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Research Article

PREPARATION AND EVALUATION OF SOFOSBUVIR POLYELECTROLYTE MICROPARTICLES

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ABSTRACT

Controlled release formulations have been widely employed to get improved therapeutic objectives with numerous drugs. Sofosbuvir is a prodrug and gets converted into its active therapeutic moiety, the triphosphate metabolite. Sofosbuvir has been approved in the treatment of hepatitis C, and is used alone or in combination with other drugs like ribavarin and ledipasvir. The microparticles of sofosbuvir were prepared by polyelectrolyte complexation technique in aqueous environment. The microparticles were evaluated for their physicochemical properties like size, surface morphology, swelling index etc. The encapsulation efficiency and *in vitro* drug release studies were carried. The results indicated that the polyelectrolyte microparticles could extend the release up to 12 hours. The drug release from all the microparticles followed first order kinetics with diffusion mechanism.

Keywords: Sofosbuvir, Polyelectrolyte microparticles, Hepatitis C and Moringa Gum.

INTRODUCTION

Polyelectrolyte complexes (PECs) are the association complexes formed between two oppositely charged particles (e.g. polymer–polymer, polymer–drug, polymer–drug–polymer). These are formed due to electrostatic interaction between oppositely charged polyions. Many researchers extensively studied on the properties of the polyelectrolytes[1] and on the formation of polyelectrolyte complexes[2-4]. The formation process of polyelectrolyte complexes may be divided into three main classes (a) primary complex formation; (b) formation process within intracomplexes; and (c) intercomplex aggregation process[5]. Polyelectrolyte complexes have gained much attention in the past few years because of their potential applications[6,7]. These can be used as membranes[8] for coating on films and fibers[9], for isolation and fractionation of proteins[10,11], for isolation of nucleic acid[12-14], for binding pharmaceutical products[15], as supports for catalyst[16] and for preparation of microcapsules for drug delivery[17,18]. The active substance can be incorporated in to the PECs by four ways[19]. In the first case, the active substance will be entrapped from the solution during precipitation of the complex. The active substance will absorb from the solution and gets incorporated into the already formed complex on contact in the second case. In the third case, the active substance may chemically bond to at least one complex partner and precipitates during complexation. In the last case, the active compound itself may act as a polyion and form a polyelectrolyte complex. The active substance from these PECs will be released either by solution equilibration or by the ion exchange mechanism or by the charge interaction and slow decomplexation as well as breakdown and dissolution of the complex.

Hepatitis C virus (HCV) infection is a major cause of end-stage liver disease and hepatocellular carcinoma (HCC)[20-22]. There have been rapid advances in treatment with the development of oral direct-acting antivirals (DAAs) such as sofosbuvir (NS5B nucleotide analogue), daclatasvir and ledipasvir (NS5A inhibitors)[23-25]. Sofosbuvir (SFB) is an oral nucleotide analogue inhibitor of non-structural 5B polymerase that has been approved for treatment of hepatitis C virus genotypes 1 to 4 (HCV)[26–28]. It is a pro nucleotide analogue, prodrug metabolized to the active antiviral agent 2'-deoxy-2'-α-fluoro-β-*C*-methyluridine-5'-triphosphate (Figure 1)[29]. The triphosphate serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase,

thus acts as an inhibitor of viral RNA synthesis. The active substance in sofosbuvir, blocks the action of an enzyme called 'NS5B RNA-dependent RNA polymerase' in the hepatitis C virus, which is essential for the virus to multiply[30-32]. This stops the hepatitis C virus from multiplying and infecting new cells. NS5B is one of the non-structural proteins essential for viral RNA replication, and has been found to be a valuable target for directly acting antiviral agents (DAAs)[33].

Microparticles are a type of drug delivery systems where the particle size ranges from one micron (one thousandth of a mm) to few mm. This microencapsulation technology allows protection of drug from the environment, stabilization of sensitive drug substances, elimination of incompatibilities, or masking of unpleasant taste. Hence, they play an important role as drug delivery systems aiming at improved bioavailability of conventional drugs and minimizing side effects[34]. In recent years, biodegradable polymeric microparticles, particularly those coated with hydrophilic polymer such as polyethylene glycol known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, and their ability to deliver proteins, peptides and genes[35-37]. Microparticles offer a method to deliver macromolecules by a variety of routes and effectively control the release of such drugs. They may also be used in the delivery of vaccines and molecules such as DNA for use in gene therapy. Microparticles offer effective protection of encapsulated agent against degradation (e.g. enzymatic), the possibility of controlled and local delivery of the drug over periods ranging from few hours to months, and easy administration (compared to alternative forms of controlled release parenteral dosages, such as macro sized implants).

The present study was planned for the application of the polyelectrolyte complexation technique in the preparation of microparticles of sofosbuvir. The microparticles were prepared by polyelectrolyte complexation technique using moringa gum and chitosan. Moringa gum is selected as natural polysaccharide. These anionic & cationic polymers have opposite charges they form complexes in the aqueous medium. Thus these complexes precipitate and help in the encapsulation of the drug. These microparticles were evaluated for the yield, percentage drug content, entrapment efficiency and morphological characters by scanning electron microscopic analysis (SEM), particle size determination by sieve method, FTIR studies, X-ray diffraction (XRD) studies and *in vitro* drug release studies.

MATERIALS AND METHODS

Sofosbuvir was obtained as free gift sample from M/s Hetero Drugs Pvt.Ltd. Hyderabad, Excipients used were Chitosan and Lactose from M/s. Qualigens, Mumbai, Glacial acetic acid from M/s. Merck specialities pvt. Ltd., Mumbai, Moringa Gum Procured from Madhava Shetty, Asst. Prof. Dept. of Botany, Sri Venkateshwara University, Tirupathi, Talc and Magnesium Stearate from M/s. Molychem, Ahmedabad.

Preparations of Moringa Chitosan Sofosbuvir (MCS) Polyelectrolyte Microparticles

In a 100 ml beaker 50ml of 0.05M phosphate buffer of pH 6.8 was taken and to this 100 mg of moringa gum, 100mg of sofosbuvir were added and mixed well using mechanical stirrer. To this mixture aqueous chitosan solution (equivalent to 15 mg of chitosan) was added slowly and continued the stirring. The mixture is allowed for phase separation and precipitation. The precipitate was collected by filtration and washed with water. Then it was allowed for drying at 50^{o} C for 1 hour. The dried mass is pulverized and passed through the sieve no#25. Different ratios of formulations were prepared by adding required amounts of gum as stated in Table 1.

EVALUATION OF MICROPARTICLES

Percentage Yield

These studies involve determination of the amount of microparticles that obtained at the end of preparation on addition of polymer and drug. It can be calculated as follow:

$$\textbf{Percentage Yield} = \frac{\textbf{Practical Yield}}{\textbf{Theoretical Yield}} \times \textbf{100}$$

Particle Size Analysis

For size distribution analysis 2gm of the microparticles of different sizes in a batch was separated by sieving using a range of standard sieves. The amounts retained on different sieves were weighed. The mean particle size of microparticles was calculated by the formulae.

$$\textbf{Mean Particle size} = \frac{\sum (\textbf{Mean Particle size of the fraction} \times \textbf{weight fraction})}{\sum (\textbf{weight fraction})}$$

Drug Content Estimation

Drug content estimation was done by dissolving the prepared microparticles in sufficient quantity of phosphate buffer pH 6.8 and diluted up to 100 ml in volumetric flask. The absorbance of the resulting solution was measured using UV-Visible spectrophotometer at 260 nm against phosphate buffer pH 6.8. From the observed absorbance drug content of sofosbuvir in microparticles was calculated.

Entrapment Efficiency

It is determined by calculating the amount of drug that is entrapped in the microparticles and the drug which is adsorbed on the surface or interior of the polymer. The amount of free, adsorbed and entrapped drug should be capable of being determined separately and this determination indicated the efficacy of the microparticles produced in terms of its active ingredients. Accurately weighed microparticles are taken in a beaker. Buffer is added to the mixture and shaken well to liberate the free drug present in the polymeric matrix. The free drug is quantified by suitable analytical method. It is calculated by:

$$\textbf{Drug loading}\ (\%) = \frac{\textbf{wt. of drug in microparticles}}{\textbf{wt. of microparticles}} \times \mathbf{100}$$

The residue left over from the extraction of the free and adsorbed drug is mixed with 5ml of 0.1m glacial acetic acid. The sample is centrifuged at 5000 rpm for 10 minutes. The supernatant is filtered through 0.45µm filter and the amount of drug entrapped is quantified by suitable analytical method

$$\textbf{Encapsulation efficiency}\ (\%) = \frac{\textbf{Actual drug loading}}{\textbf{Theoretical drug loading}} \times \mathbf{100}$$

Scanning Electron Microscopy (SEM)

It provides vital information about the porosity and micro structure of these drug delivery systems. The most common technique used is scanning electron microscopy (SEM). The sample prepared for this method should be dehydrated as vacuum field is necessary for image generation in SEM. Prior to loading the samples are coated with electron dense coating materials such as gold, palladium or a combination of both to take photomicrograph. The coating can be done by sputter coating or thermal vacuum evaporation.

Swelling and Erosion Studies

Swelling and erosion studies for microparticles were determined gravimetrically in phosphate buffer of pH 6.8 which was attached to pre-weighed glass and supported using adhesive sealant. The microparticles were weighed (W_1) and immersed separately in phosphate buffer of pH 6.8. After every 5 min time interval till 1 h, the pastille was removed from the Petri dish and excess surface water was removed carefully with blotting paper. The swollen microparticles were then reweighed (W_2) and the swelling index (SI) were calculated using the formula given in equation[38-40].

$$\textbf{Swelling Index} = \frac{\mathbf{W2 - W1}}{\mathbf{W1}} \times \mathbf{100}$$

Where, W1 = initial weight of the microparticles

W2 = final weight of the microparticles

$$\textbf{Erosion}\ (\%\ \textbf{mass loss}) = \frac{\textbf{Original Weight} - \textbf{Remaining dry weight}}{\textbf{Original Weight}} \mathbf{X100}$$

Moisture Absorption Study

The moisture absorption study indicates the relative moisture uptake of the polymers used in the pastilles formulation and also the integrity of microparticles after absorption of moisture. Moisture absorption studies have been performed using 5 % w/v agar in distilled water, which while hot was transferred to Petri plates and allowed to solidify[41]. Then microparticles were weighed and placed in desiccator overnight prior to the study to remove moisture and placed on the surface of agar plate for 2 hrs. The microparticles were weighed again and percentage of absorbed moisture was calculated using the formula.

$$\%\ \textbf{Moisture absorbed} = \frac{\textbf{Final Weight} - \textbf{Initial Weight}}{\textbf{Initial Weight}} \times \mathbf{100}$$

X-ray Diffraction (XRD)

XRD analysis was carried out to characterize the physical structure (crystallinity, amorphousness, etc.) of sofosbuvir microparticles formulation F1 using a Rigaku Miniflex instrument (Japan) with ‘‘Nifiltered’’CuK radiation. The scan was taken in the 2θ range of 5–401 with a scanning speed and step size of 11/mm and 0.011, respectively.

Fourier Transform Infrared Spectroscopy (FTIR)

To investigate any possible interaction between the drug and the utilized polymers under investigation FT-IR spectrophotometer method was used. The IR Spectra of pure drug and the combination of drug with polymers

were carried out by using FT-IR spectrophotometer on Spectrum II Perkin Elmer. The IR spectrum was recorded from 4000 cm^{-1} to 400cm^{-1}.The resultant spectra were compared for any spectral changes.

***In vitro* Drug Release Studies**

The drug release studies were performed with USP dissolution test apparatus II (Paddle method). The USP dissolution apparatus was thermostatics at the temperature of 37±0.5 ºC and stirred at a rate of 75 rpm. The prepared microparticles were added in a 900 ml of dissolution medium i.e., phosphate buffer pH 6.8 solution. The aliquots of 5 ml were withdrawn at the time interval of (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 & 12 hours) and replaced with equal volume of dissolution medium. The samples were analysed at 260 nm in a UV-Visible spectrophotometer and amount of drug release at various time intervals was calculated. The study was conducted in triplicate.

Kinetics of Drug Release

The *in vitro* dissolution profile of selected formulation were fitted to Zero order, first order, Higuchi model and Koresmeyer-Peppas model to ascertain the kinetic modeling of drug release. Correlation coefficient (R^2) values were calculated for linear curves obtained by the regression analysis of the above plot.

a. Zero-order Kinetics

Zero order release would be predicted by the following equation:

$$A_t = A_o - K_o t$$

A_t - Drug release at time‘t’
A_o - Initial drug concentration
K_o - Zero-order rate constant (hr^{-1})

When the data plotted as cumulative % drug release Vs time and the plot is linear, then the data obeys zero-order equal to K_o.

b. First order Kinetics

First order release would be predicted by the following equation:

$$\text{Log } C = \log C_o - K_t / 2.303$$

C -Amount of drug remained at time‘t’
C_o -Initial drug concentration
K -First-order rate constant (hr^{-1})

When data is plotted as log cumulative % remaining Vs time yields a straight line and then the release obeys first order kinetics. The constant ‘K’ obtained by multiplying 2.303 with the slope values.

c. Higuchi’s Model

Drug release from the matrix devices by diffusion has been described by following Higuchi’s classical diffusion equation:

$$Q = [D\varepsilon/\tau\ (2A-\varepsilon CS)\ CSt]^{1/2}$$

Q - Amount of drug released at time‘t’
D -Diffusion coefficient of the drug in the matrix
A-Total amount of drug in unit volume of matrix
CS-The solubility of drug in the matrix
ε-Porosity of the matrix
τ-Tortuosity
t-Time at which amount of drug released

When the data is plotted as Cumulative % drug released Vs square root of time yields a straight line, indicating that drug release follows diffusion mechanisms. The slope is equal to ‘K’ (Higuchi’s 1963).

d. Korsmeyer – Peppas Model

To study the mechanism of drug release, the invitro release data were fitted to the well-known exponential equation (Korsmeyer – Peppas model), which is often used to describe the drug release behavior from polymeric systems.

$$Mt/M\alpha = Kt^n$$

Mt/Mα -The fraction of drug released at time‘t’
K -Constant incorporating structural and geometrical characteristics of the drug/polymer system
N -Diffusion exponent related to the mechanism of drug release
When the data plotted as log % drug released Vs log time yields a straight line with a slope equal to ‘n’ and the ‘K’ can be obtained from y-intercept.
Mechanism of drug release as per Koresmeyer-Peppas equation / Pappas model.

RESULTS AND DISCUSSION

Percentage Yield, Drug Entrapment, Particle size and Drug Content

The microparticles were prepared by polyelectrolyte complexation technique in aqueous environment. The yield of the microparticles was found to 80.47±0.38% to 93.15±0.76% for formulation F1 to F6 (Table 2). The results indicated as the polymer concentration increased the yield also increased due to more entrapment of the drug in the polymer matrix. The size and size distribution of microparticles was studied by sieving method. The mean particle size of the microparticles was in the range of 540 ±0.16 µm to 564±0.37 µm for formulation F1 to F6 (Table 2). The results indicated that the particle size is uniform in all the formulations.

The drug content of the microparticles was shown in Table 2 indicated that the drug content for formulation F1 to F6 was found to be in the range of 90.72±0.3% w/w to 96.98±0.19 % w/w. The encapsulation efficiency values were in range of 92.30±0.5 % to 97.44±0.15% (Table 2) indicated that increase in drug to polymer concentration ratio increased the encapsulation efficiency.

Swelling Index, Erosion and Moisture absorption of sofosbuvir microparticles

The swelling index, erosion and moisture absorption are studied for sofosbuvir loaded microparticles. The results are shown in Table 3 and Figure 2 indicated that there is less percent of swelling, erosion and moisture absorption for all six formulations. Swelling index was found to be in range 0.8±0.01% to 1.4±0.21%, percent erosion was found to be in range 0.05±0.06% to 1.21±0.03% and moisture absorption was found to be in range 0.06 ±0.01% to 1.33±0.01%.

Scanning Electron Microscope

The Scanning electron microscopic images of Sofosbuvir pure drug and microparticles were shown in Figure3. The pure drug showed needle like structure. The surface of microparticles is irregular and porous in nature. This indicates that the drug is entrapped in the porous polymer matrix.

X-ray Diffraction (XRD)

X ray diffraction patterns of sofosbuvir and sofosbuvir microparticles in Figure4were obtained and confirmed the presence of crystalline structure of sofosbuvir in the sofosbuvir microparticles. sofosbuvir being entrapped into the moringa gum and chitosan offers different preferred orientation resulting in a different pattern, yet confirming its presence as a crystalline substance. The grinding of the drug loaded microparticles prior to the analysis during sample preparation may also have affected its XRD pattern.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR Spectra of chitosan, moringa gum, sofosbuvir and microparticles are shown in Figure 5. The FTIR spectra of sofosbuvir showed prominent peaks at wave numbers 1455.79 cm^{-1}(RCH_2CH_3), 1600.47 cm^{-1} (RCOO-), 827 cm^{-1} (R_2NH), 621.27 cm^{-1} for alkyl halides 1681.96 cm^{-1} (RCONH) and sofosbuvir microparticles showed prominent peaks at wave numbers 1456.55 cm^{-1} (RCH_2CH_3),1494.45 cm^{-1} (RCOO-), 768.66 cm^{-1} (R_2NH), for alkyl halides 524.04 cm^{-1} (RCONH),1601.80 cm^{-1} (RCONH). The spectra of optimized microparticles exhibited all the principle peaks present in the sofosbuvir as that of the pure drug which indicates the stable nature of the drug during encapsulation.

***In Vitro* Drug Release Studies**

In vitro drug dissolution studies were performed for pure drug as well as for all microparticle formulation using USP apparatus II in phosphate buffer pH 6.8 and the data was shown in Table 4 and Figure 6. The results indicated that the dissolution of pure drug was 93% w/w within 60 minutes. All the microparticle formulations F1 to F6 have shown an extended release of the drug over a period of 12 hours. From the graphs and data of in vitro drug release study, it was observed that formulation F-1 with lower amount of polymer showed higher percent of drug release i.e. 99.63 % w/w and formulation F-6 with higher amount of polymer showed lower percent of drug release i.e. 90.11 % w/w in 12 hours. Sofosbuvir release was decreased with increase in polymer concentration, due to an encapsulating wall thickness around the drug core. The drug release from formulation F1, F2 and F3 was more than 40% within one hour indicating the burst release of the drug from these microparticles. Formulation F5 and F6 showed 50% w/w drug release in 4 hours indicating a slow and gradual release of the drug. Further formulation F5 and F6 showed more than 90 % w/w drug release in 12 hours. And hence these two formulations F5 and F6 having drug to polymer ratio of 1:1 and 1: 1.33 can be considered as better formulations among the six. Between F5 and F6, formulation F5 with low polymer content is considered to be the best formulation.

Kinetics of Drug Release

The kinetics of drug release from the microparticles was studied by mathematical modelling the drug release to zero order, first order kinetics. The correlation coefficient values (shown in Table 5) for microparticles F1 to F6 is found to be in the range of 0.9177 to 0.9463 for zero order and 0.8971 to 0.9890 for first order respectively. These values indicated that the drug release from microparticles is favourable towards first order kinetics when compared zero order. The mechanism of drug release was studied by subjecting the data to Higuchi model and Hixson crowels model. The correlation coefficient values were found to be in the range of 0.9887 to 0.9931 for higuchi model and 0.6648 to 0.7186 for Hixon crowell model respectively. The results indicated that the mechanism of drug release from the microparticles is by diffusion. Korsmeyer-Peppas power law describes the drug release

mechanism from polymeric systems. Thus, dissolution data were fitted to these models to explain drug release mode from the prepared sofosbuvir microparticles follows peppas model (r= 0.964 to 0.979). The n value was found be in the range of 0.333 to 0.395 indicating fickian diffusion mechanism (n < 0.45) for all the prepared microparticles. The results indicated that the drug release from all the sofosbuvir microparticles followed first order kinetics with diffusion mechanism.

CONCLUSION

Sofosbuvir microparticles can be prepared by polyelectrolyte complexation technique in aqueous environment. The yield of the microparticles was found to be good. The results indicated as the polymer concentration increased the yield also increased due to more entrapment of the drug in the polymer matrix. The size and size distribution of microparticles was uniform in all the formulations. The encapsulation efficiency increased with increase in drug to polymer concentration ratio. The *in vitro* dissolution study results indicated formulation F5 found to be the best formulation with controlled release of the drug over a period of 12 hours. The drug release from all the microparticles followed first order kinetics with diffusion mechanism.

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Table 1: Formula of Sofosbuvir Polyelectrolyte Microparticles

S. No.	Ingredients	Quantity (mg or ml)					
		F-1	F-2	F-3	F-4	F-5	F-6
1	Sofosbuvir	300	300	300	300	300	300
2	Chitosan in 2 % acetic acid (mg/ml)	15	15	15	15	15	15
3	Moringa gum	75	100	150	250	300	400
4	0.05 M Phosphate Buffer pH 6.8 (ml)	50	50	50	50	50	50
5	Drug : Polymer	1:0.75	1:1	1:1.2	1:2	1:3	1:4

Table 2: Percentage Yield, Drug Entrapment Efficiency, Particle Size and Drug Content of Sofosbuvir Microparticles

Formulation	Percentage Yield (%) (Mean ± SD)	Drug Entrapment Efficiency (%) (Mean ± SD)	Particle Size (µm) (Mean ± SD)	Drug Content (%) (Mean ± SD)
F-1	80.47 ± 0.38	92.30 ± 0.5	540.78 ± 0.16	30.72±0.11
F-2	82.91 ±0.69	94.24 ± 0.32	541.67 ± 0.08	38.74±0.21
F-3	85.71 ±0.84	94.67 ± 0.7	546.68 ± 0.96	38.52±0.20
F-4	89.60 ±0.58	95.37 ± 0.02	551.37 ± 0.60	40.28±0.17
F-5	92.55 ±0.70	96.93 ± 0.11	552.43 ± 0.34	42.44±0.19
F-6	93.15 ±0.76	97.44 ± 0.15	564.69 ± 0.37	49.98±0.23

Table 3: Swelling Index, Erosion and Moisture absorption of Sofosbuvir Microparticles

Formulation	Swelling Index (%)	Erosion (% mass loss)	% Moisture absorbed
F-1	0.8	0.05	0.06
F-2	1.0	0.32	0.33
F-3	2.2	0.59	0.66
F-4	1.6	0.80	0.86
F-5	2.0	0.96	1.00
F-6	1.4	1.21	1.33

Table 4: *In Vitro* Drug release Studies of Sofosbovir Microparticles

Time (hrs)	Cumulative percent drug released (% w/w)					
	F-1	F-2	F-3	F-4	F-5	F-6
0.5	44.78	39.39	37.48	34.71	26.89	25.49
1	46.53	42.16	41.89	38.14	36.72	35.96
2	52.00	50.11	45.16	43.31	42.42	41.23
3	57.31	55.39	54.26	50.45	49.16	48.58
4	62.22	60.71	59.96	58.19	55.31	54.14
5	68.14	67.34	65.47	62.69	60.74	59.03
6	75.94	73.96	72.68	70.64	65.40	64.61
7	81.47	80.71	78.83	76.10	73.83	72.78
8	86.67	85.79	83.79	82.29	78.03	77.21
9	89.75	87.13	85.95	84.76	80.66	79.96
10	92.02	90.26	88.46	86.12	81.54	80.45
11	96.59	93.39	92.47	90.86	86.98	85.47
12	99.63	98.39	96.87	94.33	91.90	90.11

Table 5: Release Kinetics of Sofosbuvir Microparticles

Formulation	Regression Coefficient R^2					n Value
	Zero Order	First Order	Higuchi	Hixon Crowel	Peppas	
F1	0.9177	0.8971	0.9887	0.6648	0.964	0.333
F2	0.9274	0.9474	0.9931	0.681	0.981	0.357
F3	0.9331	0.9694	0.9901	0.6891	0.972	0.368
F4	0.9415	0.9848	0.99191	0.7031	0.979	0.395
F5	0.9456	0.985	0.991	0.7151	0.980	0.389
F6	0.9463	0.989	0.9904	0.7186	0.979	0.392

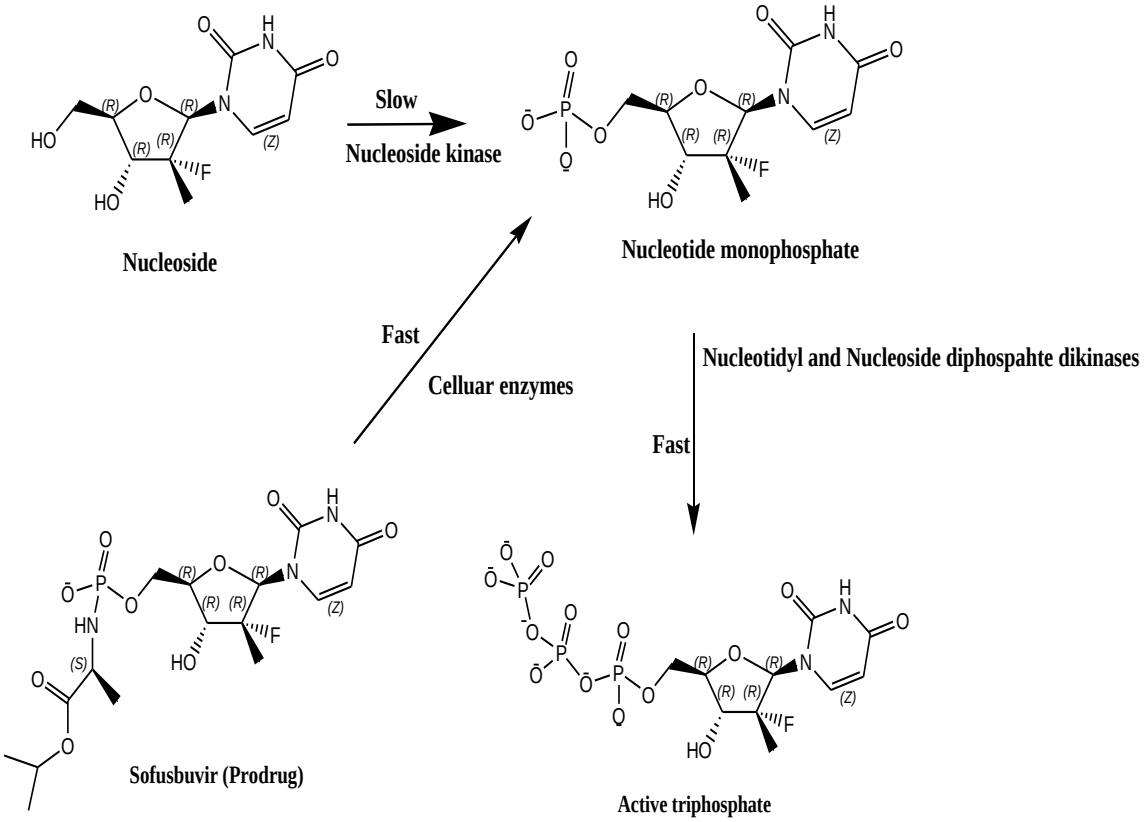
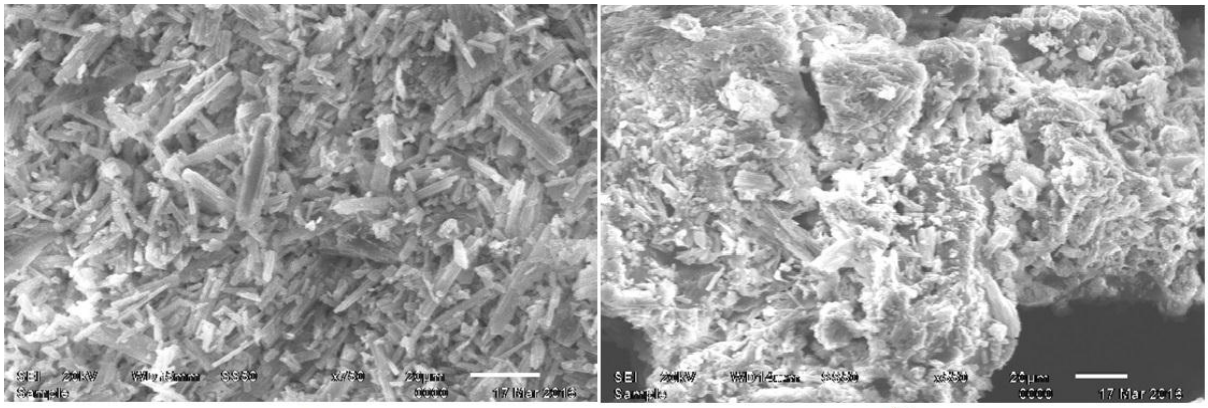


Fig. 1: The activation and metabolic pathway of Sofosbuvir

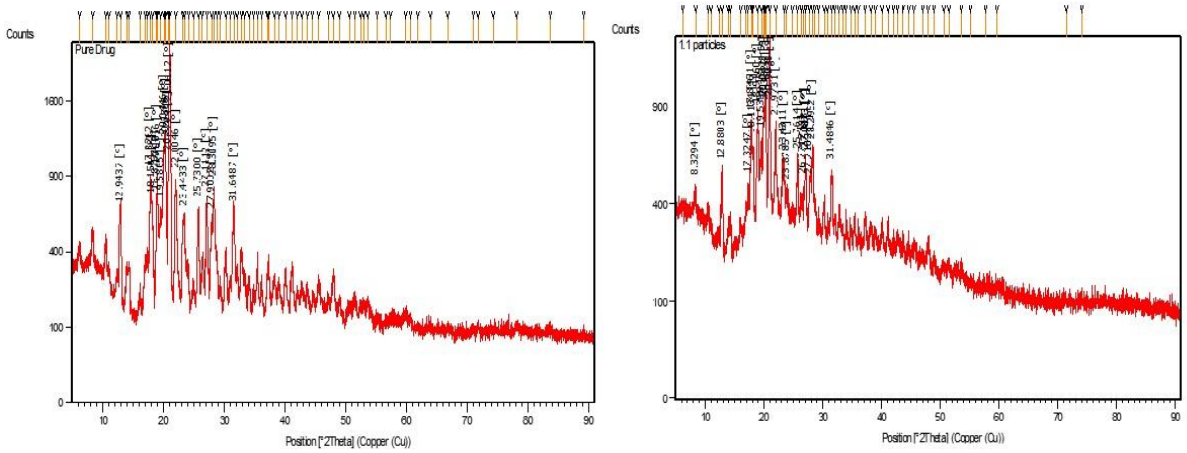
(a) (b)

Fig. 2: Microparticles (a) Before swelling (b) After swelling



(a) (b)

Fig. 3: SEM Photograph of (a) Sofosbuvirand (b) Sofosbuvir Microparticles



(a) (b)

Fig. 4: X-Ray diffraction Image (a) Sofosbuvir and (b) Sofosbuvir Microparticles

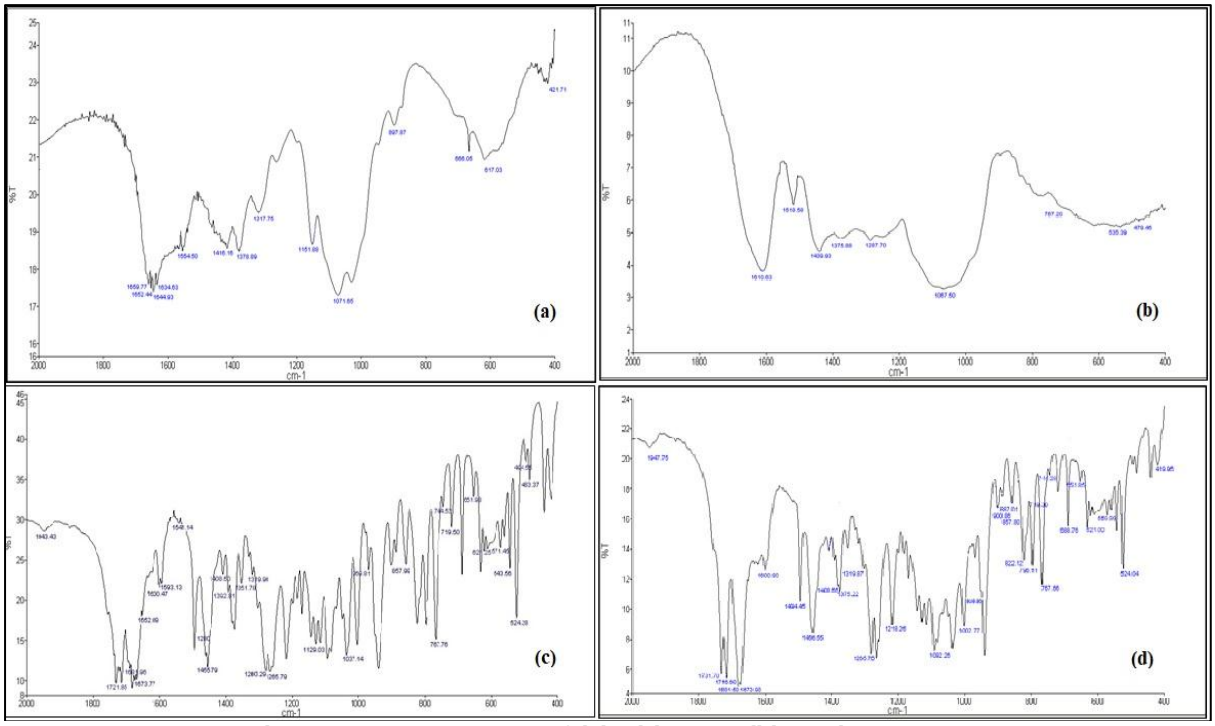


Fig. 5: FTIR Spectrums of (a) Chitosan, (b) Moringa Gum (c) Sofosbuvir and (d) Sofosbuvir Microparticles

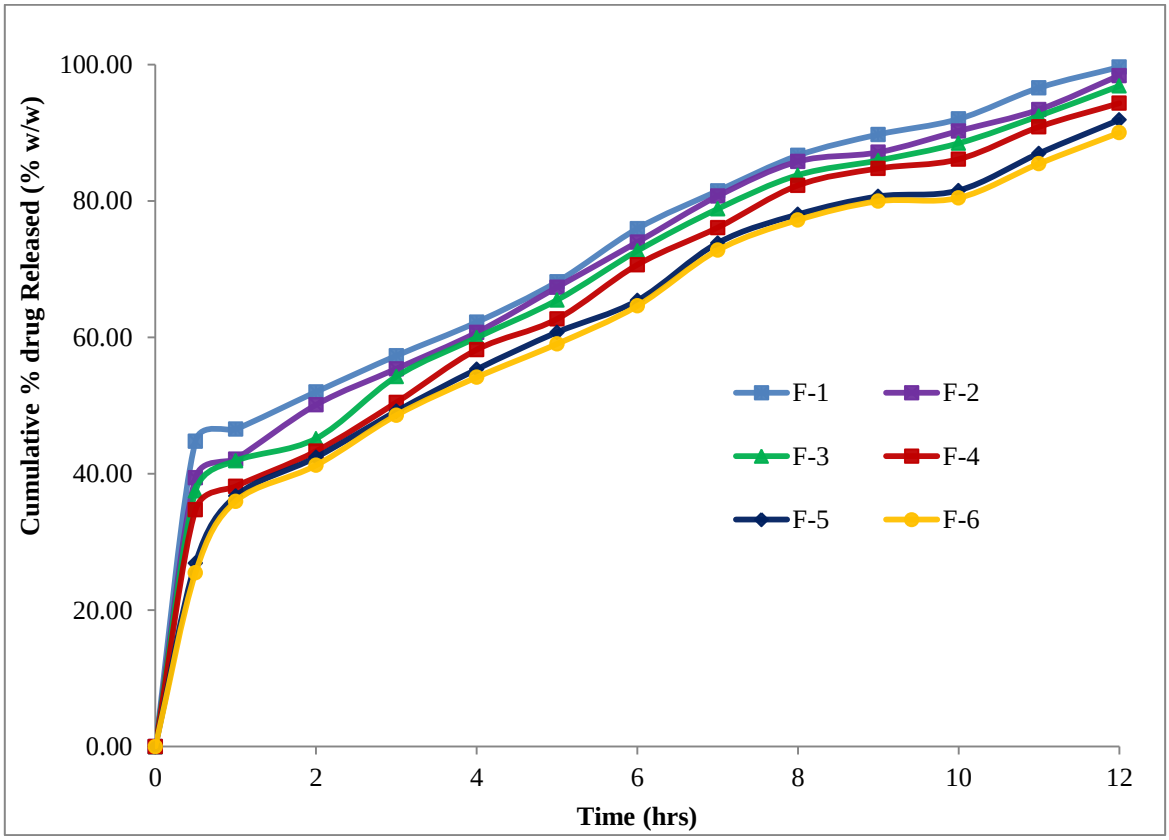


Fig. 6: Cumulative Percentage Drug Release Profiles of Formulations F-1 to F-6

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