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Thiol modification of galactoarabinan and its appraisal as controlled release carrier of sofosbuvir

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ABSTRACT

The study shows the development and optimization of thiolated and unthiolated polymer as a carrier of sofosbuvir, an anti-hepatic drug for sustained release. Successful thiolation was confirmed by the appearance of peak at 2518 cm^{-1} in FTIR and 9.07 mg of thiol group per gram of polymer (found by Ellmans reagent test). The decrease in the moisture content in thiolated polymer by Karl Fischer titration also confirmed the modification of polymer. The unthiolated and thiolated polymer was characterized by Fourier-transform infrared spectroscopy, scanning electron microscopy and thermal analysis, and evaluated for *in vitro* dissolution release studies and *in vivo* pharmacokinetics. Pre-formulation and post-formulation tests for tablets were performed. Thiolated (15% and 20%) and unthiolated (15%) polymer possessed excellent flow properties whereas; thiolated polymer (25%) showed sustained release of drug for 4 h in *in vitro* dissolution profile. Moreover, pharmacokinetic parameters including Cmax, tmax, t1/2 and AUC were determined after administering standard oral solution (SOS) and tablets of sofosbuvir at a single oral dose of 42 mg/kg body weight. Results of *in vivo* pharmacokinetics ($p < 0.0001$) have explored that Cmax increased from 503 ng/ml (SOS) to 591 and 653 ng/ml for F and TF ng/ml, tmax from less than an hour (SOS) to 5hrs. The t1/2 was increased from 1hr (SOS) to 5.15 and 7 h for F and TF and observed average AUC was 2104, 5616.5, 7199 ng/ml/hr for standard, F and TF respectively. The observed Area under the curve (AUC) for all three formulations was significantly different ($p > 0.05$), indicating the noticeable influence of the polymer and its modified form. Based on this study, it can be concluded that thiolated polymer formulation has exhibited sustained release of sofosbuvir with improved pharmacokinetic profile.

1. Introduction

Acacia nilotica (AN), commonly known as babul and Kikar, is a plant having large amount of bioactive secondary compounds. It is economically available to be used as a source of gum, tannin, fuel, fodder and timber [1]. Its gum is widely used in textile, paper, pharmaceutical and food industries [2]. Being biocompatible material, it is used in drug delivery system as carrier of drugs exhibiting anti-inflammatory, anti--cancerous and anti-oxidant properties [3,4]. Yellowish brown coloured, round crystalloid gum when dissolved in water formed an adhesive and translucent mucilaginous liquid. Structurally it consists of polysaccharides as major component containing mainly arabinose and galactose with minimal quantities of other monosaccharides. It contains phytochemicals like flavonoids, fatty acids and tannins as minor components [2–7] (Image 1 Graphical abstract).

Eco-friendly *AN* gum is a mucoadhesive polymer [6]. The mucoadhesive strength of this polymer could be enhanced by modification of polymer chain and that modified polymer can be used in drug delivery systems for slow release of drug. The –OH group in the backbone of polymer gets replaced by –SH group forming thiolated polymer by replacement reaction or simple oxidation process enhancing the mucoadhesive strength by 20–140 folds [9,10]. Thiolated polymers are extensively used in various mucoadhesive dosage forms especially in controlled release drug delivery systems [9].

Thiolated polymers have a well established mechanism of mucoadhesion [8,14,15], as the thiol moiety of the polymer can form a linkage

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with mucin protein of mucus membrane. This linkage provides an enhanced mucoadhesion strength. The mucoadhesion of a drug delivery system allows the drug to have interaction with a particular site of absorption because of increased contact time to that surface, hence providing drug with the opportunity to get absorbed and hence leading to an increase extent of drug and ultimately the bioavailability of the drug.

Sofosbuvir is a novel drug used for the treatment of hepatitis C caused by single stranded RNA hepatitis C virus (HCV). There are no common symptoms of Hepatitis C. Fatigue, loss of appetite, jaundice and nausea could be considered as major factors [10]. Recent reports and summits on Hepatitis suggested that yearly it claims lives of around millions of people [11]. For the treatment of Hepatitis C, Oral dose of 400 mg of sofosbuvir is taken on regular basis. The recovery of drug is 92%, only 8% is being consumed to treat the infection. 80% of the drug is recovered in urine, 14% in faeces and 2.5% in expired air [12].

The objective of the present work was to develop sustained release drug delivery system and enhanced bioavailability of sofosbuvir Hepatitis C medicine by use of thiol modified *galactoarabinan*. A tablet dosage form for Hepatitis C was prepared using sofosbuvir drug and thiolated *galactoarabinan* from *AN* gum (as a carrier), thereby inducing adhesion of tablet with the mucous layer. By using *galactoarabinan* from *AN* sustained release drug delivery system was formed and its thiolation led to the mucoadhesion of the drug delivery system. Due to mucoadhesion, drug gets absorbed from a particular sight of the intestine. Thus, providing enhanced contact time of the drug and increased absorption.

2. Materials and methods

2.1. Materials

Gum of *AN* was bought from herbal product shops in Lahore and purified. Sofosbuvir (drug) was gifted from CCL Pharmaceuticals, Lahore. The chemicals and reagents used were: magnesium stearate (PubChem CID:11177), lactose (PubChem CID: 440995), thioglycolic acid (PubChem CID: 1133), sodium hydroxide (PubChem CID: 14798), hydrochloric acid (PubChem CID:313), citric acid (PubChem CID:311), disodium hydrogen phosphate (PubChem CID: 24203 & 58592228), methanol (PubChem CID: 18177619). All the chemicals were used without further purification. Distilled water was used throughout this research work.

2.2. Purification of AN gum

AN gum was purified as reported earlier [3]. Gum (100 g) was dissolved in water (750 mL), filtered by vacuum filtration assembly for the removal of impurities, condensed finally to 150 mL by rotary evaporator at 30 °C and finally air dried at room temperature (~25 °C). The yield was approximately 98%.

2.3. Thiolation of AN gum

Isolated polymer (1 g) was soaked in distilled water (100 mL) for 16 h at room temperature. Thioglycolic acid (TGA, 2 g) was added in polymer solution and stirred to ensure complete mixing of polymer and TGA. HCl (5 drops) was added as catalyst with continuous stirring to form homogenized mixture. Mixture was kept in oven at 70°C for 90 min. After the stated time, the mixture was taken out and methanol was added for cooling and precipitates formation. Precipitates were washed with methanol thrice to remove excess TGA. Then they were cooled and lyophilized at −47 °C at 0.013 mbar to obtain the dried thiolated *Acacia nilotica* (*TAN*) material [13].

2.4. Determination of thiol content

The thiol content in the thiolated polymer was determined by Ellmans reagent test. 0.5% w/v dispersions of thiolated and unthiolated polymers were prepared and diluted with 0.5 M phosphate buffer of pH 8.0 to a concentration of 0.15% w/v. 0.3% Ellmans reagent solution was reacted with 5 mL sample solution. Reaction mixture was analysed through UV–Visible spectrophotometer at 450 nm [16,17].

Table 1
Composition of sustained release tablets of Sofosbuvir.

formulation	T*AN* (%)	Drug (%)	Lubricant (%)	Binder (%)	Lactose (%)
F1	10	61	1	5	23
F2	15	61	1	5	18
F3	20	61	1	5	13
F4	25	61	1	5	8

F1, F2, F3 and F4 formulations are for thiolated polymer (T*AN*) and F5, F6, F7 and F8 formulations are for unthiolated polymer (*AN*) having the same concentrations as in F1, F2, F3 and F4 formulations respectively.

2.5. Characterization

Samples were subjected to elemental analyser (LECO630-200-200) to observe the presence and quantification of carbon, hydrogen, nitrogen and sulphur. The content of water in samples was found by Karl Fischer Moisture analyser (Karl Fisher titration, Mettler Toledo SNRB746975116) [16]. Fourier-transform infrared (FTIR) spectroscopic analysis (Bruker, Alpha) was employed for the detection of functional groups.

2.5.1. Surface morphological studies

The information regarding the surface morphology was obtained using Scanning Electron Microscope Hitachi S–3400 N and Jeol JSM-6060 LV. The *AN* and *TAN* were mounted on aluminium stubs with the help of silver paint and sputter coated with gold. The images were recorded, by applying 15 kV accelerating voltage, at different magnifications.

2.5.2. Thermal analysis

SDT, Q-600 (TA instruments, USA) thermal analyser was used to perform thermogravimetric analysis (TGA) and Differential scanning calorimetry (DSC) in the range of 25 °C–800 °C at 20 °C min^{-1} heating rate under nitrogen atmosphere (100 cm^3 min^{-1} flow).

2.5.3. Swelling index

The polymer (0.10 g) was soaked in distilled water (10 mL) and wet weights were recorded after drying externally by use of a blotting paper, after every 5 min for the first hour and every hour till a constant weight was obtained. Swelling Index was calculated by the formula.

$$Swelling\ Index = [(\text{Weight of wet sample} - \text{Weight of dry sample}) / \text{Weight of dry sample})] \times 100$$

2.6. Preparation of tablets

The tablets were prepared by wet granulation method to ensure homogenous mixing of materials. The thiolated polymer, binder, lactose and drug were mixed, homogenized and granulated by water and passed through sieve. The granules were dried in oven at 40°C for 30 min. Dried granules were again passed through sieve and magnesium stearate was added as lubricant. Material was compressed under 10 kN force on a rotary tablet press. Different formulations of tablets were prepared by varying the amount of simple and thiolated polymer (Table 1).

2.7. Evaluation of tablets

2.7.1. Micrometric analysis/pre compression tests

2.7.1.1. Bulk density. The weighed amount of powder (granules) was taken in a graduated cylinder. Without tapping, the apparent volume or bulk volume (V_b) of granules was noted. The formula used to calculate the bulk density was:

$$Bulk\ density = \frac{weight\ of\ powder}{apparent\ volume\ of\ powder}$$

2.7.1.2. Tapped density. The weighed amount of powder (granules) was taken in a graduated cylinder. After tapping of counts, the volume (V_t) of granules was noted. The formula used to calculate the tapped density was:

$$\%\ \text{Drug Content} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Concentration of sample}} \times 100$$

$$Tapped\ density = \frac{weight\ of\ powder}{tapped\ volume\ of\ powder}$$

2.7.1.3. Hausner's ratio. It is defined as the proportional relationship of bulk volume to tapped volume. It describes the flow property of granules with the value to be more than 1.25 [17]. The equation used for the calculation of hausner's ratio is:

$$Hausner's\ ratio = V_b/V_t$$

2.7.1.4. Compressibility index. Flow properties of granules are also determined by compressibility index (C.I.). This method is commonly used and is easy to calculate. The equation used for compressibility index is:

$$C.I\ (\%) = \frac{100(V_b - V_t)}{V_b}$$

2.7.1.5. Angle of repose. Angle of repose of granules was measured by using funnel method. Granules were transferred through a funnel on a surface forming heap. The diameter (d) and height (h) of heap was measured [18]. For calculation for angle of repose following formula was used:

$$\tan \varnothing = \frac{2h}{d}$$

2.7.2. Post compression tests

2.7.2.1. Weight variation. Five tablets were selected randomly and weighed individually by using weighing balance (Mettler Toledo, D455203598). The average weight of tablets was noted along with the calculation of standard deviation.

2.7.2.2. Hardness. The hardness of tablets was checked by Monsanto hardness tester. Ten tablets from each formulations were selected randomly and checked for hardness.

2.7.2.3. Friability. Friability test of tablets was carried out by using Roche friability apparatus (Curio 2020). Twenty tablets from each formulations were selected randomly, weighed initially and after passing through test, the tablets were weighed again. Then the value of friability was calculated by following formula:

$$F = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100$$

2.7.2.4. Thickness and diameter. Vernier calliper was used to measure the thickness and diameter of prepared tablets. Five tablets were selected randomly from each formulation and thickness was calculated individually.

2.7.2.5. Drug content. Five tablets from each formulation were taken and crushed. An amount of powder equivalent to 90 mg drug was taken and dissolved in buffer of pH 6.8 to make total volume 100 mL. Both the solutions were measured at a wavelength of 262 nm. Drug content was calculated using the following formula.

2.8. In vitro drug release of tablets

In vitro drug release studies were carried out using USP paddle dissolution apparatus (curio DL-0708) at 37°C with 50 rpm in buffer solution of pH 6.8. After predetermined intervals sample (5 cm^3 each) was withdrawn and replaced with equal volume of sample solution. The withdrawn sample was assayed spectrophotometrically using UV visible spectrophotometer (E4-161-50-0003-S) at 262 nm. The cumulative release (percent of the drug amount in the tablet) was plotted against time [19].

2.9. In vivo pharmacokinetic studies

High performance liquid chromatography (HPLC) was used to observe the *in-vivo* pharmacokinetics of Sofosbuvir, in Albino Rabbits. The HPLC system (Shimadzu Japan) equipped with SPD-20A Prominence UV/VIS Detector, DGU-20A 5R Degassing unit, LC-20AT Prominence pump, CTO-20A Prominence oven and Lab solution software was applied to monitor and integrate output signals. Quantification was done at 262 nm, selected as lambda max. All experiments were carried out according to EU Directive 2010/63/EU for animal experiments approved by ethical and institutional research board of Pharmacy the University of Lahore ref. no. IREC-53-2019.

The animals were divided into three groups i.e., A, B and C (n = 6). All the treated and diseased animals were excluded from the studies. Individual animals were placed in separate cage with plenty of food and water *ad labitum,* and were kept for 12 h in dark and light cycles. Before the start of medication, animals were kept fastened for 12 h. The animals in all the groups were administered with single oral dose of 42 mg/kg body weight. Group 'A' was administered with dispersion of drug in distilled water and marked as control group and named as 'S', 'B' with the formulation prepared with unmodified polymer (F) and 'C' with the formulation based on thiolated polymer and named as TF respectively.

The blood samples, each of 1 ml were drawn from the marginal ear vein of the animal, initially after 30 min, and subsequently after every 60 min interval, till the 24th hour. The taken sample was added with methanol (3 ml), as de protenizer and subjected to centrifugation at 6000 RPM for 5 min. The supernatant was removed, followed by addition of triethyl ether. The sample was again centrifuged for 5 min to remove the protein completely from the sample. The procedure was

Table 2
Elemental analysis of *AN* and T*AN*.

Sample	Carbon (%)	Hydrogen (%)	Sulphur (%)	Moisture Content (%)
AN	39.208	6.2527	–	3.45
T*AN*	37.276	6.1371	3.2491	2.76

repeated twice and the supernatant was taken out, followed by its drying using water bath at 40 °C. The dried sample was reconstituted with the suitable volume of the mobile phase for quantitative analysis. The sample (20 μg) was injected to HPLC to determine the contents of the drug. PK Solver® software was used following non-compartmental model to determine the pharmacokinetic parameters. Peak plasma concentration (C_{max}) and time required to reach peak plasma concentration (T_{max}) were calculated from the visual inspection of plasma concentration-time curves. Area under the curve (AUC) was calculated by the application of 'Trapezoidal rule'. Both AUC from 0 to 24 (AUC_{0-24}) and from 0 to infinity ($AUC_{0-\infty}$, ng/ml*h) were determined. The $AUC_{0-\infty}$ was calculated with the help of AUC_{0-24} using following mathematical expression.

$$AUC_{0-\infty,} = AUC_{0-24}+C^*/k$$

Where C* = last measured concentration and k is the elimination rate constant and $t_{1/2}$ is determined as;

$$t_{1/2} = 0.693/k$$

2.10. Statistical analysis

Graphpad prism ver 7.0 was used to analyze statistically by employing analysis of variances (ANOVA) followed by Bonferroni's Multiple Comparison Test, where level of significance was set to 95% having n = 6.

3. Results and discussion

The gum isolated from *AN* was of brown color and yield was approximately 98% whereas T*AN* which was esterified by using thioglycolic acid was white in colour with 25% yield. The elemental analysis, FT-IR, monosaccharide analysis, protein analysis and GPC data conformed to those of typical samples of *AN* [2,3]. The molecular mass of the most abundant species measured using light scattering was found to be 9.06×10^5 value for *AN* [2]. According to Table 2 already reported [3], *AN* gum consists of 75% Arabinose and 25% Galactose with negligible amount of protein (0.09%). The unthiolated polymer (*AN*) completely dissolves in water in 3 h whereas the thiolated polymer (T*AN*) requires 12 h stirring to dissolve.

The percentage of carbon, hydrogen and sulphur in *AN* and T*AN* is reported in Table 2 along with the moisture content. The percentage of sulphur in T*AN* specified that thiol group has been added to the backbone of the polymer *AN*. In T*AN*, the percentage of moisture content is less due to the replacement of hydroxyl group with the thiol group in *AN*.

3.1. Determination of thiol content

Calibration curve, that was developed for the standard solutions of thioglycolic acid reaction with Ellmans reagent, showed good linearity with R^2 0.9989, and the thiol content determined in T*AN* was 9.07 mg/g of *AN*.

Table 3
IR of *AN*, T*AN*, sofosbuvir, unthiolated and thiolated polymer formulation.

Material	Hydroxyl group (O–H)	Carbonyl group (C═O)	Glycosidic linkage (C–O–C)	Thiol group (S–H)	Ester linkage (COO)	Amine group (N–H)	P=O group	Phosphate Ester bond (P–O–C)
AN	3333 cm^{-1}	1616 cm^{-1}	1244 cm^{-1}	–	1683 cm^{-1}	–	–	–
T*AN*	3274 cm^{-1}	1636 cm^{-1}	1248 cm^{-1}	2518 cm^{-1}	1697 cm^{-1}	–	–	–
Sofosbuvir	3347 cm^{-1}	–	–	–	1754 cm^{-1}	3245 cm^{-1}	1262 cm^{-1}	1183 cm^{-1}
AN polymer formulation	3345 cm^{-1}	1600 cm^{-1}	1217 cm^{-1}	–	1715 cm^{-1}	3244 cm^{-1}	1263 cm^{-1}	1183 cm^{-1}
T*AN* polymer formulation	3337 cm^{-1}	1600 cm^{-1}	1216 cm^{-1}	2510 cm^{-1}	1715 cm^{-1}	3244 cm^{-1}	1262 cm^{-1}	1182 cm^{-1}

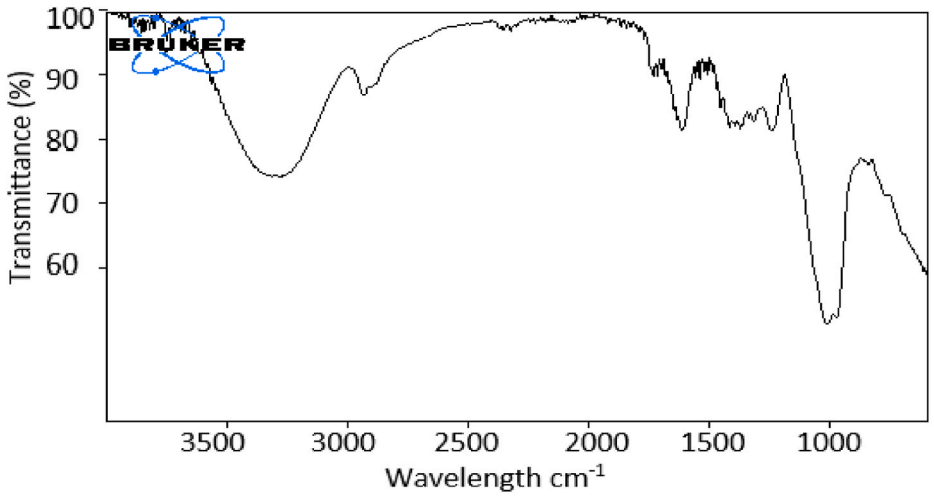

Fig. 1. FTIR spectra of *AN* polymer.

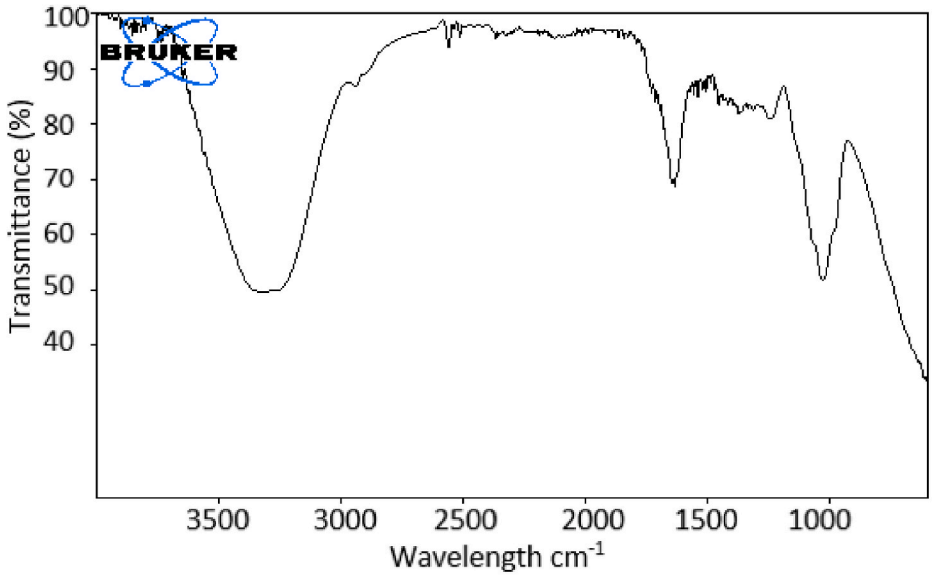

Fig. 2. FTIR spectra of T*AN*.

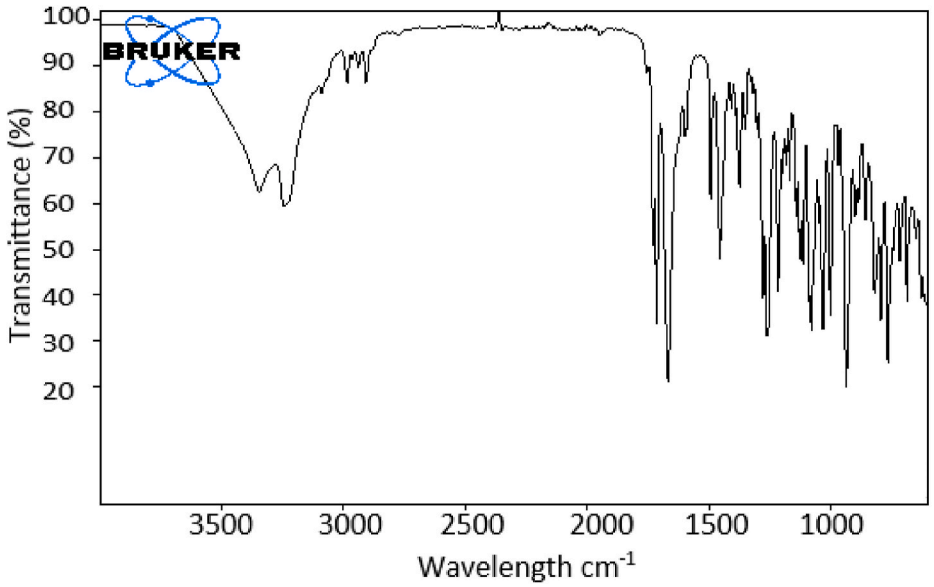

Fig. 3. FTIR spectra of Sofosbuvir.

3.2. Fourier transform infrared spectroscopy analysis

To confirm the polymeric structures, purity of *AN* and thiolation of polymer, FTIR was performed. FTIR peaks of *AN*, T*AN*, sofosbuvir, *AN* polymer formulation and T*AN* polymer formulation are reported in Table 3 and Figs. 1–5. The appearance of peak at 2518 cm^{-1} in T*AN*, confirmed the presence of thiol group as reported in the literature [13].

The FTIR spectrum of *AN* exhibited characteristic peaks at 3333 cm^{-1} (OH stretching), 2929 cm^{-1} (C–H stretching), 1616 cm^{-1} (C═O stretching), 1417 cm^{-1} and 1373 cm^{-1} (C–H bending), and 1067 cm^{-1} (glycosidic linkage, C–*O*–C) (Table 3) [20]. In the FTIR spectra of thiolated *Acacia nilotica*, the appearance of weak peak at 2518 cm^{-1} and in thiolated polymer formulation at 2510 cm^{-1} confirmed the presence of thiol group and 1636 cm^{-1} more intense stretch for COO functional group (Table 3) [21].

The FTIR spectra of Sofosbuvir showed peaks at 3347 cm^{-1} (OH stretching), 3245 cm^{-1} (NH stretching), 3090 cm^{-1} (CH stretching of phenyl group), 2985 cm^{-1} (CH stretching of alkane), 2348 cm^{-1} for COO band, 1754 cm^{-1} (C═O stretching of carboxylic ester RCOOR'), 1600 cm^{-1} and 1493 cm^{-1} (C═C stretching of $RCOO^{-}$ in phenol), 1455 cm^{-1} (C═C stretching of phenyl group), 1262 cm^{-1} (P═O stretching) and 1183 cm^{-1} (P–O–C stretching of phenyl phosphate) and 1114 cm^{-1} (C–O stretching of carboxylic ester) (Fig. 3) as reported in the literature [22].

These results indicated that the characteristic peaks of the polymers have been retained in the modified polymer and there was no new peak except for thiol group at 2518 cm^{-1} and 2510 cm^{-1}. This revealed that the polymers have not reacted with each other and retained their structural integrity. Based on these evidences the structure of the modified polymer can be proposed as shown in Fig. 6.

3.3. Drug polymer compatibility studies

The compatibility studies of pure drug and polymer were implemented by FTIR spectrophotometer. The FTIR spectra of formulations of both thiolated and unthiolated polymers showed peaks of mixture of

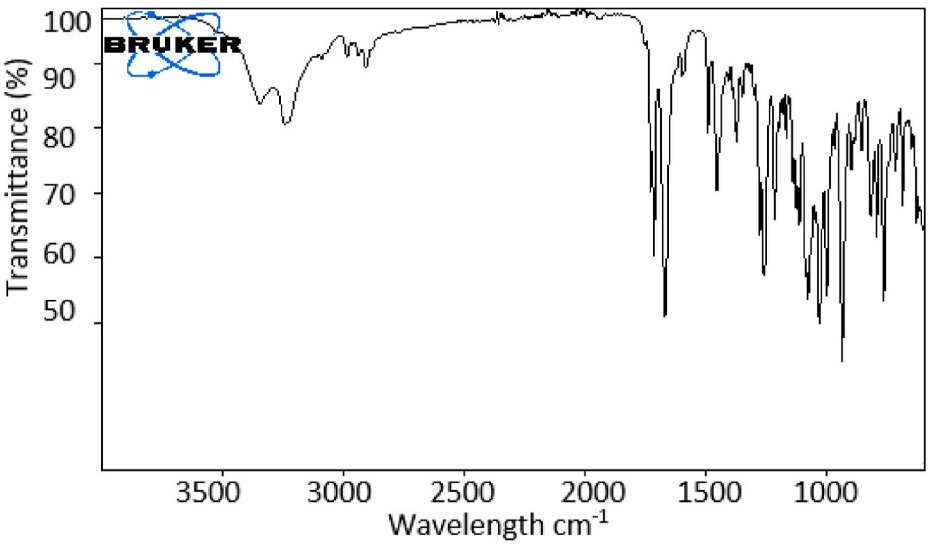

Fig. 4. FTIR spectra of *AN* formulation.

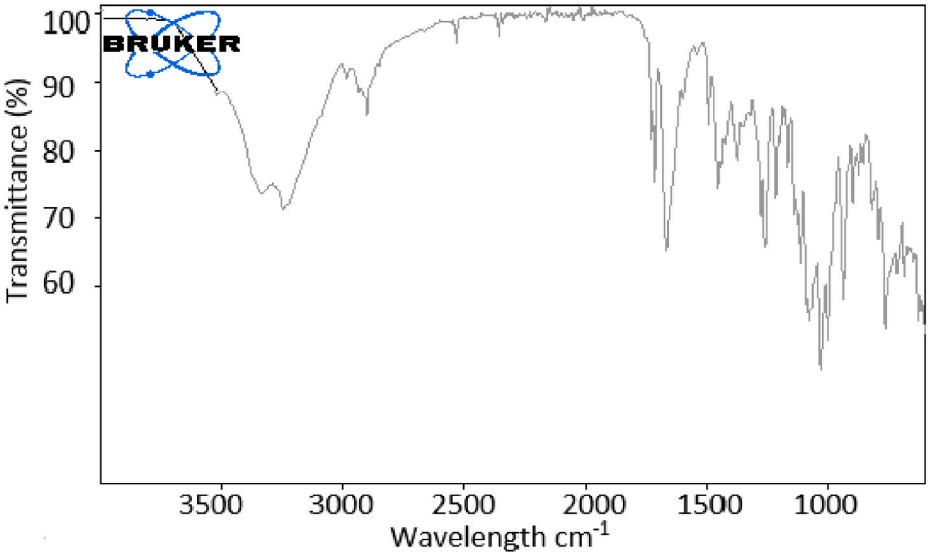

Fig. 5. FTIR spectra of TAN formulation.

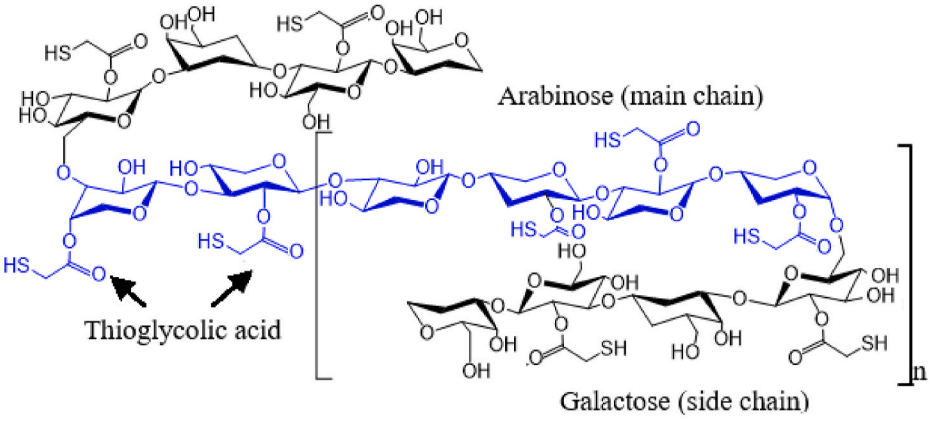

Fig. 6. Structure of the modified polymer after thiolation.

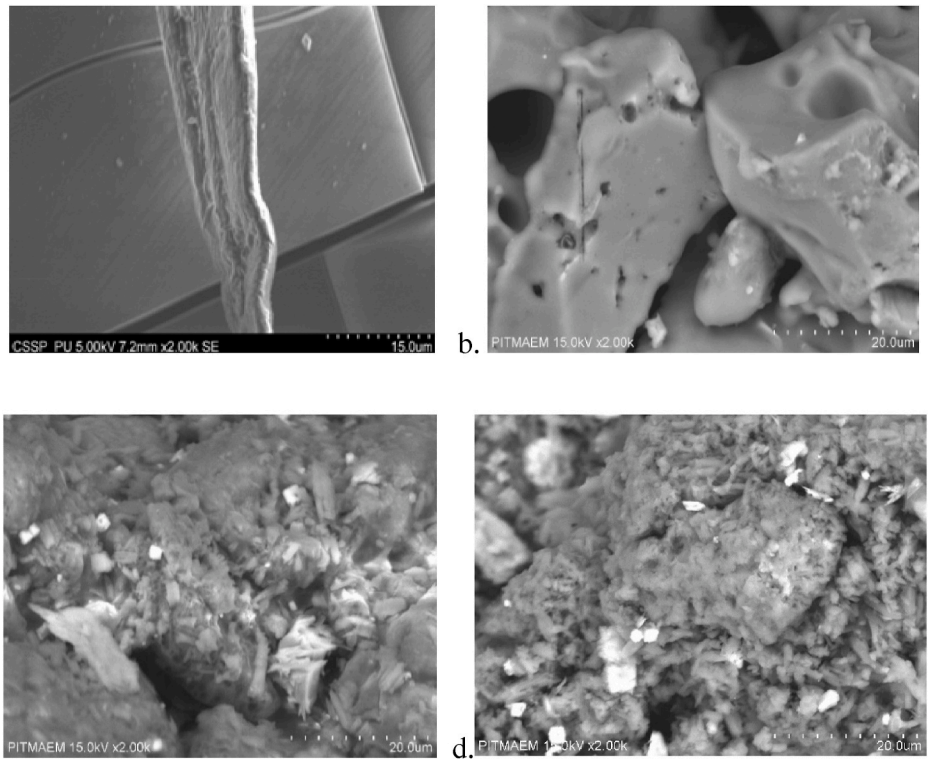

Fig. 7. SEM of a. *AN* b. T*AN* c. *AN* polymer formulation d. T*AN* polymer formulation.

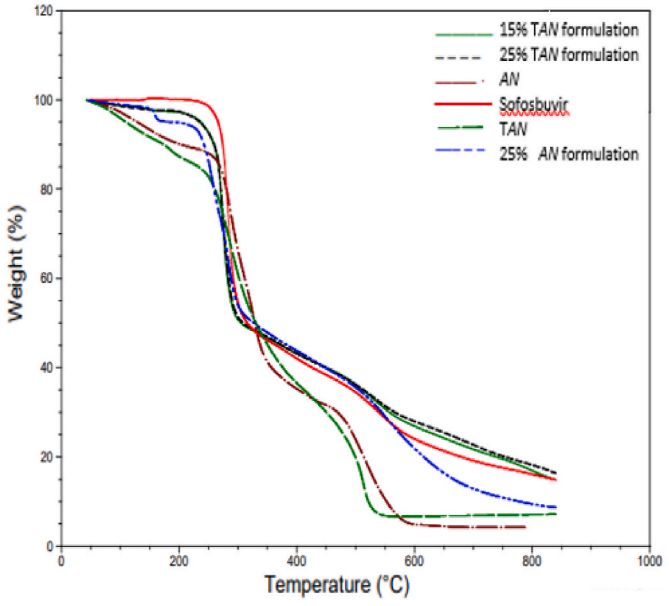

Fig. 8a. TGA Thermograms of *AN,* T*AN,* sofosbuvir, *AN* polymer formulation, T*AN* polymer formulation.

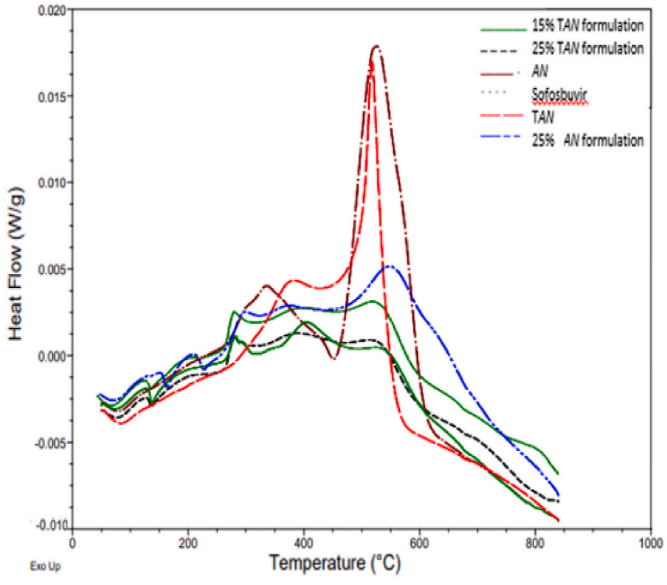

Fig. 8b. DSC Thermograms of *AN,* T*AN,* sofosbuvir, *AN* polymer formulation, T*AN* polymer formulation.

drug and polymer with small shift in values i.e. at 3347 cm^{-1} for OH band, 3244 cm^{-1} for NH band and others same peaks. No new peaks were observed that confirmed the integrity of each component in the formulation to show that no chemical change has occurred during formulation (Figs. 4 and 5).

3.4. Scanning electron microscopy

This technique is useful to study the structure of polymer and drug loading in the polymer. The results revealed that the polymer of *AN* had void and layered structure. In these voids and layers the drug molecules are encapsulated (Fig. 7).

3.5. Thermal analysis

The thermal behavior of the polymer was studied by TGA and DSC. Endothermic weight loss (15%) was exhibited by *AN* polymer from ambient to 225 °C, due to the loss of trapped moisture. Major weight loss of approximately 55% occurred in the range 250–400 °C (exothermic)

Table 4
Pre compression properties for formulations.

Formulation	Bulk density	Tapped density	Hausner's ratio	Compressibility Index	Angle of repose
F1	0.42 ± 0.03	0.48 ± 0.02	1.13 ± 0.01	11.60 ± 1.41	22°
F2	0.48 ± 0.13	0.53 ± 0.01	1.11 ± 0.12	11.20 ± 1.34	26°
F3	0.44 ± 0.19	0.47 ± 0.12	1.08 ± 0.01	8.00 ± 1.19	26°
F4	0.48 ± 0.01	0.52 ± 0.13	1.09 ± 0.18	8.75 ± 1.44	23°
F5	0.46 ± 0.03	0.52 ± 0.03	1.12 ± 0.16	10.80 ± 1.16	25°
F6	0.43 ± 0.11	0.48 ± 0.01	1.12 ± 0.01	12.30 ± 1.13	24°
F7	0.48 ± 0.03	0.53 ± 0.03	1.10 ± 0.11	9.52 ± 1.19	29°
F8	0.39 ± 0.29	0.44 ± 0.17	1.11 ± 0.01	10.0 ± 1.40	35°

due to the degradation of the structure of polymer due to detachment of side chains producing mono-/oligosaccharides (Fig. 8a and b). Third step from 475–600 °C (exothermic peak in the DSC scan) is also associated with degradation of polymer chain involved decomposition of the main polymeric chains producing monosaccharides followed by charring to produce CO_2. The ash left in both the unthiolated and thiolated polymer was less than 5%. In Sofosbuvir, the first weight loss is about 50% occurring from 210 to 300 °C. This weight loss is exothermic. Second weight loss 35% is a slow process and occurred from 300 to 800 °C, with 15% ash left at 800 °C. The thermogram of formulations of both *AN* and T*AN* are in accordance with the thermogram of drug and polymers with no further change in the weight loss showing that drug is encapsulated in the polymer. Thus, from these results, it can be concluded that *AN*, T*AN*, sofosbuvir, unthiolated and thiolated polymer formulation have thermal stability up to 210 °C.

3.6. Swelling index

The swelling index of the AN (unthiolated polymer) and TAN (thiolated polymer) was 14.4% and 20%. The swelling characteristics of these materials make them good candidates for fabrication of delivery devices. From these polymers, release of drug can be controlled by the water content and pore size. For rapid drug release high water content and large pore size is required [23].

3.7. Pre compression properties for formulations

3.7.1. Hausner's ratio

The results obtained for pre-formulation tests showed that F2, F3, F4, F7 and F8 possess excellent flow properties and F1, F5 and F6 possess good flow properties with respect to Hausner's ratio (Table 4).

3.7.2. Compressibility index

Compressibility index of F3, F4, F5, F7 and F8 possess excellent flow properties and F1, F2 and F6 possess good flow properties (Table 4).

3.7.3. Angle of repose

With respect to Angle of repose F2, F3, F5, and F7 possess excellent flow properties and.

F8 possess good flow properties (Table 4).

The results showed that F3 and F7 possess excellent flow properties with respect to Hausner's ratio, compressibility index and Angle of

Table 5
Post compression properties for formulations.

Formulations	Weight Variation (mg)	Thickness (mm)	Hardness (kg/cm^2)	Diameter (mm)	Friability Drug Content (%) (%)	Moisture content (%)
F1	0.651 ± 0.001	2.10 ± 0.3	6.0 ± 0.8	1.1 ± 0.09	0.305 ± 0.2 98 ± 2.23	3.12 ± 1.09
F2	0.649 ± 0.011	2.05 ± 0.6	8.5 ± 0.9	1.3 ± 0.07	0.236 ± 0.1100 ± 0.23	1.55 ± 1.11
F3	0.651 ± 0.001	2.95 ± 0.3	7.3 ± 0.7	1.2 ± 0.05	0.616 ± 0.9 97 ± 2.23	3.12 ± 1.03
F4	0.650 ± 0.004	2.50 ± 0.3	8.0 ± 0.8	1.3 ± 0.09	0.918 ± 0.7 95 ± 3.23	4.65 ± 1.07
F5	0.652 ± 0.001	2.45 ± 0.7	7.5 ± 0.8	1.2 ± 0.07	0.856 ± 0.2 98 ± 2.07	2.35 ± 1.09
F6	0.651 ± 0.008	2.05 ± 0.3	8.3 ± 0.6	1.1 ± 0.02	0.923 ± 0.5 95 ± 2.28	1.45 ± 1.08
F7	0.650 ± 0.001	2.10 ± 0.4	6.5 ± 0.9	1.1 ± 0.05	0.456 ± 0.3101 ± 1.22	3.14 ± 1.05
F8	0.649 ± 0.008	2.40 ± 0.3	7.3 ± 0.8	1.3 ± 0.09	0.672 ± 0.2 99 ± 1.17	3.90 ± 1.03

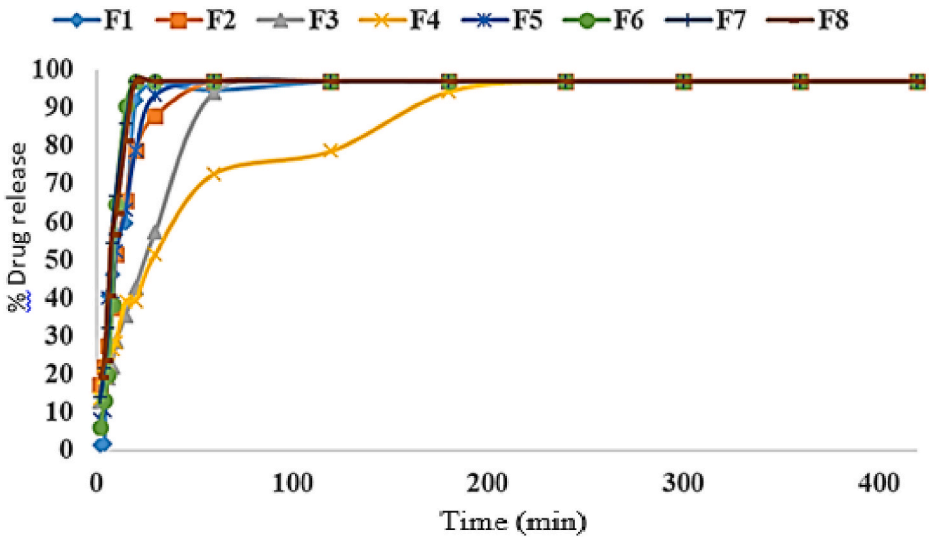

Fig. 9. Dissolution profile of Formulations.

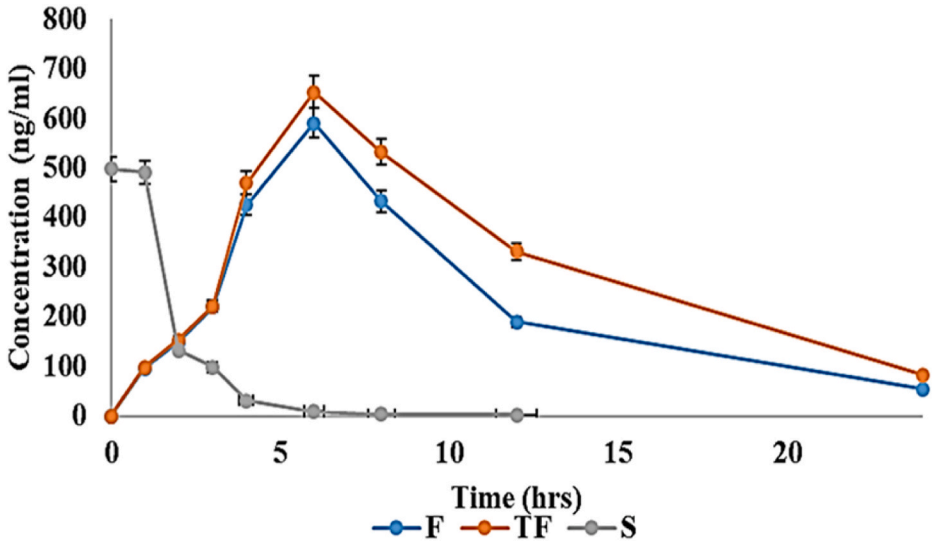

Fig. 10. Plasma concentration of Sofosbovir after administration of S, F and TF to the rabbits.

repose.

The results of pre-compression tests eg. bulk density, Tapped density and Angle of response are very similar to the values reported in literature [24] and is in accordance with US Pharmacopeia and Chinese Pharmacopeia.

3.8. Post compression properties for formulations

3.8.1. Weight variation

The average weight of each formulation was observed and calculated. Weight of tablets of each formulation ranged from 0.649 ± 0.008 to 0.652 ± 0.001 (Table 5).

3.8.2. Thickness and diameter

The thickness of all tablets was in the range of 2.05 ± 0.3 mm to 2.95 ± 0.3 mm and diameter was in range of 1.1 ± 0.09 mm to 1.3 ± 0.09 mm. According to Refs. [25,26], the diameter and thickness of tablets should not vary more than ± 5%. The results in table of thickness and diameter showed that our formulations were accurate (Table 5).

3.8.3. Hardness

The hardness of all formulations fall in the range 6.0 ± 0.8 kg/cm^2 to 8.5 ± 0.9 kg/cm^2. The value of hardness test showed that all tablets had good mechanical strength (Table 5).

3.8.4. Friability

Friability values were in range of 0.236 ± 0.1 to 0.923 ± 0.5. According to USP, the percentage of friability should be less than 1. Prepared tablets had friability value less than 1, and these results showed that these tablets can withstand pressure and stress during handling and packaging and transportation (Table 5) [24].

3.8.5. Drug content

The drug content was found to be in the range 95 ± 2.28 − 101 ± 1.22 in both thiolated and unthiolated formulations, which is well within the acceptable range of US Pharmacopeia specifications i.e.,90–110% [27].

3.8.6. Moisture content

The moisture content of tablets ranged from 1.45 ± 1.08 to 4.65 ± 1.07 (Table 5).

Table 6
Statistical data table for pharmacokinetic parameters of Sofosbovir.

Bonferroni's Multiple Comparison Test		Mean Diff.	T	Significant	*P* value	R^2
Cmax						
F vs S		3542	35.96	Yes (***)	<0.0001	0.9774
TF vs S		158.3	16.03	Yes (***)	<0.0001	0.9774
F vs TF		63.33	6.407	Yes (**)	<0.0001	0.9774
t1/2						
F vs S	4.253		82.02	Yes (***)	<0.0001	0.9996
TF vs S	6.040		116.5	Yes (***)	<0.0001	0.9996
F vs TF	1.787		34.46	Yes (***)	<0.0001	0.9996
AUC						
F vs S		3542	35.96	Yes (***)	<0.0001	0.9981
TF vs S		5059	51.34	Yes (***)	<0.0001	0.9991
F vs TF		−1518	15.40	Yes (***)	<0.0001	0.9978

3.9. In vitro drug release studies

Dissolution test was performed to study the percentage release profile of drug (Sofosbuvir). The tablets were studied for 7 h. Among the tablet formulations containing unthiolated polymer F6, F7 and F8 showed fast release of drug within 20 min and the tablets got dissolved in dissolution media. For thiolated polymer formulation, tablets remained in dissolution media for 4hrs, showing the sustained drug release. The best results for sustained release of drug were obtained from F4 formulation, which had highest percentage of thiolated polymer i.e. 25% among all other formulations and less lactose (Fig. 9).

3.10. In vivo pharmacokinetics

In vivo pharmacokinetic studies revealed that the standard drug (S), when administered orally, reached the systemic circulation rapidly, as its T_{max} was found to be less than an hour. Half-life had also been recorded as ≈1 h. However, when the drug was administered in the form of sustained release formulations i.e., F and TF, its T_{max} was noted to be around 5hrs, and $t_{1/2}$ had been found to be 5.15 and 7 h respectively. It has been confirmed by the studies, that not only in *in-vitro*, but also in *in-vivo* studies, the sustained release effect had effectively been observed. The C_{max} for standard was 503 ng/ml, that had been significantly increased in both prepared formulations i.e., 591 and 653 ng/ml for F and TF, respectively (Fig. 10). The observed AUC for all three formulations was also significantly different ($p > 0.05$), indicating the noticeable influence of the polymer and its modified form. The observed

average AUC was 2104, 5616.5, 7199 ng/ml*hr for standard, F and TF respectively. The greater values of the AUC, in the case of sustained release formulations have confirmed the greater extent of drug absorption form the prepared formulations. The findings were advocating that the natural hemicelluloses have great potential of being effective sustained release polymers. Further, the thiolation of such natural polymers might enhance the drug retarding ability leading to improved bioavailability.

Statistical analysis was performed to find out the difference between the different parameters, evaluated for *in-vivo* pharmacokinetics. The studies have confirmed that natural polymer as well as its modified form has significantly improved the studied parameters (Table 6). Hence, the unmodified as well as modified polymer could be of great interest to enhance the bioavailability of the drug leading to enhance therapeutic effectiveness of the antiviral agent.

4. Conclusion

The objectives of the study have been accomplished successfully as well as effectively, which could be observed by the successful modification of natural polymer and its appraisal as a suitable pharmaceutical carrier of the drug. A significant increase in the half-life as well as in the bioavailability was the illustration of sustained release potential of the natural excipients. The accomplished task might have opened the horizon for the researcher to utilize the safe economical, non-hazardous and easily available polymer to develop a cost effective formulation, capable of providing patient compliance. For the production of thiolated polymer at commercial level, the quality, reproducibility and the properties of thiolated polymers are very important. Other core concerns in its production are its toxicity due to residual content, impurities and regulation issues.

Ethics in publishing

All experiments were carried out according to EU Directive 2010/63/EU for animal experiments and after approval of ethical and institutional research board of Pharmacy the University of Lahore.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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