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Enhancement of solubility and dissolution rate of griseofulvin by microparticulate systems.

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Abstract
Griseofulvin is an antifungal antibiotic drug with poor aqueous solubility. Aim of the present study was to foster the solubility and dissolution rate of griseofulvin through preparation of its microcrystals and granules. Microcrystals were prepared by emulsion solvent diffusion method with various surfactants (HPMC E5 and E15; PVP K 30 and PVP K 90; β Cyclodextrin) and granulates were prepared by melt granulation technique using polymer like PEG 4000 and surfactant like poloxamer 188. Formulations were screened for apparent solubility, drug content, dissolution rate, morphology and crystallinity (XRD and DSC). Microcrystals prepared with HPMC E15 and granulates prepared with poloxamer 188 have been showed highest solubility and dissolution rate than the untreated drug. In conclusion, the results of this work suggest that these are the best technique to prepare microparticulates for enhancement of solubility and dissolution rate of griseofulvin.

Key Words
Griseofulvin, microcrystals, melt granulation, poloxamer

Introduction
The oral route remains the preferred route of drug administration due to its convenience, good patient compliance and low medicine production costs. In order for a drug to be absorbed into the systemic circulation following oral administration, the drug must be dissolved in the gastric fluids. But water insolubility has always been a key issue in pharmaceutical formulations of class II drugs, affecting formulation stability and drug bioavailability[1]. Solubility is an essential factor for drug effectiveness which is independent of administration route. It also poses a major challenge for pharmaceutical companies developing new pharmaceutical products, since nearly half of the active substances being identified through the new paradigm in high-throughput screening are either insoluble or poorly soluble in water[2,3]. The number of drugs coming from synthesis and being poorly soluble is steadily increasing. Oral bioavailability of poorly water-soluble drugs depends on their dissolution rate in the absorption site.[4] In the recent years, the interest in micron and submicron systems in pharmacy has surged. Micron system comes in range of μm and submicron in nm[5].

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There has been a considerable research interest in the area of drug delivery using particulate systems. Particulate systems used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. Based on their morphology, microparticulates are classified into microspheres, microcapsules, microemulsions, microcrystals, microsponges etc[6]. There are several methods to prepare microparticulates. Emulsion solvent diffusion method is advantageous in terms of smaller size, lower size distribution and higher encapsulation yield because of the drug rapid partitioning to the external aqueous phase[7]. The melt granulation process is simple, rapid and may be performed in one step, in contrast to conventional wet granulation. It is a good alternative to wet granulation for water-sensitive materials. As there is no solvent used in melt granulation, there are no solvent recovery issues and hence safety and environmental considerations are also reduced[8]. Griseofulvin is an antifungal agent with poor solubility and low bioavailability. It is used widely for the treatment of the dermatophytosis. It is active against Epidermophyton, Trichophyton and Microsporum species. Though griseofulvin requires long term therapy it is drug of choice for several fungal disorders. It has distinct place in therapeutic

treatment of various fungal disorders.[9] Antifungal drugs like amphotericin B, ketoconazole, griseofulvin etc. have limitations in clinical administration due to their poor solubility and other unfavorable properties. However, this molecule still interests researchers. Increasing the oral bioavailability of griseofulvin is a major goal by enhancement of its solubility and dissolution. To enhance griseofulvin bioavailability, several trials are made in order to improve both its solubility and its dissolution rate. Griseofulvin has only 30-70 % bioavailability due to its water insolubility. Its absorption is increased by taking it with fats or microfining the particles[10]. In the present investigation attempts have been made to formulate microparticulate systems of griseofulvin. The effects of different surfactants have been checked to enhance the solubility and dissolution rate of griseofulvin. Checking of the stability of the drug in the formulations is also important issue. Particulate drug delivery systems can lead to increase in dissolution profiles of Class II and Class IV drugs by increasing effective surface area several times[11]. They can also be lead to avoidance of cumulative toxicity and saving of drug due to reduced dose.

Materials and Methods

Materials

Griseofulvin was obtained as a gift sample from Cipla Ltd. (Kurkumbh, India). PVP K-30, PVP K-90, HPMC E5, HPMC E15 were supplied by Lupin Ltd. (Pune India). β-cyclodextrin, PEG 4000 and Poloxamer 188 were obtained from Cipla Ltd. (Kurkumbh, India).

Preparation of microcrystals

Microcrystals of griseofulvin were prepared by emulsion solvent diffusion method[12]. Briefly; a weighed quantity 1 g of GF was dissolved in 25 ml acetone and 25 ml ethanol. This phase was added at room temperature, under constant mechanical stirring (2000 rpm) to 100 ml aqueous solution of surfactants (Table 1). Concentration of the surfactant was optimized to reduce sticking of the drug to the stirrer blades. The lowest concentration at which problem of sticking reduced to remarkable extent were determined and used in formulation. Stirring was continued for 30 min. Crystals were collected by filtration and dried at room temperature.

Preparation of Microparticles

Granules were prepared in a porcelain dish. Firstly, the mixture of griseofulvin and polyethylene glycol 4000/ poloxamer 188 (Table 2) was dry blended for 10 min. Then, this mixture was placed in hot porcelain dish and supplied the heat around 60^0 C on temperature controlled water bath so as to melt the polymers or binders in which the drug was dispersed. The formed molten mass is then cooled to room temperature and at the end of granulation process the granules were allowed to solidify at room temperature by spreading them out in thin layers on trays. The melted granules were passed through sieve no # 60 so as to form uniform microparticles (granules). The cooled granules were stored in sealed bags for their evaluation.

Fourier Transform Infra Red Spectroscopy Characterization

FT-IR spectra of prepared batches were recorded on Shimadzu FTIR – 8400 spectrophotometer. Each spectrum was derived from single average scans collected in the region 400 – 4000 cm^{-1} at spectral resolution of 2 cm^{-1} and ratio against background interferogram. Spectra were analyzed by software supplied by Shimadzu.

Determination of Drug Content

A weighed quantity of the microcrystals\ granules were dispersed in 10 ml of distilled water. It was sonicated for 10 min and centrifuged at 2000 rpm for 10 min. The supernatant was diluted with suitable quantity of solvent and analyzed by UV-Visible Spectrophotometer.

Apparent Solubility Studies

To 10 ml of the distilled water excess quantity of sample (50 mg) was added. Apparent solubility study was performed by standardized shake flask method at 37 ^{0}C. After shaking for 48 hrs, the samples were filtered through 0.2 µm membrane filters. And the filtrate was analyzed for drug content. Saturation solubility measurements were assayed through ultraviolet absorbance determination at 296.2 nm using UV-Visible Spectrophotometer.

In-Vitro Dissolution Studies

In vitro dissolution studies were carried out using USP type II (Paddle) dissolution apparatus (LABINDIA 2000, Mumbai) at rotation speed 100 rpm was used for the study. Dissolution of the untreated drug and formulations was carried out on an equivalent of 250 mg of the GF with distilled water containing 0.54 % SLS as dissolution media. The volume and temperature of the dissolution media were 900 ml and 37 ± 0.2^0c respectively.

After fixed time intervals 5 ml of samples were withdrawn (sink condition was maintained) and assayed by using UV-Visible Spectrophotometer (Shimadzu-1700, Tokyo, Japan) by analytically validated method (r^2=0.9993). The cumulative release of griseofulvin from particulate systems was calculated.

Particle Morphology Analysis

Morphological evaluation of the optimized formulation (G7) was carried out by JSM-6400 scanning electron microscope (JEOL, Tokyo, Japan). Sample was fixed on aluminum stubs with conductive double sided adhesive tape and coated with the gold by sputter coater at 50 mA for 50 s. Particle morphology was also tested by Polariser Light Microscope (Lawrence and Mayo) of different formulations.

Powder X-Ray Diffraction (PXRD) Analysis

Crystallinity of the drug and prepared samples was determined using Philips Analytical X-RD (Model: PW 3710, Holland) with copper target. The conditions were: 40 kV voltages; 30 mA current; at room temperature. The samples were loaded on to the diffractometer and scanned over a range of 2θ values form 10 to 60^0at a scan rate of 0.05^0 C/0.4 sec.

Differential Scanning Calorimetry (DSC) Analysis

DSC is a technique for measuring the energy necessary to establish a nearly zero temperature difference between a substance and inert reference material, as two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate. Phase transition of the untreated drug and the crystals were analyzed by DSC (Universal V2.4F TA Instruments, USA, Model: SDT 2960). The samples were heated in a hermetically sealed aluminum pans. Temperature range for each sample was set from 30 to 350^0 C at a heating rate of 10^0 C /min, using nitrogen as purging gas.

Stability Studies

Optimized batches (G5, G7) were subjected for accelerated stability study as per ICH guidelines. The samples (each 10 mg, n=3) were kept for stability studies at 40 ± 2^0 C and 75 ± 5 % RH in an environmental test chamber (HMG INDIA, Mumbai, India) for a period of 3 months. Glass vials without rubber plugs were used to carry out the study. After 30, 60 and 90 days, the samples were taken out and analyzed for the drug content.

Results and Discussion

Fourier Transform Infrared Spectroscopy

State of drug molecules with excipients was determined using FT-IR. Fig. 1 depicts IR spectra of Batch G, G5 and G7 respectively. There is no shift of peaks after interaction with excipients of the drug substances; indicating no change in chemical structure of drug. GF showed sharp peaks at 3030, 3000, 2931, 1705, 1658, 1616, 1597, 1580, 1501, 1222, 1213, 800 cm^{-1}. Hence The FT-IR spectra of the prepared batches showed that no changes occurred in the chemical nature and did not present a great fingerprint difference.

Drug Content

For untreated drug the drug content was considered to be 100 %; however drug content of formulations (Batch G1 to Batch G5) was found to be in the range of 91.76 to 96.38 %. The drug content of Batch G6 and G7 was 96.11 %, 95.26 % respectively. Drug content of all batches are given in Table 3. The batches of microcrystals showed high drug content with little incorporation of surfactant on their surfaces.

Apparent Solubility

Results of solubility studies are presented in Table 4. GF/ HPMC E15 microcrystals (Batch G5) and granules of GF/Poloxamer (Batch G7) showed greater solubility (0.01259 mg/ml and 0.01280 mg/ml respectively) as compared to the untreated drug (0.00487 mg/ml) and microcrystals prepared with other surfactants/polymers. The increased saturation solubility is an inverse function of particle size. Based on Noyes- Whitney equation an increase in saturation solubility leads to an increase in dissolution velocity[13].

The solubility of drug increased due to:

- Hydrophilic nature of polymers.
- Slight reduction in crystallinity.
- Reduction in particle size.
- Increased wettability due to adsorption on the surface.

In Vitro Drug Release Study

Tables 5 depict the results of dissolution studies. The release pattern from various formulations showed some variations (Fig. 2). As compared with pure drug, formulations show increase in dissolution rate. Amongst the all formulations of griseofulvin Batch G1 (GF\β

Cyclodextrin microcrystals), Batch G2 (GF\PVP K30 microcrystals) and Batch G3 (GF\PVP K90 microcrystals) showed about 84.3 % to 87.3 % cumulative % drug release as compared to the 75.3 % drug release of untreated griseofulvin within 90 minutes of testing period. That increased drug release profile showed that β Cyclodextrin, PVP K30 and PVP K90 were present on newly formed surfaces of drug particles and responsible for the considerable increase in dissolution. Cyclodextrin is mostly used for inclusion complex whereas PVP is well known stabilizer in the solubility and dissolution rate enhancement experiments. Batch G4 (GF\HPMC E5 microcrystals) and Batch G5 (GF\HPMC E15 microcrystals) showed 93.7 % and 96.3 % drug release in 90 minutes testing period. The increased dissolution is an inverse function of particle size. This could be due to high mass transfer caused by increase in surface area of drug particles. HPMC E5 and HPMC E15 are low viscosity grade polymers having hydrophilic nature; hence lead to enhanced dissolution profile. HPMC is also good stabilizer used in different experiments. Batch G6 (GF\PEG 4000 granulates) and Batch G7 (GF\Poloxamer 188 granulates), those granulates showed 94 % and 98.1 % drug release in 90 minutes respectively. Thus melt granulation technique showed best results of dissolution rate. So in the optimization of biopharmaceutical characteristics of drug, polymers play an important role.

Particle Morphology

Microphotographs of different formulations (Fig. 3) showed needle/ rod shaped microcrystals, rough shaped particles of Griseofulvin. SEM microphotograph of batch G7 (Granules of GF/Poloxamer 188) showed about 200 µ particles with rough surfaces indicating presence of polymer/surfactant on their surfaces.

XRD Study

Investigation of the X-ray diffractograms (Fig. 4) revealed a number of changes in the location of the peaks (appearance and disappearance) of the drug substance GF and optimized batches G5, G7 respectively. A few diffuse peaks or decreases in crystallinity were observed in formulations which may indicate a slight physical interaction of the drug substance with the used excipients. X-ray diffractogram of GF showed intense peaks at 21.750 (86), 24.595 (128), 35.795 (79) and 40.095 (114) 2θ angles respectively indicating the crystalline nature of drug GF. Batch G5 showed less intense peaks at 24.500 (98), 35.680 (26) and 39.900 (42) 2θ angles respectively indicating slight reduction in crystalline nature of drug. Batch G7 showed further decrease in intensity of peaks at 24.690 (77), 39.660 (36) and 35.780 (31) 2θ angles respectively indicating reduction in crystallinity of griseofulvin. Those differences were attributed to the presence of HPMC and Poloxamer; the change in the nature of particles was due to dilution of the particles with surfactants rather than particle size. Polymorphic form of drug remains unchanged.

DSC Analysis

DSC is a technique for measuring the energy necessary to establish a nearly zero temperature difference between a substance and inert reference material, as two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate. Fig. 5 reports the DSC scans of treated drug, Batch G5 and Batch G7 respectively. Thermal analysis completely reconfirmed the previously reported XRD findings. Untreated GF showed a melting endothermic peak onset at 222.80^0 C comparative to GF/ HPMC E15 microcrystals (222.32^0 C) and granules of GF/Poloxamer (221.78^0 C). The DSC study revealed that slightly decrease in melting endothermic peak compared to GF. These modifications are clearly attributed to the presence of HPMC E15 and Poloxamer 188. The decreased peak area of granules of GF\Poloxamer 188 indicated the incorporation of griseofulvin in poloxamer. The presence of HPMC E15 did not influence the thermogram. The changes in peaks were due to changes in internal energy of drug, presence of polymer and reduction in particle size.

Stability Studies

GF/ HPMC E15 microcrystals (Batch G5) and granules of GF/Poloxamer (Batch G7) were screened for accelerated stability studies and the drug content was observed to be 92.77 %, 94.14 % after 30 days, 91.33 %, 93.70 % after 60 days and 90.07 %, 91.30 % after 90 days respectively. Thus these batches were quite stable at accelerated storage conditions for three months. That improved stability of the drug ascribed to the presence of surfactants/polymer.

Conclusions

Griseofulvin is an important agent in the treatment of dermatophytosis. However the optimal clinical use of griseofulvin is limited due to its poor aqueous solubility. Results of present work lead to a conclusion that reduced size of the drug particles with presence of the surfactants and polymers on the surface of griseofulvin microparticles are responsible for meteoric rise in solubility and dissolution velocity. Surfactants play an important role in the solubility of the drugs by increasing the wettability due to adsorption on the surfaces. No interaction was happened with drugs and excipients. In this study prepared griseofulvin granulates with Poloxamer 188 exhibited excellent physicochemical properties, solubility, dissolution rate and stability when compared with pure drug. If this process can be scaled-up to manufacturing level, this technology has the potential to provide long-term stability, increased dissolution and solubility to Class II drugs.

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References

1. Kipp J., The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs, Int. J. Pharm., 2004, 284, 109–122.
2. Patravale VB, Date AA, Kulkarni RM et al, Nanosuspensions: a promising drug delivery strategy. J. Pharm. Pharmacol., 2004, 56, 827–840.
3. Burger's Medicinal Chemistry and Drug Discovery, 6th edi., Drug Development by Donald J. Abraham, Vol.2, 649-682.
4. Sung-Hyun P., Hoo-Kyun C., The effects of surfactants on the dissolution profiles of poorly water-soluble acidic drugs, Int. J. Pharm., 2006, 321, 35-41.
5. Vyas SP., Khar RK., Targeted and controlled drug delivery, 1st edi., CBS Publishers and distributors, New Delhi, 2002, 331-385.
6. Jia-You F., Chia-Lang F., Chi-Hsien L., Yu-Han S., Solid Lipid Nanoparticles versus Nanostructured Lipid Carriers, Euro. J. Pharm. Biopharm. 2008, 70, 633-640.
7. Brown DM. Drug Delivery Systems in Cancer Therapy. Totowa, NJ. Humana Press, 2004, 117-126.
8. Ginity JW, Koleng JJ, Repka MA, Hot melt extrusion technology, Encyclopedia of pharmaceutical technology, Swarbrick J., Boylon JC., Editor. Marcel Dekkar, Inc.: Newyork, 2000, 203-226.
9. KD Tripathi, Essentials of Medical Pharmacology, 6th edi., JAYPEE Publishers (P) Ltd.,760-761.
10. Zohra Z., Souad S., Hatem F., Preparation and characterization of poly-caprolactone nanoparticles containing griseofulvin, Int. J. of Pharm., 2005, 294, 261-267.
11. Kapsi, SG, Ayres JW, Processing factors in development of solid solution formulation of itraconazole for enhancement of drug dissolution and bioavailability, Int. J. Pharm. 2001, 229, 193–203.
12. Nighute AB, Bhise SB, Preparation and evaluation of microcrystals of cefuroxime axetil, Int.J. of PharmTech Research, 2009, 1, 424-430.
13. Muller RH, Keck CM, Drug nanocrystals of poorely soluble drugs produced by high pressure homogenization, Eur. J. Pharm. Biopharm. 2006, 62, 3-16.

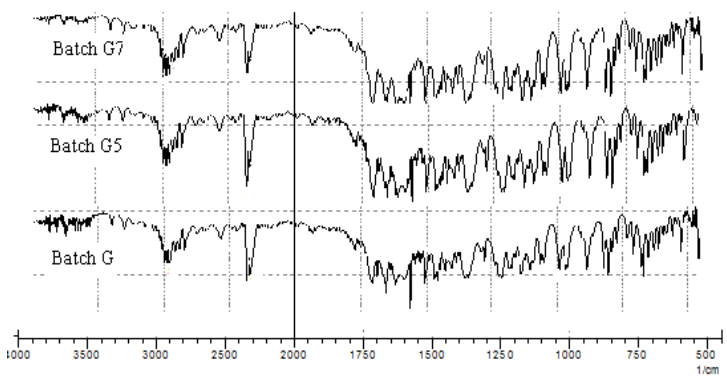

Fig. 1: IR spectra of Batch G, G5 and G7.

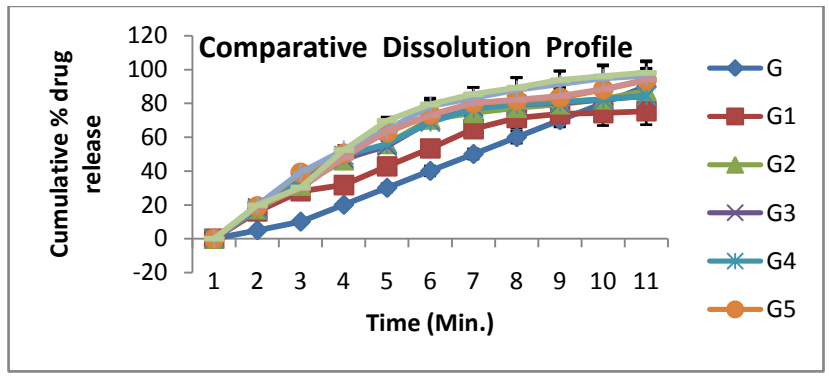

Fig. 2: Comparative Dissolution Profile.

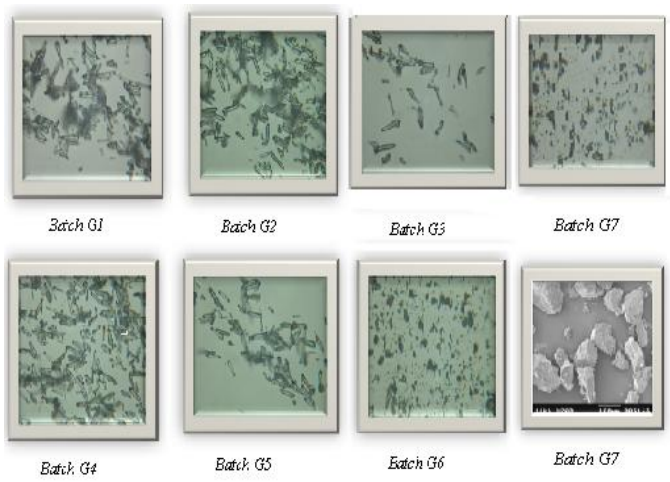

Fig. 3: Microphotographs of Different Batches of Griseofulvin.

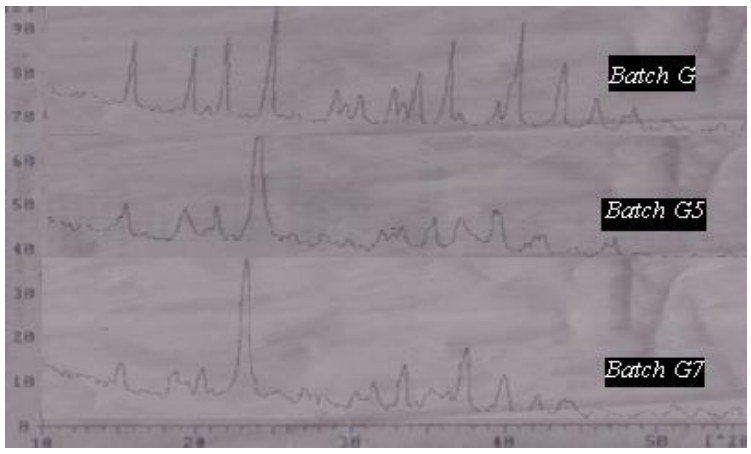

Fig. 4: X-ray diffraction pattern of Batch G, G5 and G7.

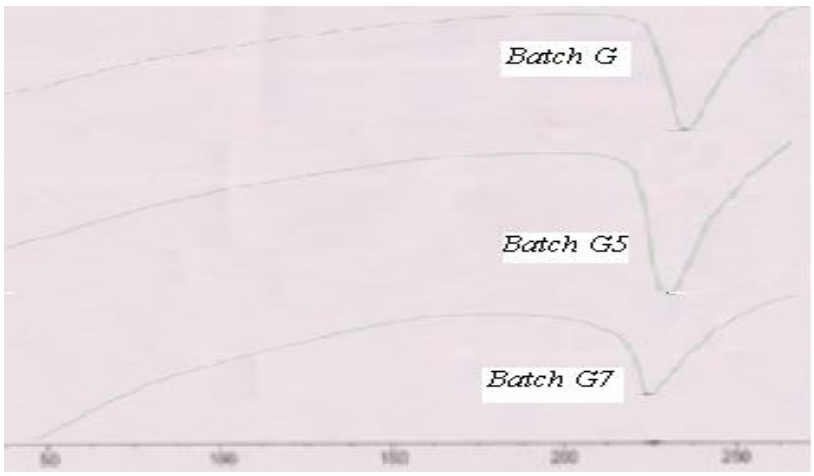

Fig. 5: DSC thermogram of Batch G, G5 and G7.

Table 1: Surfactants used with its Concentrations.

Sr.No.	Batch	Batch ID	Conc. of Surfactants (% w/v)
1	GF	G	-
2	GF/β-CD	G1	0.20
3	GF/PVP-K30	G2	0.20
4	GF/PVP-K90	G3	0.20
5	GF/HPMC E5	G4	0.10
6	GF/HPMC E15	G5	0.10

Table 2: Drug: Excipient Ratio of different Batches.

Sr. No.	Batch	Batch ID	Drug: Excipient Ratio
1	GF/PEG 4000	G6	1:1
2	GF/Poloxamer 188	G7	1:1

Table 3: % Drug Content.

Batch	% Drug Content
G	100
G1	96.38
G2	93.74
G3	91.76
G4	96.31
G5	95.84
G6	96.11
G7	95.26

Table 4: Apparent Solubility.

Batch	Conc. (µg/ml)	Conc. (mg/ml)
G	4.8750	0.00487
G1	6.1316	0.00613
G2	8.1163	0.00811
G3	8.2800	0.00880
G4	11.1011	0.01110
G5	12.5976	0.01259
G6	11.8750	0.01187
G7	12.8036	0.01280

Table 5: Comparative Dissolution Profile.

Time (min.)	Cumulative % Drug Release							
	G	G1	G2	G3	G4	G5	G6	G7
0	0	0	0	0	0	0	0	0
5	16.0	17.4	18.3	17.4	19.1	19.9	19.3	19.8
10	28.0	31.3	33.7	33.9	38.9	39.2	29.7	30.3
20	31.7	46.3	47.4	49.1	50.2	52.5	47.8	52.4
30	42.7	55.9	54.7	55.9	62.8	64.4	63.7	69.8
40	53.4	70.4	69.3	68.8	73.3	78.4	72.8	79.3
50	64.8	74.3	77.8	76.7	80.2	83.3	80.1	85.4
60	71.3	77.3	79.4	79.3	81.7	88.0	82.0	89.3
70	73.7	79.4	80.1	80.7	83.3	91.3	84.3	93.7
80	74.3	82.3	82.3	82.4	88.3	94.7	88.3	96.0
90	75.3	87.3	84.3	84.3	93.7	96.3	94.0	98.1

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