RESEARCH PAPER

Tenofovir Alafenamide-Loaded Chitosan Nanoparticles: Development, Characterization, and Evaluation as a Long-Acting Anti-HIV Delivery System

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ABSTRACT

Objective: Tenofovir Alafenamide (TAF) is a powerful nucleotide reverse transcriptase inhibitor that has better renal and bone safety profiles than the traditional antiretroviral therapy. Its clinical utility is however constrained by low aqueous solubility, low bioavailability as well as the inconvenience of having to take frequent doses, reducing patient compliance and enhancing chances of viral resistance.

Methods: The aim of this research would be to design and fine-tune TAF-loaded chitosan (CH) nanoparticles through a spray-drying method and ionic gelation to realize sustained drug delivery and enhanced therapeutic effects. Nanoparticles were developed at a different concentration of chitosan and sodium tripolyphosphate (TPP).

Results: The optimum formulation was characterized by a particle size of 156.3±8.2 nm, polydispersity index (PDI) of 0.247+0.031, and zeta potential of +21.5±2.1 mV and a percent encapsulation of 87.4±3.6%. Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) were used to confirm the presence of the polymeric encapsulation of the drugs. The *drug* release tests proved that TAF released steadily in 12 hours after applying Higuchi kinetics model, showing that about 99.7% of the drug was released. The scanning electron microscopic analysis demonstrated agglomerated but uniform-shaped spherical nanoparticles of smooth surface.

Conclusion: These findings indicate that TAF-loaded chitosan nanoparticles are a novel promising delivery system is the treatment of HIV, as it may be possible to administer prolonged dosing periods and provide better patient adherence.

Keywords: Tenofovir alafenamide, nanoparticles, chitosan, HIV treatment, sustained release, encapsulation efficiency, antiretroviral therapy

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INTRODUCTION

Human immunodeficiency virus (HIV) is a worldwide-level health issue that has 39.9 million individuals with HIV/AIDS as of 2023, although it has made big strides in antiretroviral therapy (ART) [1]. Treatment of HIV infection has turned into a chronic disease due to highly active antiretroviral therapy (HAART) which was introduced but full cure of the virus as a disease has been impossible because of the reservoirs of the virus and the new development of the drugs resistant to the virus [2]. Although effective conventional oral antiretroviral drugs are associated with a number of critical issues such as low bioavailability, inefficient tissue penetration, systemic toxicity, and the need to be administered daily or multiple times a day, which pose significant challenges to patients adherence and effective treatment results [3].

One of the next-generation nucleotide reverse transcriptase inhibitors, tenofovir alafenamide (TAF), has received significant clinical attention in that it exhibits superior pharmacological properties when compared to the first-generation tenofovir disoproxil fumarate (TDF) [4]. TAF shows significantly high intracellular concentrations of tenofovir-diphosphate (the active form) and 90 percent lower plasma concentrations than TDF, which leads to better renal and bone safety outcomes [4]. Clinical trials and real-world research have proven that TAF based regimens are similar or even more effective than the conventional antiretroviral combinations and

have good tolerability profiles [5]. Moreover, tenofovir alafenamide/emtricitabine/bictegravir (TAF/E/ B) has become different regimen of choice in the international guidelines on the management of HIV infection because it is highly resistant to resistance, has limited drug-drug interactions, and has a single-tablet formulation [6].

However, despite these advantages, conventional oral TAF formulations continue to present significant limitations for long-term management of HIV. The necessity to administer it daily impairs compliance, especially in environments that are low-resource so that it is difficult to access healthcare and pharmacy services [2]. Additionally, poor penetration of antiretroviral drugs into viral sanctuary sites, including the central nervous system, lymphoid tissues, and macrophages, allows for continued viral replication and the development of latent reservoirs [7]. The introduction of nanotechnology-based drug delivery systems has become a new revolutionary way of addressing these shortcomings, which includes enhancement of drug solubility, stability, bioavailability, and also targeted delivery to infected cells [8].

Chitosan is a natural biocompatible and biodegradable polysaccharide that is a crustacean shell, and has gained a lot of interest as a nanocarrier of antiretroviral drugs delivery systems [9]. The chitosan nanoparticles have several benefits such as improved mucoadhesivity, programmed drug release, intrinsic antimicrobial activity and facilitate cellular uptake by associating with negatively charged cell surfaces [10]. Past research has shown that different antiretroviral drugs such as lopinavir, darunavir and efavirenz can be successfully encapsulated into chitosan nanoparticles and the therapeutic effect of the encapsulated drug is better when it is encapsulated than when it is not encapsulated [11].

The current research aimed at the formulation of TAF-loaded chitosan nanoparticles through the application of spray-drying method along with ionic gelation methods. We aimed at designing a long delivery system that would be able to sustain the release of TAF, enhance cellular uptake and lowering dose frequency without altering therapeutic levels of the drug. This strategy will empirical in filling in some crucial gaps within existing HIV treatment regimens and offer a platform on which injectable long-acting regimens can be developed in the future.

MATERIALS AND METHODS

Materials

Tenofovir Alafenamide (TAF) was acquired with the Sigma-Aldrich (St. Louis, MO, USA). Chitosan (molar weight, 50-190 kDa, de-acetylation level, above 75) was obtained at Himedia Laboratories (Mumbai, India). All other reagents, were purchased from Sigma Aldrich & Merck KGaA (Darmstadt, Germany).

Preparation of Nanoparticles

The chitosan nanoparticles loaded with TAF were prepared using modified spray-drying method with ionic gelation as already mentioned above [1], [9]. Concisely, chitosan (50-150 mg) was added (1% v/v acetic acid) into 10 mL solution under constant magnetic stirring to make a clear solution that took 2 hours to become clear at room temperature. TAF (25 mg) was mixed with the chitosan solution to dissolve with absolute ethanol. The mixture was left to mix further (1 hour) to become homogeneous. To carry out the ionic cross-linking and formation of nanoparticles the drug-polymer solution was dropwise added to a sodium tripolyphosphate solution (1-3% w/v in water) and continuously stirred at 500 rpm. This was then sprayed in Mini Spray Dryer B-290 (Buchi, Flawil, Switzerland) with the temperature inlet of 90°C, outlet temperature set at 50-55°C, aspirator setting at 100% and pump rate at 3 mL/minute through the use of nitrogen as carrier gas. The nanoparticles were dried by spray drying and stored in sealed containers stored at 4°C until their further characterization.

Formulation Design

The parameters of the formulation were optimized using a factorial design (3 x 3 x 3). They were three independent variables: chitosan concentration (X_1: 50, 100, 150 mg), TPP concentration (X_2: 1, 2, 3 w/v), and the ratio of drugs to polymer (X_3: 1:2, 1:3, 1:4). Particle size (Y1), polydispersity index (Y2), zeta potential (Y3) and the encapsulation efficiency (Y4) were the dependent variables of the measurement. There were nine formulations prepared and assessed (Table 1).

TABLE 1: Formulation composition of TAF-loaded chitosan nanoparticles

FORMULATION	CHITOSAN (MG)	TPP (% W/V)	DRUG:POLYMER RATIO (TAF:CH)
F1	50	1	1:2
F2	50	2	1:2
F3	50	3	1:2
F4	100	1	1:4
F5	100	2	1:3
F6	100	3	1:4

F7	150	1	1:3
F8	150	2	1:4
F9	150	3	1:3

CHARACTERIZATION METHODS

Particle Size Determination and Zeta Potential Determination.

A Malvern Zetasizer Nano ZS (Malvern Panalytical, Malvern, UK) was used to measure the size of the nanoparticles, polydispersivity index (PDI) and zeta potential of the nanoparticles using dynamic light scattering (DLS) technology [12,13]. Suspensions of nanoparticles (1 mg/mL in ultrapure water) were diluted 10 times and measured at 25ºC. To measure the zeta potential, nanoparticles were suspended in 10 mM NaCl solution at pH 7.4 and the Smoluchowski equation was used. The measurements were conducted in triplicate.

Morphological Characterization

Gold coating was applied and Scanning electron microscopy (SEM) on a Hitachi S-4800 SEM (Hitachi High-Technologies, Tokyo, Japan) at accelerating voltage of 5 kV was performed.

Encapsulation Efficiency and Drug Loading

A UV-Visible spectrophotometric method was used to determine the drug loading capacity (DLC percentage) and encapsulation efficiency (EE percentage) of TAF loaded nanoparticles. In summary, the 10 mL nanoparticle suspension was centrifuged 12,000 rpm, 15 minutes, 4ºC to separate the nanoparticles and the free (unentrapped) drug. The transparent supernatant with the unentrapped Tenofovir Alafenamide (TAF) was collected carefully after centrifugation. A UV-Visible spectrophotometer was used with 260 nm of the wavelength (λmax of TAF) to analyze the presence of free drug in the supernatant. A normal calibration curve had been previously prepared at different concentration such as 1-100 µg/mL of TAF solutions in water: methanol (40:60 v/v). The concentration of drug that was not entrapped was calculated by the calibration curve equation on the values of absorbance. The amount of free drug to the total drug initially added on formulation was used to determine the encapsulated drug concentration.

Encapsulation % & drug loading capacity were determined by using below equations:

$$\boldsymbol{EE}(\%) = \frac{(\text{Total TAF} - \text{Free TAF})}{\text{Total TAF}} \times 100$$

$$\textbf{DLC}\ (\%) = \frac{\text{Entrapped TAF}}{\text{Nanoparticle mass}} \times 100$$

Fourier transform infrared spectroscopy (FTIR)

The attenuated total reflectance (ATR) mode of FTIR spectrophotometer PerkinElmer Spectrum Two FTIR spectrophotometer (PerkinElmer, Waltham, MA, USA) was used to perform the FTIR spectroscopy. All samples were scanned at the range of 500 to 4000 cm^{-1} at scan rate 32 with 4 cm^{-1} resolution range. Pure TAF and blank chitosan nanoparticles as well as TAF-loaded nanoparticles were spectrophotometers in order to determine possible interactions among the drug and polymer.

X-ray Powder Diffraction (XRD)

XRD The X-ray diffractometer (Rigaku corporation, Tokyo, Japan) was used to obtain the XRD with 40 kV and 15 mA of Cu K alpha radiation (λ =1.54056 A). The samples were scanned on a 2θ value of 5-50º with a rate of 2º/minute and a step size of 0.02º. The crystallinity index was determined using the diffraction patterns.

Differential Scanning Calorimetry (DSC)

A PerkinElmer Diamond DSC (PerkinElmer, Waltham, MA, USA) was used as the method of thermal analysis. Samples (5-10 mg) were added in capped aluminum pans and heated between 30ºC and 300ºC at a heating rate of 10ºC /minute under the flow of nitrogen (20 mL/minute). As a reference, a blank pan was provided.

EVALUATION METHODS

***In Vitro* dissolution studies**

In vitro drug dissolution carried out by using dialysis bag on TAF loaded chitosan nanoparticles [14]. Dialysis (TAF equivalent of a nanoparticle suspension, 2 mL) was performed in a dialysis membrane (cutoff 12,000 Da, Spectrum Labs, Rancho Dominguez, CA, USA) using a 50 mL phosphate buffered saline (PBS, pH 7.4) at 37ºC with continued stirring. The release medium 1mL was removed at regular time intervals (0.5h, 1h, 2h, 3h, 4h, and continue until 11h and 12h) and 1mL of fresh PBS was put into this solution to maintain a sink state. The amount of TAF released was determined using UV Visible spectrophotometric method. The percentage of drug release determined against time.

***In Vitro* Release Kinetics**

The data of release was also correlated with different models of kinetic release such as zero-order, first-order,

Higuchi, and Korsmeyer-Peppas models in order to identify the drug release mechanism [14], [15].

RESULTS AND DISCUSSION

Formulation optimization and characterization of particle

They were nine nanoparticle formulations prepared and characterized (Table 2). The size of the particle was between 127.4 ± 6.8 nm and 198.6 ± 11.3 nm with the most suitable formulation (F5) with a particle size of 156.3 ± 8.2 nm. Polydispersity index varied between 0.208 ± 0.018 to 0.287 ± 0.039, which means that all formulations were relatively homogenous in size distributions. The values of zeta potential were reported to be positive, having the value of +12.3 ±1.8mV to +24.6 ±2.8mV that is because of amino groups as base present in chitosan. The increased values of zeta potential indicate greater colloidal stability and diminished inclination to aggregation of nanoparticles [7,16].

TABLE 2: Physicochemical characterization of TAF-loaded chitosan nanoparticles

FORMULATION	PARTICLE SIZE (NM) ± SD	PDI ± SD	ZETA POTENTIAL (MV) ± SD	EE (%) ± SD	DLC (%) ± SD
F1	127.4 ± 6.8	0.208 ± 0.018	+12.3 ± 1.8	68.2 ± 4.1	6.8 ± 0.5
F2	142.8 ± 7.5	0.231 ± 0.025	+14.6 ± 2.2	75.3 ± 3.8	7.5 ± 0.6
F3	165.2 ± 9.1	0.256 ± 0.028	+16.8 ± 2.5	78.1 ± 4.3	7.8 ± 0.7
F4	148.6 ± 8.3	0.219 ± 0.020	+15.4 ± 2.1	76.5 ± 3.9	7.7 ± 0.6
F5	156.3 ± 8.2	0.247 ± 0.031	+21.5 ± 2.1	87.4 ± 3.6	8.7 ± 0.7
F6	172.4 ± 9.8	0.267 ± 0.034	+19.2 ± 2.6	82.3 ± 4.1	8.2 ± 0.8
F7	181.5 ± 10.2	0.276 ± 0.037	+21.7 ± 2.8	84.6 ± 4.5	8.5 ± 0.8
F8	198.6 ± 11.3	0.287 ± 0.039	+24.6 ± 2.8	79.8 ± 4.2	8.0 ± 0.8
F9	165.8 ± 8.9	0.241 ± 0.029	+20.3 ± 2.4	83.5 ± 4.0	8.4 ± 0.7

Presenting data as mean ± SD (n=3), EE: Encapsulation Efficiency; DLC: Drug Loading Capacity

The values of encapsulation efficiency were between 68.2 ± 4.1% (F1) and 87.4 ± 3.6% (F5), where formulation F5 showed the best encapsulation efficiency. Likewise maximum drug loading capacity in formulation F5 identified 8.7 ± 0.7. The difference in the encapsulation efficiency and drug loading capacity between formulations can be explained by the different amounts of chitosan and TPP ratio, which affect the level of ionic cross-linking and the drug entrapment in polymeric matrix [7]. The high level of encapsulation and the ability of the particles to be of a good size is one of the reasons why Formulation F5 was chosen to be further characterized with a concentration of chitosan of 100 mg, a concentration of TPP of 2% w/v, and the ratio of the drug to the polymer of 1:3.

Morphological characterization

Analysis through SEM and the obtained result revealed the formation of spherical nanoparticles with a fairly smooth morphology and mostly of a spherical shape. The micrographs also indicated that there was a high and

compact structural arrangement, which implies that there was effective crosslinking of the chitosan and TPP. There were few irregularities on the surface of the nanoparticles indicating uniformity in formation of the polymer matrices on the process of the ionic gelation. Nevertheless, slight particle agglomeration was obtained which can be explained by interparticle electrostatic forces and partial aggregation of the samples preparation due to drying (Fig. 1). The measurements were reliable because the range of particle size was similar to that of DLS (150-170 nm) which is the range of the SEM image. The resemblance of the two methods ensures reliability and reproducibility of the process of nanoparticle characterization.

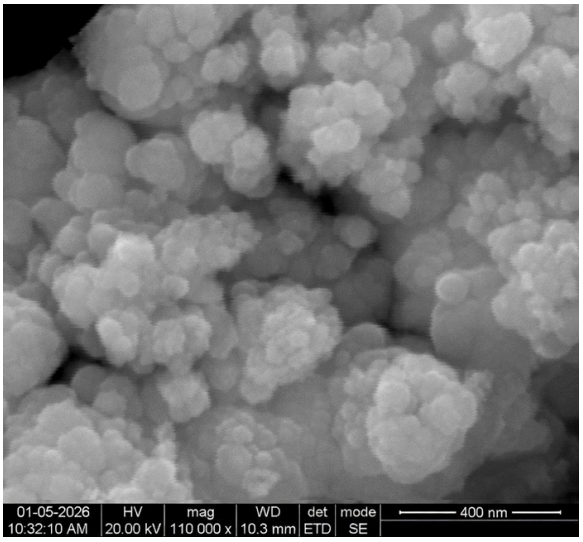

Fig. 1: SEM image of TAF-loaded chitosan nanoparticles

Spectroscopic and thermal analysis

To determine the interaction of TAF with chitosan in the process of nanoparticles formation, the FTIR spectroscopy was used (Fig. 2). The FTIR spectrum of pure chitosan had some typical peaks of 3358 & 3297 cm^{-1} (NH stretching & O-H stretching), C-H stretching at 2870 cm^{-1}, amide I band, C=O stretching at 1650 cm^{-1}, and amide II band, N-H bending at 1586 cm^{-1} [14,17,18]. Characteristic peaks were observed at 3556-3270 cm^{-1} represent N-H stretching, C-H stretching at 2100 cm^{-1}, 1868 cm^{-1} is represented C=O stretching & C-O stretching represent at 1200-1500 cm^{-1} in the spectrum of pure TAF. Spectrum of pure chitosan and TAF-loaded chitosan nanoparticles were maintained with minor shifts in their positions, which means that pure chitosan was encapsulated without any covalent conjugation between TAF and the polymer. The lack of new peaks and the maintenance of traditional functional group ones indicate that TAF still retains its chemical integrity in the nanoparticle matrix.

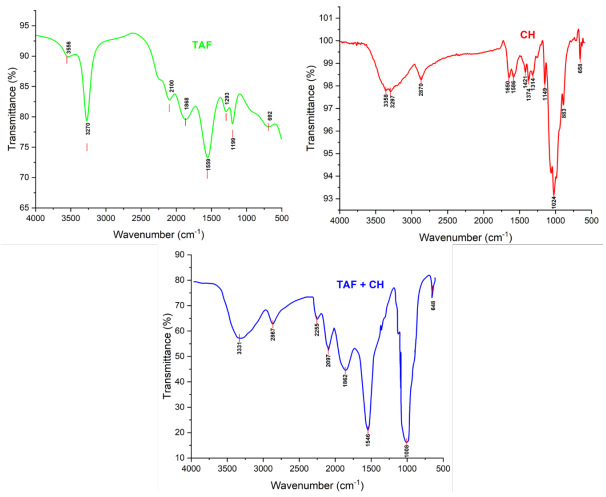

Fig.2: FTIR spectra of pure TAF, chitosan & TAF loaded in chitosan nanoparticles

XRD used to determine the crystallinity of TAF to show that there were notable differences between the crystalline nature of the material before and after encapsulation (Fig. 3). Pure TAF XRD pattern revealed sharp, intense diffraction peaks at 2θ of 2.5°,10.5°, 13.2°, 17.5°, 20.3°, 23.3° and 26.8°, which is highly crystalline. Conversely, pure chitosan XRD pattern shows a peak at 2θ ≈ 3.2°, 10° and 15.4° which represent the semi-crystalline structure of chitosan [15,19,20]. Analysis of XRD pattern of nanoparticles loaded with TAF (F5) revealed that the amorphous structure was mainly present and large amounts of sharp peaks of crystalline TAF were reduced or eliminated with the loading of TAF into the polymer matrix, which indicating successful encapsulation and conversion of crystalline to amorphous TAF. This amorphous transformation is beneficial because it normally improves the bioavailability and solubility of drugs [14].

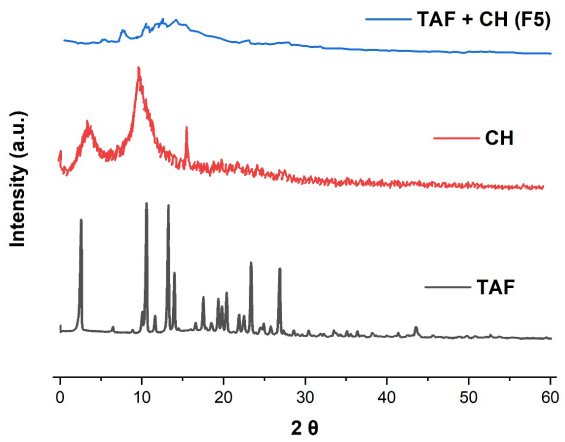

Fig. 3: XRD pattern of pure TAF, chitosan & TAF loaded chitosan nanoparticles (F5)

To determine the thermal stability, as well as the interactions, of the nanoparticles, different scanning calorimetry (DSC) was carried out (Fig 4). Pure TAF was subjected to the DSC thermogram which displayed an endothermic peak at 132°C which was represent melting temperature. Chitosan was purified & showed an endothermic peak at about 58°C, as a result of loss of water and exothermic degradation peak at about 317°C [15,21] followed by an endothermic peak at 421°C. The DSC thermogram of TAF-loaded nanoparticles indicated attenuation or elimination of the typical melting peak of TAF which once again proved amorphous encapsulation of the drug into the chitosan structure. The thermal stability profile was comparable to blank nanoparticles, and this shows that the encapsulation of drugs does not affect thermal integrity of the polymer matrix.

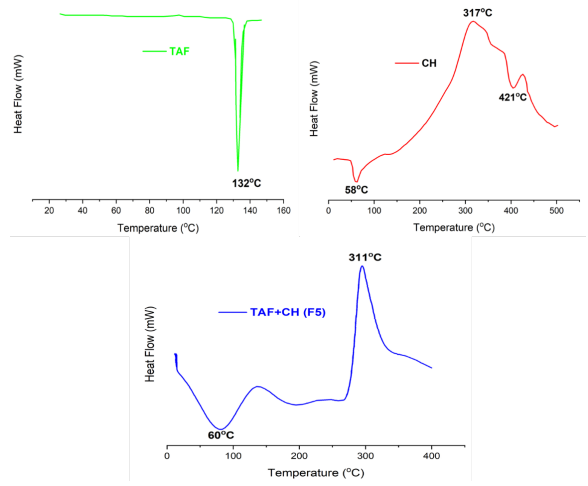

Fig. 4: DSC curves of TAF, chitosan and TAF-loaded chitosan nanoparticles (F5) with modified thermal behavior indicating drug encapsulation

***In vitro* drug release kinetics**

In vitro drug release kinetics of TAF-loaded chitosan nanoparticles (F5) showed a biphasic release profile (Fig. 5). The release was initially burst with a percentage of about 58.4 ± 3% in the first 4 hours, and this can be attributed the drug molecules present on the surface of nanoparticles. This was succeeded by a sustained release stage that had a slower rate of release in the next 12 hours. The percentage of TAF discharged at 12 hours was 97.5 ± 1.5% which is good retention of the drug in the polymeric framework [14,22]. The prolonged release pattern is beneficial in the realization of long-term therapeutic level of drug dosing that may be reducing dosing frequency.

The release kinetics analysis gave the best fit using the Higuchi model (R^2= 0.9847) indicating that drug release took place through diffusion of the nanoparticle matrix. Korsmeyer-Peppas model provided an n value of 0.42 which is in agreement with the Fickian diffusion (n < 0.5) which means that the release of the drug is not due to polymer swelling or making, but it is due to diffusion through the polymer matrix [14], [15]. This knowledge of the release kinetics is essential in the prediction and optimization of the *in vivo* study.

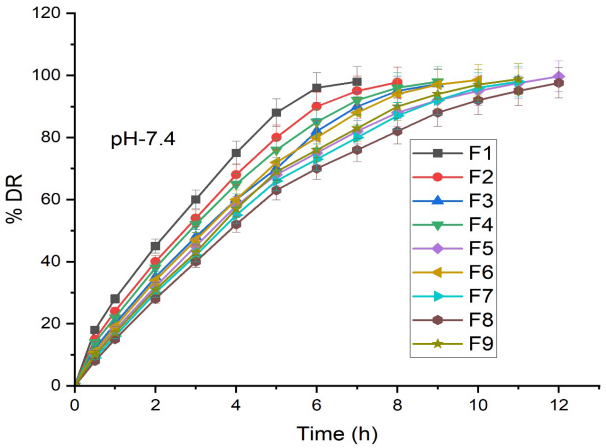

Fig. 5: *In vitro* drug release for TAF loaded chitosan nanoparticles (Formulation F1-F9)

FORMULATION	R^2 (ZERO-ORDER)	R^2 (FIRST-ORDER)	R^2 (HIGUCHI)	R^2 (KORSMEYER–PEPPAS)

F1	0.9301	0.9874	0.9753	0.9299
F2	0.9186	0.9904	0.9797	0.9281
F3	0.8420	0.9883	0.9322	0.9196
F4	0.9154	0.9753	0.9288	0.9404
F5	0.8615	0.9797	**0.9847**	0.9405
F6	0.8243	0.9322	0.9273	0.9105
F7	0.9028	0.9288	0.9195	0.9365
F8	0.8114	0.9273	0.9365	0.9195
F9	0.8872	0.9104	0.9404	0.9290

DISCUSSION

This research paper has managed to produce and characterize TAF-loaded chitosan nanoparticles through a spray-drying method together with ionic gelation. Optimized formulation (F5) produced the particle sizes of optimal range (156.3 ± 8.2 nm) in the parenteral administration and high encapsulation efficiency (87.4 ± 3.6%) and positive zeta potential +21.5 ± 2.1 mV to guarantee the colloidal stability [9]. The attributes are essential in developing injectable formulations that are long acting with success [23-25].

TAF nanoparticle represents crystalline nature to amorphous form which was confirmed by the XRD analysis is likely to increase the drug solubility and bioavailability [15]. It has been shown in the past that amorphous drug formulations tend to have better dissolution and absorption than crystalline counterparts and may, therefore, result in a faster therapeutic plasma concentration [14]. The release pattern was biphasic *in vitro*, release which began with a burst release that was followed by sustained release, which is typical of polymeric nanoparticles and is beneficial to achieving immediate therapeutic effect with long-term drug action. The Higuchi fit (R 2 = 0.9847) that indicates Fickian diffusion, indicates that the polymer matrix diffusion is the main controlling factor that leads the release of drug from the formulation. This knowledge will be important in forecasting the *in vivo* performance as well as optimizing future formulations based on longer-release or long-acting parenteral depots [14], [7].

Compared with traditional TAF preparations, the nanoparticulate system has a number of unique benefits [26,27]: (1) amorphous encapsulation of drugs in nanoparticles enhance solubility and bioavailability, (2) prolong drug release kinetics and reduce dosing frequency, (3) specific targeting of HIV-contaminated cells (macrophages and lymphocytes), and (4) the systemic toxicity may be reduced due to lower plasma drug exposure. Moreover, this chitosan platform can be used to build more sophisticated formulations, such as surface-functionalized nanoparticles carrying specific targeting ligands or combination formulations with complementary antiretroviral agents.

CONCLUSION

This research work helps to successfully developed TAF-loaded chitosan nanoparticles having the best physicochemical characteristics, release kinetics, high biocompatibility and desirable stability properties. The optimized formulation F5 resulted in a particle size of 156.3 ± 8.2 nm, an encapsulation efficiency of 87.4 ± 3.6% and a TAF release of 12 hours based on Higuchi diffusion kinetics. It is anticipated that transformation of crystalline TAF into amorphous structure in the polymeric structure will increase the bioavailability of the drug in the treatment of HIV. These findings are significant indications that TAF-loaded chitosan nanoparticles are a viable platform to use in the development of reduced-frequency or longer-acting parenteral preparations that would greatly enhance patient compliance and treatment outcomes in the management of HIV.

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ABBREVIATIONS

Tenofovir Alafenamide (TAF), Chitosan (CH), Sodium tripolyphosphate (TPP), Polydispersity index (PDI), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Human immunodeficiency virus (HIV), Antiretroviral therapy (ART), Highly active antiretroviral therapy (HAART), Tenofovir disoproxil fumarate (TDF), Bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF), Scanning electron microscopy (SEM), Drug loading capacity (DLC), Encapsulation efficiency (EE), Different scanning calorimetry (DSC)

AUTHORS CONTRIBUTION

The authors declare that this publication is derived from the Ph.D. thesis work of Mrs. Lavanya Mandapati who

performed the preliminary research, collected the data, executed all experiments, and drafted the complete manuscript. P V Madhava Reddy served as the supervisor and contributed by revising the text and validating the data presented in this study.

CONFLICT OF INTERESTS

No conflict of interest among the authors.

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