, grade: LH-21), and mixture thereof.

In accordance with preferred embodiment of present invention, the disintegrant is preferably Low-substituted Hydroxypropyl Cellulose (L-HPC, grade: LH-21) having a hydroxypropoxyl content of about 10.0 - 12.9%, which has been found to further enhance the dissolution by way of reducing the disintegration with faster drug release.

In accordance with present invention, the dried powder obtained after adsorption or spreading on filler is lubricated to compress to tablets for film coating with a compound selected from a group comprising colloidal silicon dioxide (Aerosil 200) and magnesium stearate, and mixture thereof.

Accordingly, in one embodiment, the present invention also relates to a method for preparation of drug delivery solid dosage formulation of sirolimus comprising steps of:-

a) preparing solutions of sirolimus in a solvent and of complexing agent in another solvent;

b) mixing solutions from step-a) and adding thereto a wetting agent resulting in formation of a complex of sirolimus, complexing agent and wetting agent; and
c) adsorbing the complex from step-b) in dissolved state on a filler and allowing to dry to a powder form which is compressed to a tablet form;

wherein, complexing agent is hydroxypropyl β - cyclodextrin; and wetting agent is selected from a group comprising polysorbate 80, sodium lauryl sulphate [SLS], propylene glycol, polyoxyl 40 hydrogenated castor oil (cremophor RH 40) and mixture thereof.

In accordance with method of present invention, the solution of Sirolimus is prepared in an organic solvent, preferably in acetone.

In accordance with method of present invention, the solution of complexing agent is prepared in a solvent selected from group comprising polyethylene glycol and propylene glycol.

In accordance with preferred embodiment of method of present invention, the solvent for complexing agent is preferably propylene glycol, which has been found to act not only as solvent for complexing agent, but also act as wetting agent. This dual purpose of propylene glycol has been found to improve the dissolution of Sirolimus in the formulation of present invention.

In accordance with present invention, the wetting agent is preferably propylene glycol or polyoxyl 40 hydrogenated castor oil (Cremophor RH40), more preferably polyoxyl 40 hydrogenated castor oil (Cremophor RH40).

In accordance with present invention, the filler is selected from a group comprising microcrystalline cellulose, powdered cellulose, polyols, binders and mixture thereof. In accordance with present invention, the binder acts as filler as well as binder.

In accordance with present invention, the formulation may optionally comprise disintegrants.

In accordance with preferred embodiment of present invention, the binder is selected from a group comprising hydroxypropyl cellulose (Klucel LF Pharm), polyvinyl pyrrolidone, and hydroxypropyl methyl cellulose, and mixture thereof.

In accordance with preferred embodiment of present invention, the binder is preferably hydroxypropyl cellulose (Klucel LF Pharm), which has been found to enhance the compressibility of the dosage from to tablet.

In accordance with preferred embodiment of present invention, the polyols is selected from group comprising pearlitol, sorbitol, and mannitol, particularly the α-form of spray dried mannitol, and mixture thereof.
In accordance with preferred embodiment of present invention, the polyols is preferably pearlitol, which is more preferably pearlitol SD 200, which has been found to further enhance the flowability of Powder to compress to a tablet as well as it adsorb complex solution for improved dissolution.

In accordance with present invention, the dried powder obtained after adsorption or spreading on filler is lubricated to compress to tablets for film coating with a compound selected from a group comprising colloidal silicon dioxide (Aerosil 200) and magnesium stearate, and mixture thereof.

In accordance with one of the preferred embodiments of the present invention, the tablet form from step - c) is further processed for film coating with opadry containing hydroxypropyle methyl cellulose having a viscosity of about 5 to 6 mps of 2 % w/v aqueous solution.

Presently, the mechanism of complexation is not known. It is believed that molecules of Sirolimus get entrapped in molecules of complexing agent and its surface treatment with wetting agent, due to judicious selection of complexing agent, solvent for complexing agent and wetting agent, has been surprisingly and unexpectedly found to result in formulation having enhanced in-vitro dissolution and in-vivo bioavailability.

The in-vivo bioavailability studies of present formulation, when prepared in accordance with method of present invention, performed on male and female albino rat surprisingly and unexpectedly demonstrated better in-vivo bioavailability as compared to Rapamune (the Sirolimus formulation) of prior art.

The film tablet obtained in accordance with present invention has been surprisingly and unexpectedly found to have improved dissolution and easy to swallow with water.

As it is evident from the foregoing description, the drug delivery solid dosage formulation of Sirolimus is easy to be manufactured on mass scale production.

In accordance with preferred embodiment of present invention, Sirolimus and the complexing agent - hydroxypropyl β - cyclodextrin (Kleptose HPB) are taken in a weight ratio varying from about 1 : 0.5 to about 4 : 2 w/w.

In accordance with preferred embodiment of present invention, Sirolimus and the wetting agent, which in accordance with present invention is preferably Cremophor RH 40 are taken in a weight ratio varying from about 0.5 : 2.25 to about 3 : 13.5 w/w.

In accordance with preferred embodiment of present invention, Sirolimus, complexing agent [hydroxypropyl β - cyclodextrin (Kleptose HPB)] and the wetting
agent [cremophor RH 40] are respectively taken in a weight ratio varying from about 0.5 : 0.25 : 2.25 to about 4 : 2 : 18 w/w.

In accordance with preferred embodiment of present invention, the complexing agent [hydroxypropyl β-cyclodextrin (Kleptose HPB)], the wetting agent [Cremophor RH 40] and the solvent for complexing agent [propylene glycol] are respectively taken in a weight ratio varying from about 0.5 : 4.5 : 10 to about 4 : 36 : 40 w/w.

In accordance with one of the embodiments of present invention, Sirolimus, the wetting agent [Cremophor RH 40], the complexing agent [hydroxypropyl β-cyclodextrin (Kleptose HPB)] and the solvent [propylene glycol] are mixed, respectively, in a weight ratio of about 1 : 4.5 : 0.5 : 10 w/w.

In accordance with one of the preferred embodiments of present invention, the combination of binder to disintegrant, where the binder is hydroxypropyl cellulose (Klucel LF Pharm) having a viscosity of 75 to 150 cps at 25°C in a 5% aqueous solution and disintegrant is low-substituted hydroxypropyl cellulose (L-HPC grade-LH-21) having a hydroxypropoxy content of about 10.0 - 12.9% is in the weight ratio of about 0.33 : 0.47 to about 4:5.64 w/w.

In accordance with one of the preferred embodiments of present invention, microcrystalline cellulose may vary from about 40% to about 60% of the formulation.

In accordance with one of the preferred embodiments of present invention, Polylol, which is preferably Pearlitol SD 200 may vary from about 10% to 30% w/w of the formulation.

In accordance with one of the preferred embodiments of present invention, Sirolimus acts as immunosuppressant drug.

In accordance with one of the preferred embodiments of the present invention, the formulation comprises complex of Sirolimus as immunosupprasant drug in acetone, hydroxypropyl β-cyclodextrin as complexing agent in propylene glycol used as solvent as well as wetting agent.

In accordance with most preferred embodiment of present invention, the formulation comprises complex of Sirolimus as immunosupprasant drug in acetone, hydroxypropyl β-cyclodextrin as complexing agent in propylene glycol, Cremophor RH 40 as wetting agent.

It has been found that formulation comprising complex of Sirolimus as immunosupprasant drug in acetone, hydroxypropyl β-cyclodextrin as complexing agent in propylene glycol used as solvent as well as wetting agent [Formulation - I]
does demonstrate better *in-vitro* dissolution and better *in-vivo* bioavailability, however the formulation comprising complex of Sirolimus as immunosupprasant drug in acetone, hydroxypropyl β - cyclodextrin as complexing agent in propylene glycol, Cremophor RH 40 as wetting agent [Formulation - II], surprisingly and unexpectedly demonstrate further enhanced *in-vivo* bioavailability, confirming surprising and unexpected results for the formulations [Formulation - I and Formulation - II] of the present invention particularly for the formulation comprising complex of Sirolimus as immunosupprasant drug in acetone, hydroxypropyl β - cyclodextrin as complexing agent in propylene glycol, Cremophor RH 40 as wetting agent [Formulation - II], and of presently provided method of preparation thereof.

It has also been found that drug Sirolimus of formulations of present invention immediately binds the RBCs.

Further, the preliminary analysis of Sirolimus in plasma was not detected up to about 4 hours after dosing in rats.

Further, no mortality or any signs of intoxication have been observed with formulations of present invention for a period upto day 14 at a dose of 2.0 mg per animal.

Therefore, it can be concluded that the formulations of present invention are safe for use.

**Experimental Studies:**

The following four formulations [Table - I] of present invention were prepared in accordance with method of present invention as described hereinabove.
Table - I

<table>
<thead>
<tr>
<th>Name of Ingredient</th>
<th>Exp.-01 (mg/tablet)</th>
<th>Exp.-02 (mg/tablet)</th>
<th>Exp.-03 (mg/tablet)</th>
<th>Exp.-04 (mg/tablet)</th>
<th>Exp.-05 (mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sirolimus</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hydroxypropyl β – Cyclodextrin</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>--</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>--</td>
</tr>
<tr>
<td>Cremophor RH 40</td>
<td>7</td>
<td>9</td>
<td>9</td>
<td>--</td>
<td>9</td>
</tr>
<tr>
<td>Microcrystalline cellulose (Avicel 102)</td>
<td>--</td>
<td>155.50</td>
<td>182</td>
<td>191</td>
<td>182</td>
</tr>
<tr>
<td>Pearlitol SD 200</td>
<td>144</td>
<td>58</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Hydroxypropyl cellulose (Klucel LF Pharm)</td>
<td>--</td>
<td>28</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Low-substituted Hydroxypropyl Cellulose (L-HPC, grade: LH-21)</td>
<td>6</td>
<td>22</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Powdered Cellulose (Arbocel - M80)</td>
<td>60</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Hydroxypropyl cellulose (Klucel EF Pharm)</td>
<td>5</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Aerosil – 200</td>
<td>--</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>2</td>
<td>1.5</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Opadry containing HPMC</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Total (weight) per Tablet</td>
<td>256 mg</td>
<td>312 mg</td>
<td>332 mg</td>
<td>332 mg</td>
<td>311 mg</td>
</tr>
</tbody>
</table>

All the above five formulations in the form of film coated tablet of present invention comprising Sirolimus having modified surface by forming a complex with complexing agent (except formulation of Exp. 05) and wetting agent propylene glycol (formulation of Exp. 04) with or without Cremophor RH 40 (formulations of Exp. 01, 02 and 03) were found to have a consistent improvement in *in-vitro* dissolution in the recommended media of purified water with 0.4% Sodium Lauryl Sulphate [SLS]. The *in-vitro* dissolution of formulations of Exp. 03 and 04 was found to be about 90 to 95% after about two hrs. When a blank formulation without complexing agent and wetting agent of present invention was studied, it was found to have poor dissolution of about 63% confirming surprising and unexpected results on use of Sirolimus having modified surface by forming a complex with complexing agent and wetting agent propylene glycol with or without Cremophor RH 40. The formulation comprising complex of Sirolimus as immunosuppressant drug in acetone, hydroxypropyl β -
cyclodextrin as complexing agent in propylene glycol, Cremophor RH 40 as wetting agent, which is the most preferred embodiment of the present invention, demonstrated, surprisingly and unexpectedly, consistent improvement in in-vitro dissolution [Table - II] which indicates that it is expected to have improved bioavailability.

<table>
<thead>
<tr>
<th>Time in Minute</th>
<th>Rapamune D66317 - US (2 mg)</th>
<th>Present invention Exp. 3 (2 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>10</td>
<td>91.07</td>
<td>86.69</td>
</tr>
<tr>
<td>20</td>
<td>95.87</td>
<td>91.27</td>
</tr>
<tr>
<td>30</td>
<td>98.32</td>
<td>92.09</td>
</tr>
<tr>
<td>45</td>
<td>97.89</td>
<td>92.3</td>
</tr>
<tr>
<td>60</td>
<td>99.81</td>
<td>92.9</td>
</tr>
<tr>
<td>120</td>
<td>99.90</td>
<td>94.84</td>
</tr>
</tbody>
</table>

It has also been observed that formulations of Exp. 02, 03 and 04 comprising hydroxypropyl cellulose (Klucel LF Pharm) as binder demonstrated better dissolvability and binding strength as compare to formulations of Exp. 01 comprising hydroxypropyl cellulose (Klucel EF Pharm) as binder.

The present studies also demonstrated that formulation of Exp. 01 comprising powdered cellulose as filler demonstrated poor dissolution.

**Bioavailability Studies:**

For the comparison purpose, the formulation of Exp.03 comprising complex of Sirolimus as immunosuppressant drug in acetone, hydroxypropyl β - cyclodextrin as complexing agent in propylene glycol, Cremophor RH 40 as wetting agent - the most preferred embodiment of the present invention; and formulation of Exp.04 comprising complex of Sirolimus as immunosuppressant drug in acetone, hydroxypropyl β - cyclodextrin as complexing agent in propylene glycol used as solvent as well as wetting agent - one of the preferred embodiments of the present invention were compared for the in-vivo bioavailability, using rat model of male and female gender, with that of Rapamune [of Batch No. D66317 of Wyeth Pharmaceuticals Ltd].

For experimental studies, three male and three female rats were administered formulations of Exp. 03, Exp. 04 and Rapamune [of Batch No. D66317 of Wyeth Pharmaceuticals Ltd]. A blank - placebo was also performed without drug. For these studies, one tablet of the formulation was converted into powder with the help of mortar and pestle. The powder was suspended in 2.0 ml of water. This 2.0 ml
suspension was administered orally to each rat. A 0.5 ml blood was withdrawn before dosing the animals. A 0.5 ml of blood was withdrawn after 30 minutes, and 1, 2, 4 hours interval of time. These blood samples were further treated for the extraction and HPLC analysis.

5 Extraction of Sirolimus from Blood:

A 0.5 ml of blood was collected with EDTA as an anticoagulant in each of above experiment. In a screw capped plastic test tube a 0.5 ml of whole blood, 1.0 ml of ZnSO₄ (50 gms/lit) and 1.0 ml of acetone was added. It was mixed with Vortex Mixer for 20 seconds and then centrifuged at 2600g at room temperature for 5.0 minutes. The supernatant was collected in a glass centrifuge tube. A 200 μl NaOH (200 mmol / lit) was added to the supernatant. The tube was mixed with Vortex Mixer for 30 seconds. A 2.0 ml of 1-chlorobutane was added to it and again mixed with Vortex Mixer for 1.0 minute. The supernatant was collected and was dried under nitrogen for 30 minutes. The dry extract was reconstituted with 150 μl of Mobile Phase and 500 μl hexane. The content was mixed with Vortex Mixer for 30 seconds and then centrifuged at 2600g at Room Temperature for 2.0 minutes. The hexane layer was discarded and the content of the tube was dried under nitrogen for 1.0 minute to remove any residue of hexane. The 20 μl extract was then injected to the HPLC column. The mobile phase used was Acetonitrile : Water in the ratio of 95:05 with flow rate of 1.0 ml/ minute. The analysis was conducted at 278 nm. The peak was detected at 4.73 to 4.87. The study revealed different concentrations of the drug in blood samples of male and female rats. The pharmacokinetic experimental data for formulation of Exp. 03 and of reference formulation - Rapamune are given in Table III; and for formulation of Exp. 04 and of reference formulation - Rapamune are given in Table IV, wherein CI is Clearance, AUC is Area Under Curve, VSS is Volume of distribution at steady state, Cmax is concentration maximum in blood and Tmax is time maximum for reaching maximum concentration in blood.
From the above experimental data it is observed that:

1. The formulation of Exp. 03 of present invention containing 2.0 mg Sirolimus when dosed in 100 gm male rat showed a Cmax of 35.03 µg/ml at 1.0 hour, and the clearance calculated was 5.709 µg/min/kg in male.

2. The formulation of Exp. 03 of present invention containing 2.0 mg Sirolimus when dosed in 100 gm female rat showed a Cmax of 6.4 µg/ml at 1.0 hour, and the clearance calculated was 31.25 µg/min/kg in female.

3. The formulation of Exp. 04 of present invention containing 2.0 mg Sirolimus when dosed in 100 gm male rat showed a Cmax of 24.56 µg/ml at 1.0 hour, and the clearance calculated was 8.141 µg/min/kg in male.
4. The formulation of Exp. 04 of present invention containing 2.0 mg Sirolimus when dosed in 100 gm female rat showed a Cmax of 3.2 µg/ml at 0.5 hour, and the clearance calculated was 82.918 µg/min/kg in female.

5. The reference formulation - Rapamune of prior art containing 2.0 mg Rapamycin when dosed in 100 gm male rat showed of 11.7 µg/ml at 1.0 hour, and the clearance calculated was 17.094 ug/min/kg in male.

6. The reference formulation - Rapamune of prior art containing 2.0 mg Rapamycin when dosed in 100 gm Female rat showed a Cmax of 2.87 µg/ml at 0.50 hour, and the clearance calculated was 92.507 µg/min/kg in female.

These experiments confirm that formulations of Exp. 03 and of Exp. 04 of the present invention are better than that of Rapamune formulation which confirms the surprising and unexpected results for the formulations of present invention comprising complex of Sirolimus as immunosupprasant drug in acetone, hydroxypropyl β - cyclodextrin as complexing agent in propylene glycol as solvent for complexing agent as well as wetting agent without or without Cremophor RH 40 as wetting agent, and of presently provided method of preparation thereof.

These experiments also confirm that the formulation of Exp. 03 demonstrates even better in-vivo bioavailability than the formulation of Exp. 04 of the present invention confirming surprising and unexpected results for the formulation of present invention comprising complex of Sirolimus as immunosupprasant drug in acetone, hydroxypropyl β - cyclodextrin as complexing agent in propylene glycol, Cremophor RH 40 as wetting agent.

The above studies further confirm that drug Sirolimus of formulations of present invention immediately binds the RBCs particularly in respect of formulation of Exp. 03 and formulation of Exp. 04 of present invention.

Further, the preliminary analysis of Sirolimus of formulations of present invention in plasma was not detected up to 4 hours after dosing in rats.

Further, there was no mortality or any signs of intoxication in case of formulations of present invention upto day 14 at a dose of 2.0 mg per animal.

Therefore, it can be concluded that the formulation of Exp. 03 and formulation of Exp. 04 of present invention are safe for use.

It has been observed from the above experimental studies that Rapamune may have demonstrated better in-vitro dissolution, but its in-vivo bioavailability is much.
poorer than the formulations of present invention, particularly the formulations of Exp 03 and Exp 04 of the present invention.

The present invention has been described with reference to foregoing examples. It is obvious for a person skilled in the art that deviations therefrom are possible without deviating from the scope of present invention. Therefore, in one embodiment such deviations are included in the scope of present invention.
Claims

1. A drug delivery solid dosage formulation of Sirolimus as immunosuppressant drug, wherein the formulation has improved *in-vitro* dissolution and *in-vivo* bioavailability, and is independent of fat content in the physiological system, and wherein the sirolimus is surface treated with a complexing agent and wetting agent in dissolved state, and wherein the complexing agent is hydroxypropyl β-cyclodextrin and the wetting agent is selected from a group comprising polysorbate 80, sodium lauryl sulphate [SLS], propylene glycol, polyoxy 40 hydrogenated castor oil (cremophor RH40) and mixture thereof.

2. A formulation as claimed in claim 1, wherein solvent for sirolimus is an organic solvent.

3. A formulation as claimed in claim 2, wherein organic solvent is acetone.

4. A formulation as claimed in any one of the preceding claims, wherein solvent for complexing agent is selected from a group comprising polyethylene glycol, ethyl alcohol and propylene glycol.

5. A formulation as claimed in claim 4, wherein solvent is propylene glycol.

6. A formulation as claimed in any one of the preceding claims, wherein wetting agent is selected from propylene glycol or polyoxy 40 hydrogenated castor oil (Cremophor RH40).

7. A formulation as claimed in any one of the preceding claims, wherein wetting agent is polyoxy 40 hydrogenated castor oil (Cremophor RH40).

8. A formulation as claimed in any one of the preceding claims, wherein formulation further comprises filler selected from a group comprising microcrystalline cellulose, powdered cellulose, polyols, binders and mixture thereof.

9. A formulation as claimed in claim 8, wherein binder is selected from a group comprising hydroxypropyl cellulose (Klucel LF Pharm), polyvinyl pyrrolidone, hydroxypropylmethyl cellulose, and mixture thereof.

10. A formulation as claimed in any one of the claims 8 or 9, wherein binder is hydroxypropyl cellulose (Klucel LF Pharm) having a viscosity of 75 to 150 cps at 25°C in a 5% aqueous solution.
11. A formulation as claimed in any one of the preceding claims 8 to 10, wherein polyols is selected from group comprising pearlitol, sorbitol, mannitol, and mixture thereof.

12. A formulation as claimed in claim 11, wherein mannitol is a form of spray dried mannitol.

13. A formulation as claimed in any one of the preceding claims 8 to 12, wherein polyols is pearlitol, which is pearlitol SD 200.

14. A formulation as claimed in claim 8, wherein microcrystalline cellulose vary from about 40% to about 60% of the formulation.

15. A formulation as claimed in any one of the claims 8 to 14, wherein polyols vary from about 10% to 30% w/w of the formulation.

16. A formulation as claimed in any one of the preceding claims, wherein formulation further comprises disintegrant.

17. A formulation as claimed in claim 16, wherein disintegrant is selected from a group comprising hydroxypropyl Cellulose and low-substituted Hydroxypropyl Cellulose (L-HPC, grade: LH-21), and mixture thereof.

18. A formulation as claimed in claim 16 or 17, wherein disintegrant is Low-substituted Hydroxypropyl Cellulose (L-HPC, grade: LH-21) having a hydroxypropoxyl content of about 10.0 - 12.9%.

19. A formulation as claimed in any one of the preceding claims, wherein formulation further comprises lubricant.

20. A formulation as claimed in claim 19, wherein lubricant is selected from a group comprising colloidal silicon dioxide (Aerosil 200) and magnesium stearate, and mixture thereof.

21. A formulation as claimed in any one of the preceding claims, wherein Sirolimus and complexing agent are taken in a weight ratio varying from about 1 : 0.5 to about 4 : 2 w/w.

22. A formulation as claimed in any one of the preceding claims, wherein Sirolimus and wetting agent are taken in a weight ratio varying from about 0.5 : 2.25 to about 3 : 13.5 w/w.

23. A formulation as claimed in any one of the preceding claims, wherein Sirolimus, complexing agent and wetting agent are respectively taken in a weight ratio varying from about 0.5 : 0.25 : 2.25 to about 4 : 2 : 18 w/w.
24. A formulation as claimed in any one of the preceding claims, wherein complexing agent, wetting agent and solvent for complexing agent are respectively taken in a weight ratio varying from about 0.5 : 4.5 : 10 to about 4 : 36 : 40 w/w.

25. A formulation as claimed in any one of the preceding claims, wherein Sirolimus, wetting agent, complexing agent and solvent are mixed, respectively, in a weight ratio of about 1 : 4.5 : 0.5 : 10 w/w.

26. A formulation as claimed in any one of the preceding claims, wherein when combination of binder is hydroxypropyl cellulose (Klucel LF Pharm) having a viscosity of 75 to 150 cps at 25°C in a 5% aqueous solution and disintegrant is low-substituted hydroxypropyl cellulose (L-HPC grade-LH-21) having a hydroxypropoxy content of about 10.0 - 12.9%, the binder and disintegrant are taken in the weight ratio of about 0.33 : 0.47 to about 4:5:64 w/w.

27. A formulation as claimed in any one of the preceding claims, wherein it comprises complex of Sirolimus as immunosuppressant drug in acetone, hydroxypropyl β - cyclodextrin as complexing agent in propylene glycol used as solvent as well as wetting agent.

28. A formulation as claimed in any one of the preceding claims, wherein it comprises complex of Sirolimus as immunosuppressant drug in acetone, hydroxypropyl β - cyclodextrin as complexing agent in propylene glycol, Cremophor RH 40 as wetting agent.

29. A method for preparation of drug delivery solid dosage formulation of sirolimus as claimed in any one of the preceding claims 1 to 28, wherein the formulation is prepared by comprising steps of:-

a) preparing solutions of sirolimus in said solvent and of complexing agent in said another solvent;

b) mixing solutions from step-a) and adding thereto a wetting agent resulting in formation of a complex of sirolimus, complexing agent and wetting agent; and

c) adsorbing the complex from step-b) in dissolved state on said filler and allowing to dry to a powder form which is compressed to a tablet form; wherein, complexing agent is hydroxypropyl β - cyclodextrin; and wetting agent is selected from a group comprising polysorbate 80, sodium lauryl sulphate
[SLS], propylene glycol, polyoxyl 40 hydrogenated castor oil (cremophor RH 40) and mixture thereof.

30. A method as claimed in claim 29, wherein dried powder obtained in step - c) is lubricated to compress to tablets for film coating with a compound selected from a group comprising colloidal silicon dioxide (Aerosil 200) and magnesium stearate, and mixture thereof.

31. A method as claimed in any one of the claims 29 or 30, wherein tablet form from step - c) is further processed for film coating.

32. A method as claimed in claim 31, wherein said coating is achieved with opadry containing hydroxypropyle methyl cellulose having a viscosity of about 5 to 6 mps of 2 % w/v aqueous solution.

33. A drug delivery solid dosage formulation of Sirolimus substantially as herein described with reference to foregoing examples.

34. A method for preparation of drug delivery solid dosage formulation of Sirolimus substantially as herein described with reference to foregoing examples.