American Journal of Advanced Drug Delivery
http://www.imedpub.com/advanced-drug-delivery/

Original Article

Formulation and Evaluation of Acyclovir Sodium Solid Lipid Microparticles

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Date of Receipt- 13/12/2016
Date of Revision- 15/12/2016
Date of Acceptance- 27/12/2016

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ABSTRACT

Acyclovir sodium is an antiviral drug used to treat herpes, chicken pox and herpes skin infections. Acyclovir sodium potential as an antiviral drug is limited by its low oral bioavailability (20-30%) with short half-life (2-3 hours) with poor plasma protein binding. There is an opportunity to utilize Acyclovir sodium as an antiviral drug by enhancing the bioavailability by formulation technology. In this paper solid microparticle (o/w) of Acyclovir was prepared by melt dispersion technique. The characterization of drug using scanning electron microscopy, FT-IR, particle size, percentage yield, drug loading capacity, hausner's ratio and carr's index, bulk density and tapped density. *In vitro* drug release studies using phosphate buffer has shown as formulation F5 sustained release for 17 hrs.

Keywords: Acyclovir sodium, Sustain release, Solid lipid micro particles.

INTRODUCTION

Acyclovir is an antiviral drug which slows the spread and growth of herpes virus. It is also used to treat genital herpes, cold sores, shingles and chicken pox. Acyclovir has a low bioavailability of 20-30% and excreted in unchanged in urine both by filtration and secretion. Hence it is prescribed 3-4 times a day which has negative effect on patient compliance[1]. Acyclovir may also cause side effects like bleeding, purple or red pinpoint spots under skin, no urinating , painful or difficult urination, swelling in feet or ankles, feeling tired or short of breath. In order to enhance the efficacy and to improve safety formulation of solid lipid micro particles is carried out. Solid lipid micro particles are having size between in the range of 1-1000 µm with drug being dissolved, entrapped and encapsulated in micro particle matrix[2].

The drug entrapment got upto 80% which are easily compatible with living systems since SLMs system based of biomaterials. In SLMs drug is protected from degradation as it is sealed in the biomaterial matrix. The leaching of the drug is passive mechanism and is independent of concentration of drug. As the micro particle continues get dissolved leading to slow sustained release of the drug in the stomach. The suitability of solid lipid micro particles for sustained release has been established by several authors[3-5]. SLMs can be prepared by Spray-drying, Spray congealing, O/W melt dispersion technique, Double-emulsion solvent evaporation, solvent evaporation, High pressure homogenization, w/o melt dispersion technique[6]. The improvement in patient can be dosing schedule can be reduced one or two times. In this paper SLMs of Acyclovir sodium of different concentrations of bio lipids are prepared using stearic acid by o/w melt dispersion techniques. The various formulations are evaluated for suitability of formulation of sustained released preparation[7].

MATERIALS AND METHODS

Material

All the reagents and chemical used are of analytical grade doubly distilled water was used in making preparation in this study. Acyclovir sodium (Helix Pharma Pondicherry, India), Stearic acid (Lipidchem Johor, Malaysia), Tween 80 (Thomas baker Mumbai, India), Dimethyl sulfoxide (SD fine chemicals, Mumbai, India).

Preparation of Solid Lipid Micro Particles containing Acyclovir Sodium

Two different methods were used for preparation of solid lipid micro particles with Dimethyl sulfoxide.

In method 1 SLMs were prepared in o/w melt preparation technique. Stearic acid was melted on a water bath with a temperature limit of 72˚C. The drug particles grounded to the fine size was dispersed with molten stearic acid aqueous phase consisting of water and tween 80 at 72˚C was the molten phase was slowly added to the aqueous phase by steering and the emulsification was assisted by homogenizer for 15 minutes[8]. The dispersion rapidly cools down at 20˚C by immersing the solution in ice bath. The formed micro particles were separated by the filtration by this formulation F1, F2, F3 and F4 were prepared. In another method formulation F7 and F8 were prepared by same method. However the Dimethyl sulfoxide is used instead of water and tween 80 to dissolve finally grounded acyclovir sodium (Table 1).

Characterization of SLMs of Acyclovir Sodium: The characterization of Acyclovir was carried out using FT-IR spectrum and Scanning Electron Microscopy[10].

FT-IR spectrum: The KBr disc technique was employed for preparation of sample. Pellet of 1 mg Acyclovir sodium and 100 mg dried spectroscopic grade KBr was prepared in a die with the application of pressure and pellet was analyzed using FT-IR Spectrophotometer.

Scanning electron microscopy: Scanning electron microscopy (SEM) was used to verify uniformity of particle shape and size. The samples were previously fixed on a brass stub using double-sided adhesive tape and were then made electrically conductive by coating with a thin layer of gold and palladium alloy (180-200Å) using a fine coat ion sputter (SUK PHY) (JEOL, fine coat ion sputter JFC-1100). The surface morphology of the sample was observed under a scanning electron microscope JSM-6400 (Joel, Japan) operated at 10kev pulse at different resolutions[11].

Evaluation of SLMs

Particle size: The particle sizes of the microparticles were determined by using optical microscopy method. Approximately 100 microparticles were counted for particle size analysis by using calibrated optical microscope[12,13].

$$\text{Least count}=\frac{\text{Stage Micrometer}}{\text{Optical Micrometer}}, \quad \text{Particle size}=\frac{\sum nd}{\sum n}$$

Here, $\sum n$ = no. of particles

$\sum nd$ = average diameter

Percentage (%) yield and drug loading capacity: The prepared microparticles were collected and weighed. The measured weight was divided by the total weight of all the excipients and drug. To calculate the drug loading capacity, weighed 10 mg drug was added to 10 ml of phosphate buffer (pH 6.8) to facilitate the microparticles to get dissolved and heated at 70˚C,then the resultant solution centrifuged at 8000 rpm for 10 min. Samples were measured at an absorbance of 252 nm in double beam U.V Spectrophotometer. Percentage yield and drug loading capacity of microparticles were determined by the following equations:

$$\text{Percentage yeild}=\frac{\text{Total formulation weight}}{\text{Total weight of excipient+drug}}\times 100$$

$$\text{Loading capacity}=\frac{\text{Amount of drug encapsulated}}{\text{Weight of lipid}}\times 100$$

Angle of repose: Angle of repose of different formulations was measured according to fixed funnel standing method.

Here, θ=angle of repose, r=radius, h=height

$$\theta = \tan^{-1}\ h/r$$

Bulk density and tapped density: Bulk and tapped densities were measured by using 10 ml of graduated cylinder. The sample was poured in cylinder, tapped mechanically for 100 times, then tapped volume was noted down to calculate bulk density and tapped density. Each experiment for micromeritic properties was performed in triplicate manner, by using following equation.

$$\text{Bulk density}=\frac{\text{Mass}}{\text{Untapped Volume}}$$

$$\text{Tapped density}=\frac{\text{Mass}}{\text{Tapped density}}$$

Hausner's ratio and Carr's index: Hausner's ratio of microparticles was determined by comparing the tapped density to the bulk density using the equation. Carr's index value of microparticles was computed according to the following equation:

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

$$\text{Carr's index}(\%) = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100$$

In Vitro Dissolution Studies

In vitro drug release study was carried out using USP Dissolution apparatus (Type II). The formulations equivalent to weight containing 200 mg acyclovir sodium were immersed in dissolution medium. Phosphate buffer (pH 6.8) (900 ml) was used as the dissolution medium and maintained at 37 ± 0.5°C at a rotation speed of 50 rpm. Samples were withdrawn and the same amount was replaced using the same dissolution medium. The amount of the drug was quantified using double beam U.V spectrophotometer at 252 nm[14].

Drug Release Kinetic Studies

Raw data obtained from *in vitro* release studies was analyzed,and it was fitted to different equations and kinetics model to calculate the percent drug release and release kinetics of acyclovir sodium from microparticles. The kinetic models used were zero-order equation, first-order, higuchi's model and korsmeyer-peppas equation[15].

RESULTS

Characterization and Evaluation of SLMs

FT-IR spectrum: Peaks were evident at 3572.41 cm^{-1} (O-H stretching), 3342.62 (O-H stretching), 2915.43 (aliphatic C-H stretching), 2873.38 (aliphatic C-H stretching symmetric), 1632.41 (0-H deformation), 1459.09 (aliphatic C-H deformation), 1278.57 (C-O stretching) for Acyclovir sodium showed in Figure 1. So presence of these characteristic peaks of Acyclovir sodium in physical mixture reveals that the drug remains intact in formulation F5 and there is no interaction between drug and stearic acid used Figure 2.

Scanning electron microscopy: Scanning electron microscopy was performed for lipid microparticles of formulation F5 to obtain more information on the particle size and morphology. The photos of lipid microparticles shows that the formulated acyclovir sodium microparticles were of spherical shape, which were shown in Figure 3. Moreover the microparticles were observed with smooth surface, which may contribute to its release of the drug in a sustained manner compared to the microparticles having rough surface.

Evaluation of SLMs

Particle size, percentage yield and drug loading capacity: The reason behind selecting solid lipid microparticles was their ability to have better particle size (7 µm), percentage yield and to better drug entrapment. Drug loading capacity is considered as an important parameter as improper entrapment leads to the initial burst release of the drug, which hinders its sustained release property. Acyclovir sodium solid lipid microparticles of formulation F5 has shown 100% drug loading capacity as depicted in Table 2.

Bulk density and tapped density: The bulk density and tapped density of the formulations were in range of 0.18 to 0.31 and 0.25 to 0.39 respectively. Formulation F5 was having Bulk density=0.31 and Tapped density=0.39 as depicted in Table 3.

Hausner's ratio and Carr's index: Hausner' ratio were found to be in the range 1.07 to 1.38. Carr's Index was in the range of 6.89 to 28. Formulation F5 was having Hausner's ration 1.25 and Carr's index 20.5 as depicted in Table 3.

Angle of repose: Angle of repose values of formulations F1 to F8 were found in range 30 to 37. The formulation F5 was having angle of repose 30 as depicted in Table 3.

In vitro Drug Release

The percentage drug release from acyclovir sodium with formulation F5 was observed by using USP Dissolution tester (Type II). These were compared with the pure acyclovir sodium drug as shown in Figure 4. The *in vitro* drug release showed that the solid lipid microparticles containing acyclovir sodium have sustained the drug release. Formulation F5 showed better sustained release of drug. *In vitro* release of formulation F5 was 75% after 17hrs shown in Table 4.

DISCUSSION

Acyclovir sodium formulations with DMSO and stearic acid and without DMSO has produced solid lipid micro particles with desirable flow properties and formulation F5 was found to be

most suitable. The FT-IR studies indicated no chemical interactions between Acyclovir and stearic acid. The Tapped density (0.31 and 0.39), Carr's and Hausner's ratio (20.5 and 1.25), Angle of repose (30) is suitable for punching and capsulation of the Acyclovir sodium solid lipid micro particles. *In vitro* dissolution studies has established the SLMs release the Acyclovir sodium in a sustained manner of zero Oder release which was statically with high or values. Slow sustained release of micro particles is going to enhance the bioavailability of drug and suitable for the design of sustained released formulation. The sustained release shall be able to do away with dosing schedules with acyclovir sodium. Sustained release preparation would belief improve the patient compliance can be improve the outcomes to herpes infection treatment.

ACKNOWLEDGEMENT

The authors are thankful to Dr Sarvesh Malvia Jain, Director, Oniosome Healthcare Pvt. Ltd, Mohali, Punjab for providing the assistance in carrying out the research work.

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Table 1: Formulation details[9]

Formulation	Drug (gm)	Lipid stearic acid (gm)	Tween80 (ml)	Dimethyl Sulfoxide (ml)	Water q.s (ml)
F1	0.2	1	0.1	-	100
F2	0.2	2	0.1	-	100
F3	0.2	3	0.1	-	100
F4	0.2	4	0.1	-	100
F5	0.2	3	0.1	2	100
F6	0.2	3	0.1	3	100
F7	0.2	3	0.1	4	100
F8	0.2	1	0.1	3	100

Table 2: Particle size, percentage yield and drug loading capacity

Formulation code	Particle size (µm)	Percentage yield	Drug loading capacity
F1	4.96	39.7	9.6
F2	4.92	54.6	14.4
F3	4.61	76.9	57.3
F4	4.18	83.8	11.4
F5	7.67	97.8	100
F6	6.20	87.5	94.6
F7	6.72	78.1	50
F8	4.36	26.7	1.2

Table 3: Bulk density, tapped density, hausner's ratio, carr's index and angle of repose

Formulation code	Bulk density	Tapped density	Hausner's ratio	Carr's index	Angle of repose
F1	0.29	0.39	1.34	25.6	33
F2	0.26	0.33	1.26	21.2	37
F3	0.28	0.36	1.28	22.2	36
F4	0.29	0.36	1.24	19.4	32
F5	0.31	0.39	1.25	20.5	30
F6	0.28	0.35	1.25	20	32
F7	0.18	0.25	1.38	28	32
F8	0.27	0.29	1.07	6.89	33

Table 4: Drug release of acyclovir sodium solid lipid microparticles for F5 formulation

Time (min)	Log time	S.R time	% cumulative release	Log % cumulative release	% cumulative remaining	Log % cumulative remaining
0	0	0	0	0	100	2
30	1.477121255	5.477225575	5.497826	0.740190991	94.502174	1.975441799
60	1.77815125	7.745966692	6.032196	0.780475444	93.967804	1.972979078
120	2.079181246	10.95445115	7.525848	0.876555443	92.474152	1.966020357
180	2.255272505	13.41640786	8.443978	0.926547093	91.556022	1.961686915
240	2.380211242	15.49193338	9.676163	0.985703176	90.323837	1.955802378
300	2.477121255	17.32050808	11.38905	1.0564875	88.61095	1.947487393
360	2.556302501	18.97366596	17.27123	1.237323268	82.72877	1.917656567
420	2.62324929	20.49390153	22.76862	1.357336709	77.23138	1.887793795
480	2.681241237	21.9089023	29.15253	1.464676251	70.84747	1.850324346
540	2.73239376	23.23790008	36.52177	1.562551817	63.47823	1.802624809
600	2.77815125	24.49489743	41.35568	1.616535166	58.64432	1.768225955
660	2.819543936	25.69046516	46.97753	1.671890179	53.02247	1.724459955
720	2.857332496	26.83281573	50.74688	1.705409346	49.25312	1.692433747
780	2.892094603	27.92848009	55.40079	1.743515958	44.59921	1.649327166
840	2.924279286	28.98275349	58.78808	1.769289277	41.21192	1.615022848
900	2.954242509	30	68.7334	1.837167827	31.2666	1.495080658
960	2.982271233	30.98386677	73.4071	1.865738067	26.5929	1.4247657
1020	3.008600172	31.93743885	75.8359	1.879874845	24.1641	1.383170624

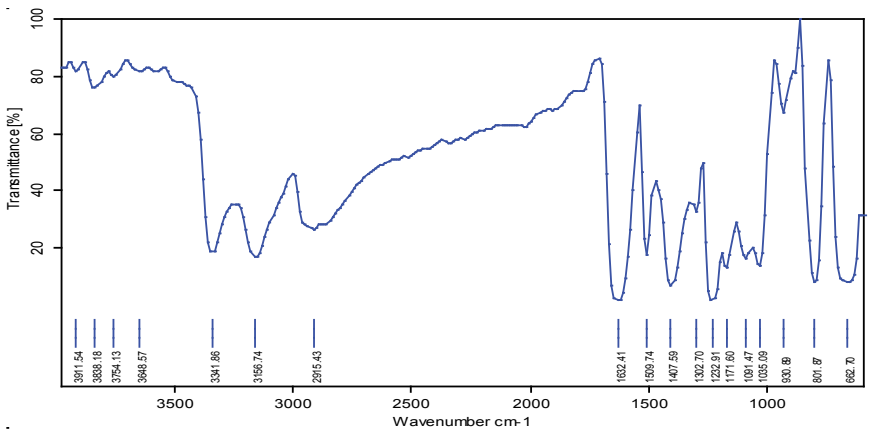

Figure 1. FT-IR spectra of acyclovir sodium

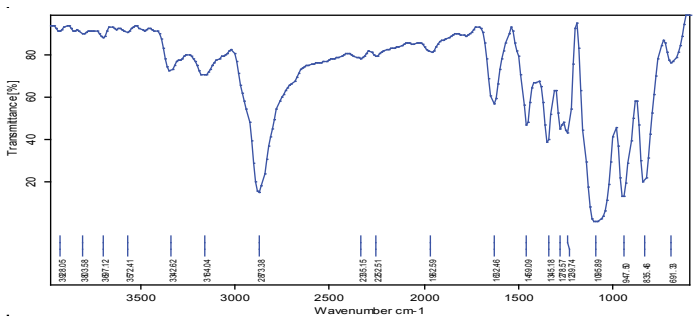

Figure 2. FT-IR spectra of physical mixture of acyclovir sodium, stearic acid and di-methyl sulfoxide of formulation F5

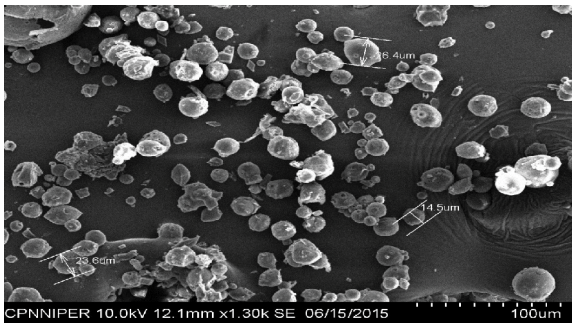

Figure 3. SEM image of acyclovir sodium SLMs of formulation

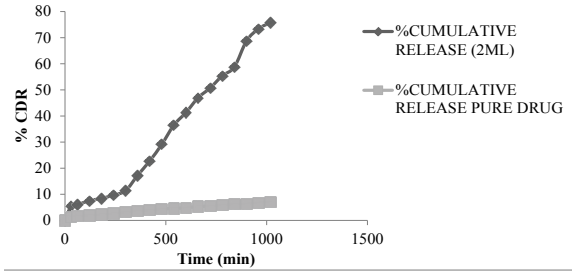

Figure 4. *In vitro* drug release profile of formulation F5 with pure acyclovir sodium