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PREPARATION AND CHARACTERIZATION OF SOFOSBUVIR LOADED CHITOSAN-TRIPOLYPHOSPHATE MUCOADHESIVE MICROSPHERES

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ABSTRACT

Hepatitis C virus (HCV) infection is a major cause of end-stage liver disease and hepatocellular carcinoma. The advent of direct-acting antiviral treatments for chronic hepatitis C infection has dramatically increased rates of cure. The aim of the present study was to develop sofosbuvir loaded chitosan microspheres by the ionic gelation method using sodium tripolyphosphate (Na-TPP) as the crosslinking agent. The use of ionotropic gelation avoids the possibility of the occurrence of the toxic and undesirable effects associated with the use of glutaraldehyde, a chemical crosslinking agent. The prepared microspheres were evaluated for mean particle size and particle size distribution, drug loading, encapsulation efficiency and in-vitro drug release. FT-IR spectroscopic analysis was performed to ascertain drug polymer interaction. The release profiles showed zero-order release behavior up to 12 hours where the highest drug release was 99.78 % of the sofosbuvir loaded in the chitosan microspheres, indicating a strong crosslinking between chitosan and TPP anions. The surface morphology of the prepared microspheres was studied by SEM. With an increase in the crosslinking density the rate of drug release decreased. From the results of the present investigation it may be concluded that drug loaded chitosan microspheres can be prepared by a simple technique which avoids the use of complex apparatus and special precautions.

Keywords: Hepatitis C virus, Sofosbuvir, Microspheres, Chitosan, Sodium tripolyphosphate

INTRODUCTION

The hepatitis C virus (HCV) is a flavivirus with 6 major genotypes that currently infects approximately 150 million people worldwide [1]. Untreated chronic HCV infection often leads to progressive liver fibrosis and cirrhosis with the potential for hepatic decompensation and/or hepatocellular carcinoma. Globally, nearly half a million people die annually from liver disease related to chronic HCV infection[2]. Providentially, HCV is curable. The first available treatment regimen was prolonged interferon based therapy, first without and later with ribavirin, which was associated with substantial side effects and a relatively low rate of cure [3]. The first direct-acting antiviral agents (DAAs) were approved in 2011 in the form of two protease inhibitors, telaprevir and boceprevir, each combined with pegylated interferon and ribavirin for genotype 1 infection [4,5]. Subsequent rapid clinical development of new all-oral DAA regimens has dramatically increased overall cure rates to over 95% and this sustained virologic response confers an overall mortality benefit with reduced risk of complications in patients with advanced fibrosis or cirrhosis [6].Most of the drugs in the clinical phase fail to achieve clinical outcomes due the absence of

ability to reach the target site. An effective approach to overcome this failure is by the improvement of controlled drug release systems that release drugs or bioactive compounds at the targeted sites. One of the issues to be solved in is the extreme changes of pH in the human digestive system. Drugs that do not survive the changing pH along its path to reach the targeted site would not produce effective medications[7]. A controlled drug release system consists of three components: a therapeutic agent, a targeting moiety and a carrier system. A wide range of materials such as natural or synthetic polymers, lipids and surfactants have been employed as drug carriers [8]. Among the natural polymers used as drug carriers, chitosan has received significant attention due to its abundant availability, unique mucoadhesivity, biodegradability, non-toxicity, low-immunogenicity and biocompatibility[9-10]. Chitosan, a linear amino polysaccharide is obtained by deacetylation of chitin a natural polysaccharide found in the exoskeleton of crustaceans such as crab and shrimp. Chitosan beads or microspheres prepared using the complexation reaction between anions and chitosan has been developed by many researchers due to its relatively easy process. This anion complexation usually called ionotropic gelation method used a wide variety of anions to crosslink chitosan to produce chitosan-drug matrix in the form of microspheres or nanospheres. Among the anions that can be used in this method, sodium tripolyphosphate is a low molecular weight crosslinker produces chitosan-drug microspheres with the strongest mechanical resistance, as well as release profile curves approaching the zero-order curve[11]. The non-toxic multivalent tripolyphosphate forms a gel with chitosan due to ionic interactions between the positively charged amino groups on chitosan and a negatively charged tripolyphosphate anion. This ionic interaction is dependent on the pH of the solution [12].Sofosbuvir 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)[13-15]. It is a pro nucleotide analogue, prodrug metabolized to the active antiviral agent 2'-deoxy-2'-α-fluoro-β-C-methyluridine5'-triphosphate [16]. 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[17-19]. 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 [20]. The objective of the present study was to prepare drug loaded chitosan microspheres by a simple technique which avoids the use of complex apparatus and special precautions based on a slight modification of the ionic gelation method using Na-TPP as the crosslinking agent.

MATERIALS AND METHODS

Materials

Sofosbuvir was obtained as free gift sample from Aurobindo Pharma Ltd; Excipients used were Chitosan from Himedia, Mumbai. Glacial acetic acids were purchased from Merck; Sodium tripolyphosphate was purchased from Lobachemipvt Ltd (Mumbai, India). All other chemicals and reagent used were of analytical grade. Ultrapure water was used throughout the study.

Preparation of mucoadhesive microspheres

Chitosan solution of 0.5 to 1.5% wt/vol concentration was prepared in acetic acid using homogenizer (Remi motors, Mumbai) at 5000rpm for about 30 minutes. then drug was added to chitosan solution. Microspheres were formed by dropping the bubble-free dispersion of chitosan –drug solution through a disposable syringe (10 ml) onto a gently stirrer (100 rpm) at room temperature in 5% or 10% wt/vol (Sodium tripolyphosphate) TPP solution add Glutaraldehyde as cross linking agent [21]. Chitosan microspheres were separated after 2 hours by filtration and rinsed with distilled water and then they were vacuum dried. The composition of formulations given in table 1.

Table 1 Formulations of the mucoadhesive microspheres

Formulation Code	Sofosbuvir (mg)	Chitosan (%)	% Sodium tripolyphosphate (TPP)
F1	10	0.5	5
F2	10	1.0	5
F3	10	1.5	5
F4	10	0.5	10
F5	10	1.0	10
F6	10	1.5	10

Evaluation of microspheres

Percentage yield

The prepared microspheres with a size range of 200-300nm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

$$\% \textbf{ Yield } = \frac{\textbf{Actual weight of product}}{\textbf{Total weight of drug and polymer}} \boldsymbol{x}\ \mathbf{100}$$

FTIR spectroscopic analysis

FT-IR spectroscopic studies of Sofosbuvir (pure drug), chitosan (polymer), blank (unloaded) microspheres and Sofosbuvir loaded chitosan microspheres were done by recording the respective FT-IR spectra in a JASCO, Model 4200 Spectrophotometer (Japan) over a wave number range of 400 – 4000 cm-1 .

Drug entrapment

The various formulations of the mucoadhesive microspheres were subjected for drug content. 10 mg of mucoadhesive microspheres from all batches were accurately weighed and crushed. The powder of microsphere was dissolved in 10 ml 0.1 N HCl and centrifuge at 1000 rpm. This supernatant solution is than filtered through whatman filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 0.1 N HCl. The percentage drug entrapment was calculated using calibration curve method.

Measurement of mean particle size

The mean size of the microspheres was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer(Horiba Instruments) at a scattering angle of 90°. A sample (0.5mg) of the microspheres suspended in 5 ml of distilled water was used for the measurement[22].

Determination of zeta potential

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Horiba Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate.

Shape and Surface characterization of microspheres by scanning electron microscopy (SEM)

From the formulated batches of microspheres, formulations (F4) which showed a suitable balance among the percentage releases were examined for surface morphology and shape using scanning electron microscope Jeol Japan 6000. Sample was fixed on carbon tape and fine gold sputtering was practical in a high vacuum evaporator. The acceleration voltage was set at 10KV during scanning. Microphotographs were taken on dissimilar magnification and higher magnification (200X) was used for surface morphology[23].

***In-vitro* release studies**

The drug release rate from mucoadhesive microspheres was passed out using the USP type II (Electro Lab.) dissolution paddle instrument. A weighed amount of mucoadhesive microspheres equivalent to 100 mg drug were dispersed in 900 ml of 0.1 N HCI (pH=1.2) maintained at 37 ± 0.5°C and stirred at 55rpm. One ml sample was withdrawn at predetermined intervals and filtered and equal volume of dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. The collected

samples analyzedspectrophotometrically at 264 nm to determine the concentration of drug present in the dissolution medium[24].

RESULTS AND DISCUSSION

Percentage yield of different formulation was determined by weighing the microspheres after drying. The percentage yield of different formulation was in range of 75.65– 81.25%. The drug entrapment efficacies of different formulations were in range of 65.56- 75.65% w/w. This is due to the mucoadhesion characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of sofosbuvir microspheres Table 2.

On the basis of the maximum percentage yield and drug entrapment was found to be formulation F4 in mucoadhesive microspheres so formulation F4 was further studies. The results of measurement of mean particle size of optimized formulation F4 of mucoadhesive microsphere was found 178.5.

Table 2 Percentage yield and drug entrapment for different formulation

Formulation	Percentage Yield	Drug entrapment (% w/w) of prepared microsphere
F_1	78.98±0.25	68.60±0.25
F_2	79.98±0.12	70.25±0.56
F_3	76.56±0.36	69.65±0.47
F_4	81.25±0.14	75.65±0.58
F_5	76.56±0.25	70.12±0.65
F_6	75.65±0.56	65.56±0.84

Results of zeta potential of optimized formulation F4 of mucoadhesive microsphere was found -33.6mV. Shape and surface characteristic of sofosbuvir microspheres examine by Scanning Electronic Microscopy analysis. Surface morphology of formulation examines at two different magnifications 55X which illustrate the smooth surface of microspheres. The drug release rate from mucoadhesive microspheres was passed out using the USP type II (Electro Lab.) dissolution paddle instrument. A weighed amount of mucoadhesive microspheres equivalent to 100 mg drug were dispersed in 900 ml of 0.1 N HCI (pH=1.2) maintained at 37 ± 0.5°C and stirred at 55rpm. From the graphs (Fig 1) and data (Table 3) of in vitro drug release study, it was observed that formulation F 1 has low drug release and maximum amount of drug release found in formulation F4. The kinetics of drug release from the microspheres was studied by mathematical modelling the drug release to zero order, first order kinetics.

Table 3 Releasestudy data of formulation F1-F6

Time (hr)	% of Drug Release					
	F1	F2	F3	F4	F5	F6
0.5	43.25	40.25	38.65	26.65	20.21	15.56
1	56.65	50.32	48.98	39.98	36.65	28.89
2	78.89	68.98	65.45	45.65	40.54	38.14
4	98.21	85.56	80.25	59.98	50.25	45.65
6		99.89	89.98	72.45	68.98	65.54
8			99.74	85.56	75.56	72.25
10	-	-		94.56	82.45	79.98
12	-	-		99.78	85.65	83.21

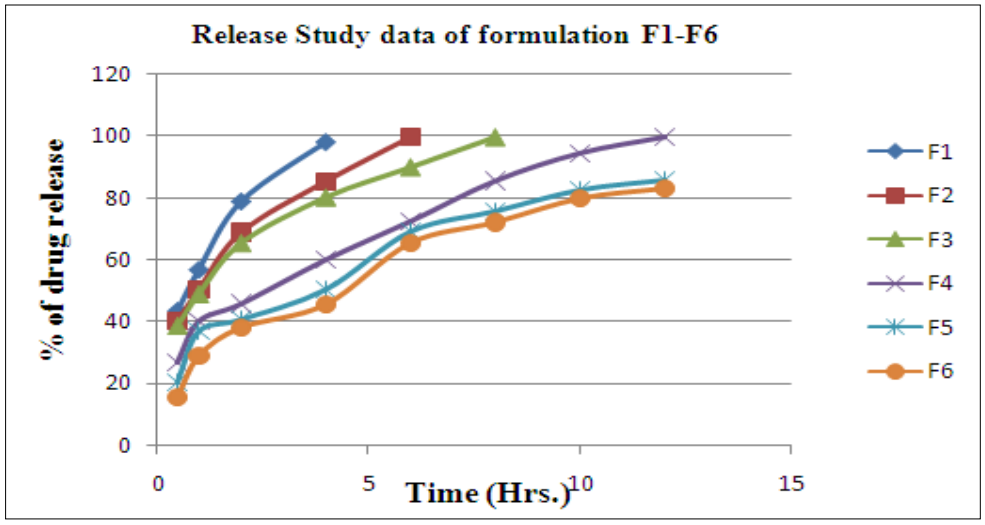
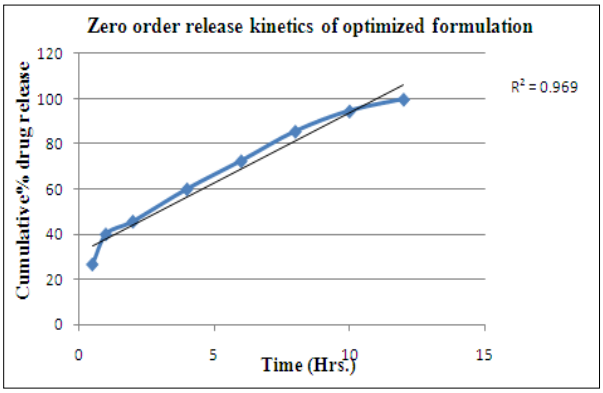


Fig.1 Graph of release study of formulation F1-F6

Table 4 Release kinetics of optimized formulation of microsphere F4

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Released	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	26.65	1.426	73.35	1.865
1	1	0	39.98	1.602	60.02	1.778
2	1.414	0.301	45.65	1.659	54.35	1.735
4	2	0.602	59.98	1.778	40.02	1.602
6	2.449	0.778	72.45	1.860	27.55	1.440
8	2.828	0.903	85.56	1.932	14.44	1.160
10	3.162	1	94.56	1.976	5.44	0.736
12	3.464	1.079	99.78	1.999	0.22	-0.658



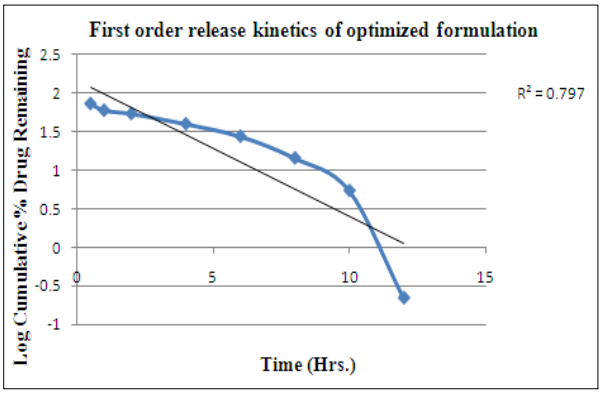


Fig 3 Zero order release kinetics graph **Fig. 4 First order release kinetics graph**

The *In vitro* drug release data of the optimized formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation, in order to determine the mechanism of drug release. When the regression coefficient values were compared, it was observed that 'r' values of microsphere was maximum zero order i.e 0.969 hence indicating drug release from formulations was followzero order Table 5.

Table 5: Comparative study of regression coefficient for selection of optimized Formulation F4

Release Kinetics		Zero order	First order
R^2	Mucoadhesive Microsphere	0.969	0.797

According to ICH guidelines, 3 months accelerated stability study at 40±2°c and 75±5% RH optimized formulations (F4) was carried out. It showed negligible change over time for parameters like appearance, drug content, dissolution and assay etc., No significant difference observed in the drug content between initial and formulations stored at 40±2°c & 75±5% RH for 3 months.

CONCLUSION

There are a myriad techniques available for entrapment and controlled release of important drugs, but only a few pass all the requirements laid down for safe, effective and targeted drug delivery to humans. Chitosan is a very attractive alternative for use in human system because it has long been used as food additive. Controlled drug delivery systems aim to ensure sustained release of drugs in their therapeutic range and chitosan based microspheres are being increasingly used. In case of chitosan/TPP based controlled drug release preparations, chitosan and TPP concentration, pH of TPP and drug concentration are very important parameters for formation of non-fragile, spherical microspheres with good drug encapsulation and tunable in vitro drug release behavior. The sofosbuvir loaded chitosan microspheres

were prepared by ionic gelation using Na-TPP as the crosslinking agent. The in vitro dissolution study results indicated formulation F4 found to be the best formulation with controlled release of the drug over a period of 12 hours. The drug release from the microspheres followed zero order kinetics.The results of all studies have been shown to be satisfactory Thus, Chitosan-TPP based release formulations are an attractive alternative to traditional drug delivery systems.

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