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Development Of Nanocapsules Of Tenofovir Alafenamide Fumarate For Reduced Protein Binding And Enhanced Bioavailability.

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ABSTRACT

Chronic hepatitis B affects around two billion people worldwide, causing both acute and chronic liver diseases. Tenofovir Alafenamide Fumarate (TAF), an oral tenofovir prodrug, is widely prescribed at a 25 mg daily dose to treat Chronic hepatitis B . However, TAF's protein binding limits its bioavailability in the systemic circulation. Hence the aim of the present study is to prepare and evaluate Polymeric coated TAF nanocapsules by coacervation phase separation technique to prevent protein binding and to enhance bioavailability. Nanocapsules were produced successfully in the range of 870 nm to 2500 nm. The pure drug has a protein binding of up to 73.5% within 60 minutes while a significant reduction of up to 16.0% in protein binding has been observed in case of poloxamer coated nanocapsule formulation. FTIR spectrum of pure and nanocapsule formulation of TAF evidenced no incompatibility.

Keywords: Tenofovir alafenamide fumarate, coacervation, Polymeric coated nanocapsules, reduced protein binding,

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INTRODUCTION

Chronic hepatitis B (CHB) is a persistent HIV infection affecting two billion people worldwide. It is a viral infection that attacks the liver and can cause both acute and chronic disease. WHO estimates that 296 million people were living with chronic hepatitis B infection in 2019, with 1.5 million new infections each year. In 2019, hepatitis B resulted in an estimated 820 000 deaths, mostly from cirrhosis and hepatocellular carcinoma1 (primary liver cancer).

Hepatitis B is primarily transmitted through mother-to-child or horizontal transmission, particularly during the first five years of life. It can also be spread through needle stick injury, tattooing, piercing, and exposure to infected blood and body fluids. In highly endemic areas, prenatal transmission occurs most frequently, while horizontal transmission occurs from an infected child to an uninfected child during the first five years of life [1-3].

There is no specific treatment for acute hepatitis B. Chronic hepatitis B can be treated with medicines. Care for acute hepatitis B should focus on making the person comfortable. They should eat a healthy diet and drink plenty of liquids to prevent dehydration from vomiting and diarrhoea [4]. Treatment can slow the advance of cirrhosis, reduce cases of liver cancer improve long term survival. Most people who start hepatitis B treatment must continue it for life. Hepatitis B can be prevented by vaccines that are safe, available and effective.CHB patients face a 15-40% lifetime risk of complications.

Current treatments for hepatitis B fall into two general categories including the use of Immune modulator Drugs and Antiviral Drugs. Among the Oral Antivirals (Nucleotide Analogues) Tenofovir Alafenamide Fumarate (TAF) is an oral tenofovir prodrug approved by the FDA in 2016. It is a fumarate salt prepared from Tenofovir Alafenamide by reaction of one molecule of fumaric acid for every two molecules of Tenofovir Alafenamide.

But the main drawback of TAF is, it is liable for protein binding to the extent of 80% when it enters the blood circulation, limiting its bioavailability in the systemic circulation5. Polymeric coating of the drug by coacervation phase separation to convert it into nanocapsules may be a promising technique to reduce protein binding and enhance bioavailability [5-8]. The production of poloxomer coated nanocapsules in nano size may have added advantage to exploit them as injectable formulation with reduced protein binding and enhanced bioavailability. Therefore the main aim and objectives of the present research work is to prepare the development of injectable poloxamer coated nanocapsules of tenofovir alafenamide fumarate, evaluation at *in-vitro* level and to assess protein binding reduction studies for enhanced bioavailability.

Preparation of poloxamer coated nanocapsules of tenofovir alafenamide fumarate

Poloxamer coated nanocapsules of tenofovir alafenamide fumarate were prepared by coacervation phase separation technique [9-11] using poloxamer as a coating agent. For the preparation, acetone and tween 20 were used as solvents and liquid paraffin was an encapsulating vehicle. Tenofovir alafenamide fumarate drug and poloxamer were taken in 1:2 ratio and the composition of trial formulations are shown in **Table 1**. Different trials were tried by changing stirring speed as shown in the table. During the method of preparation, poloxamer was dissolved in acetone and TAF was dispersed as particles in liquid paraffin that contained 0.1 ml of tween 20. The polymer solution was added slowly to the drug dispersion by means of a burette. The mixture was agitated using magnetic stirrer at room temp (25°c) until the acetone (polymer solvent) was evaporated. The rate of stirring was kept constant from beginning to end. The drug and polymer are used in 1:2 ratios. The liquid paraffin was decanted and the nanocapsules were collected, washed with petroleum ether to remove any remaining oil phase and dried under reduced pressure for at least 12 hrs. Each trial was prepared to contain 10 doses since single dose is 25mg.

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Table 1: Ingredients of nanocapsules of tenofovir alafenamide fumarate.

S.NO	Formulation	Composition	Stirring speed
1	TAF NC 1	TAF -25mg Poloxamer -500mg Acetone -15 ml Liquid paraffin -25 ml Tween 20 –0.1ml	1000
2	TAF NC 2		1200
3	TAF NC 3		1250
4	TAF NC 4		1300
5	TAF NC 5		1350
6	TAF NC 6		1400

Evaluation of tenofovir alafenamide fumarate nanocapsules

Trial formulations of nanocapsules of TAF Viz., TAF NC 1 to TAF NC 6 that were produced as discrete particles were evaluated for particle size, percentage yield, entrapment efficiency, angle of repose, protein binding studies and Drug excipient interaction studies by FTIR.

Particle size determination by Zeta sizer

Particle size of prepared TAF nanocapsules was measured by Zeta sizer. A suspension of formulated TAF nanocapsules was prepared by adding it to 10ml of distilled water. Ensured the particles are well-dispersed to avoid aggregation, which can affect the results. The measurement was carried out at a temperature of 27^0C and the scattering angle was set to 90^0. Polydisperse index were also denoted from the data obtained from zeta sizer (PDI = 1: Indicates a mono disperse sample where all particles or molecules are of uniform size or weight. PDI > 1: Indicates a poly disperse sample with a broader distribution of sizes or molecular weights. The larger the PDI, the more heterogeneous the sample).

Percentage yield of nanocapsules of TAF

The percentage of practical yield is calculated to know about the efficiency of the method, it helps in the selection of a suitable method of production. The yield of nanocapsules of TAF was determined by comparing the total weight of nanocapsules with the combined weight of the polymer and drug.

Encapsulation efficiency

The efficiency of encapsulation of TAF was determined by measuring the total amount of TAF present in sample of the nanocapsules and comparing this value with the expected amount of TAF in each of the samples based on the drug loading during the preparation. Nanocapsules equivalent of 25 mg of TAF was taken and dissolved in 10 ml of phosphate buffer of pH 7.4 and centrifuged at 1000 rpm for 5 min. Then the sample was analyzed by UV visible spectroscopic method under at 260nm.

Flow property-The angle of repose

Flow properties of powder are generally assessed by determining the angle of repose. Angle of repose is defined as the maximum angle possible between the surface of a pile of powder and the horizontal plane. The angle of repose is determined by allowing powder to flow freely through an orifice from a certain height and form a conical heap on the horizontal plane. The angle which the heap forms with the horizontal surface is the angle of repose and is determined by the formula:

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

Where, θ is the angle of repose, h is the height of heap of powder and r is the radius of the base of heap of powder.

Table 2: Specifications for Angle of repose

The angle of repose (Degrees)	Flow property
≤20	Excellent
20-30	Good
30-34	Passable
≥40	Very poor

Assessment of protein binding of TAF and its nanocapsules at *in- vitro* level

Preparation of phosphate buffer of pH 7.4

Phosphate buffer of pH 7.4 was prepared by adding 2.38 gm of disodium hydrogen phosphate and 0.19 gm of potassium dihydrogen phosphate were taken and to which 8g of sodium chloride dissolved in 1000 ml of distilled water.

Preparation of 40µM solution of egg albumin solution

0. 140 gm of albumin flakes were dissolved in 50 ml of buffer prepared and shaken well and kept aside.

Protein binding study

One egg was taken in a china dish and Con. HCl was added slowly so that the egg membrane was separate. The egg membrane was tied at the neck of one side of the boiling tube having open ends on both both sides. On the hand 25 mg of tenofovir alafenamide fumarate was dissolved in egg albumin solution and this prepared egg albumin solution with TAF was poured through the other opening end of boiling tubeand this was kept in slightly contact with buffer of pH 7.4 which is taken in a beaker and arranged to the stand.

A sample solution of 1ml was collected by using pipette for every 10 mins at 10,20,30,40,50 and 60 mins time interval from the beaker and fresh buffer was replaced.

The collected samples were assessed under UV spectrophotometer at 260nm, and the absorbances were noted. The procedure was repeated for nanocapsule preparations TAF NC 5 and TAF NC 6 and determined protein binding [12].

Calculations:

$$\text{Concentration} = \frac{\boldsymbol{Absorbance}}{\boldsymbol{slope}}$$

$$\text{Total amount of drug} = \frac{\boldsymbol{Amount\ of\ drug\ taken}}{\boldsymbol{Molecular\ weight\ of\ drug}} \times \frac{\mathbf{1000}}{\boldsymbol{Dissolution\ fluid\ volume}}$$

$$\text{Unbound drug} = \frac{\boldsymbol{Concentration\ of\ drug}}{\boldsymbol{Molecular\ weight\ of\ drug}} \times \frac{\mathbf{1000}}{\boldsymbol{Dissolution\ fluid\ volume}}$$

$$\text{Drug bound} = Total\ drug - Unbound\ drug$$

$$\%\ \text{Drug bound} = \frac{\boldsymbol{Drug\ bound}}{\boldsymbol{Total\ drug}} \times \mathbf{100}$$

Fourier Transform Infrared Spectroscopy Studies

To check compatibility of drug with formulation ingredients used for preparation of nanocapsules, IR analysis was carried out for pure TAF and nanocapsules of TAF using IR spectrophotometer (BRUKER,

Japan) KBr disc method. The samples were thoroughly blended with dry powdered potassium bromide crystals, compressed to form a disc, placed in a sample holder and then the spectrum was recorded from 4000 to 400 cm-1.

RESULTS AND DISCUSSION

Trial formulations of nano capsules, TAF NC 1 to TAF NC 6 produced by coacervation phase separation technique were appeared as free flowing discrete particles and were are all evaluated for particle size, percentage yield, angle of repose, entrapment efficiency, FTIR and protein binding studies.

Determination of particle size of nanocapsules of TAF

Particle size of values of prepared nanocapsules, TAF NC 1 to TAF NC 6 are shown in Table 3 and the relevant figures obtained from Zeta Sizer are presented in Fig. 1 to Fig. 6. As per the results it is observed that the present method is successful in producing the drug in the form of nano capsules ranging in size from 870 nm to 2500 nm. Poly Disperse Index (PI) data of the tenofovir alafenamide nanocapsules ranged from 1.605 to 1.062 shows uniformity in size of nanocapsules in each trial. These results shows that the ingredients and process parameters used for preparation of nanocapsules of TAF in the present work are successful in producing the product in injectable nano particles.

Table 3: Particle size data of TAF nanocapsules

S.NO	Formulation	Particle size (nm)	PI
1	TAF NC 1	878.5	1.605
2	TAF NC 2	840.5	1.012
3	TAF NC 3	2596.3	1.091
4	TAF NC 4	1837.4	1.017
5	TAF NC 5	1023.9	1.006
6	TAF NC 6	1055.8	1.062

Figure 1: Particle size of TAF NC 1

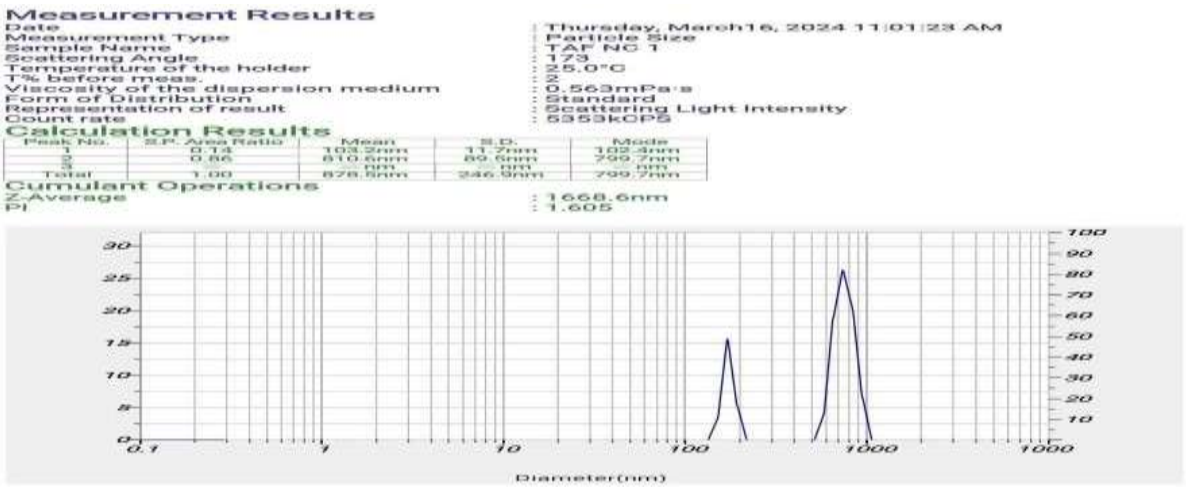

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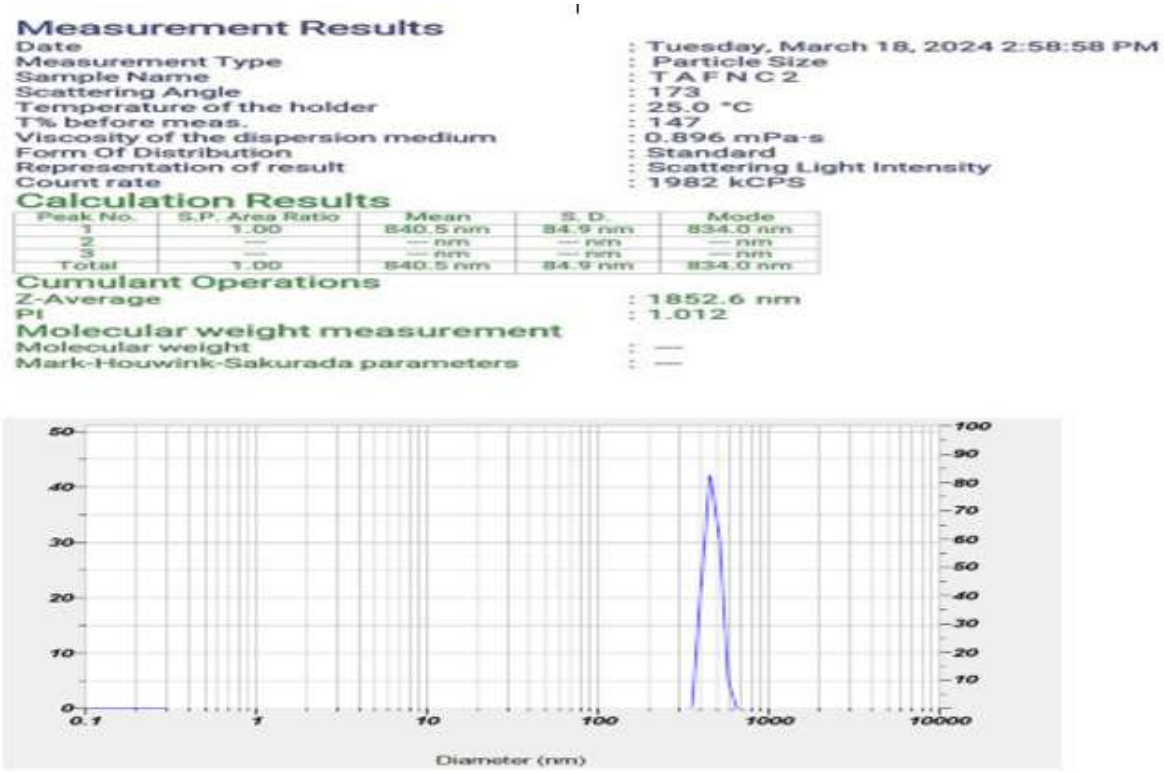
Figure 2: Particle size of TAF NC 2.



Measurement Results

Date	: Tuesday, March 18, 2024 2:58:58 PM
Measurement Type	: Particle Size
Sample Name	: TAF NC 2
Scattering Angle	: 173
Temperature of the holder	: 25.0 °C
T% before meas.	: 147
Viscosity of the dispersion medium	: 0.896 mPa·s
Form Of Distribution	: Standard
Representation of result	: Scattering Light Intensity
Count rate	: 1982 kCPS

Calculation Results

Peak No.	S.P. Area Ratio	Mean	S. D.	Mode
1	1.00	840.5 nm	84.9 nm	834.0 nm
2	—	— nm	— nm	— nm
3	—	— nm	— nm	— nm
Total	1.00	840.5 nm	84.9 nm	834.0 nm

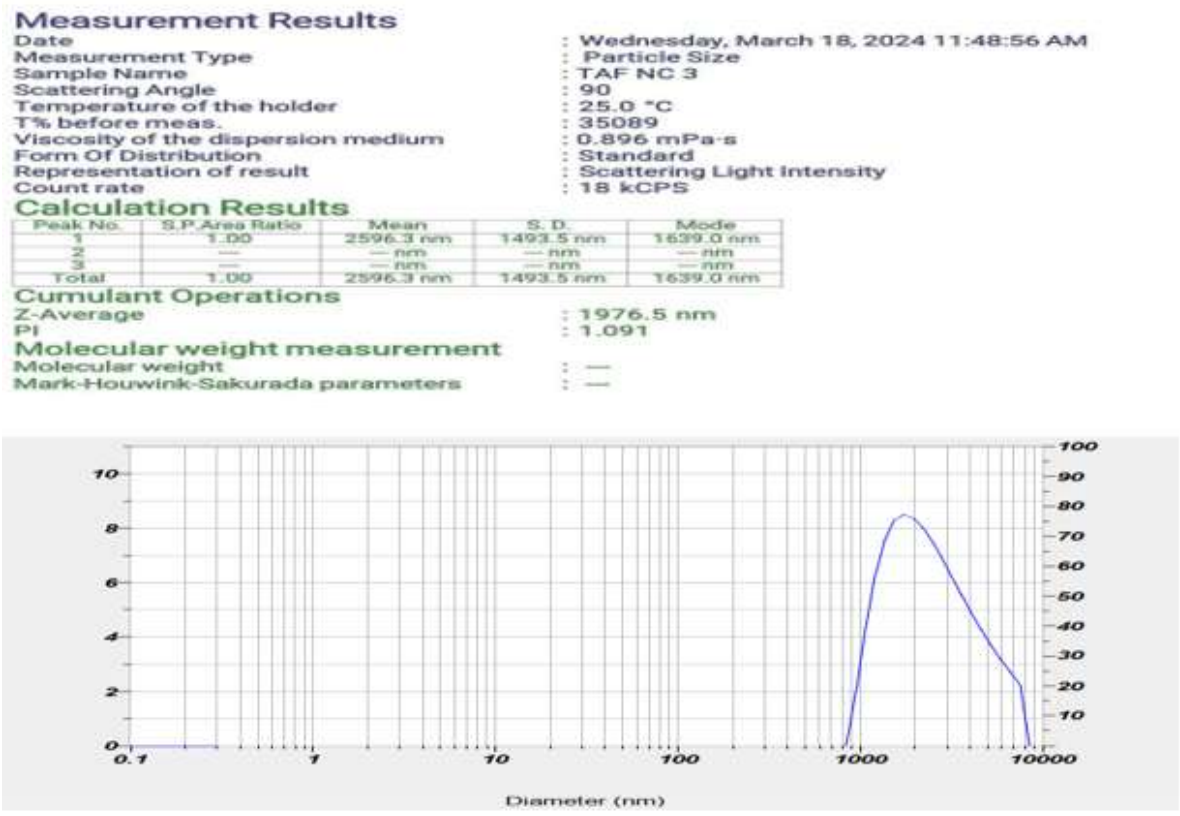
Cumulant Operations

Z-Average	: 1852.6 nm
PI	: 1.012

Molecular weight measurement

Molecular weight	: —
Mark-Houwink-Sakurada parameters	: —

Figure 3: Particle size of TAF NC 3.



Measurement Results

Date	: Wednesday, March 18, 2024 11:48:56 AM
Measurement Type	: Particle Size
Sample Name	: TAF NC 3
Scattering Angle	: 90
Temperature of the holder	: 25.0 °C
T% before meas.	: 35089
Viscosity of the dispersion medium	: 0.896 mPa·s
Form Of Distribution	: Standard
Representation of result	: Scattering Light Intensity
Count rate	: 18 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	2596.3 nm	1493.5 nm	1639.0 nm
2	—	— nm	— nm	— nm
3	—	— nm	— nm	— nm
Total	1.00	2596.3 nm	1493.5 nm	1639.0 nm

Cumulant Operations

Z-Average	: 1976.5 nm
PI	: 1.091

Molecular weight measurement

Molecular weight	: —
Mark-Houwink-Sakurada parameters	: —

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Figure 4: Particle size of TAF NC 4.

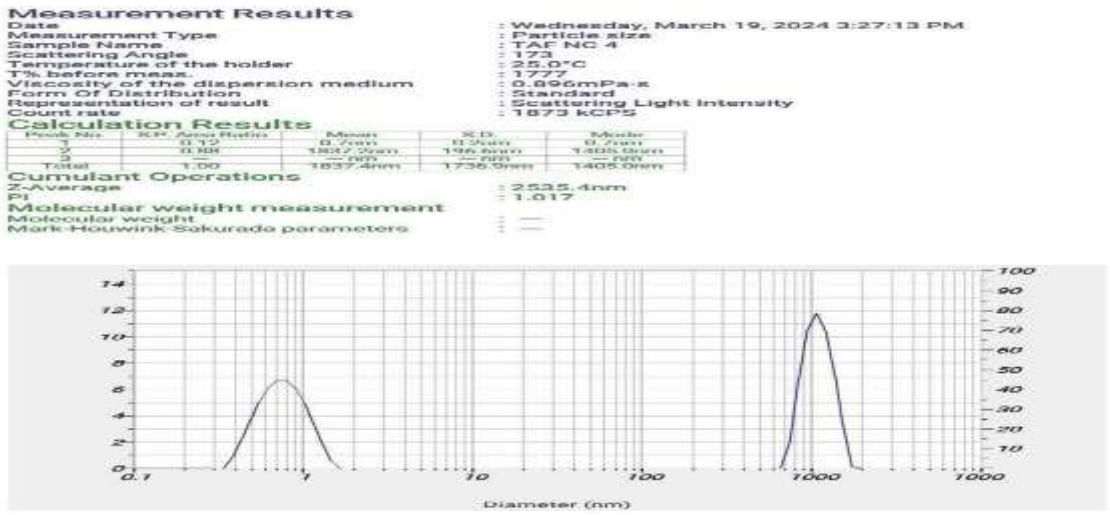


Figure 5: Particle size of TAF NC 5.

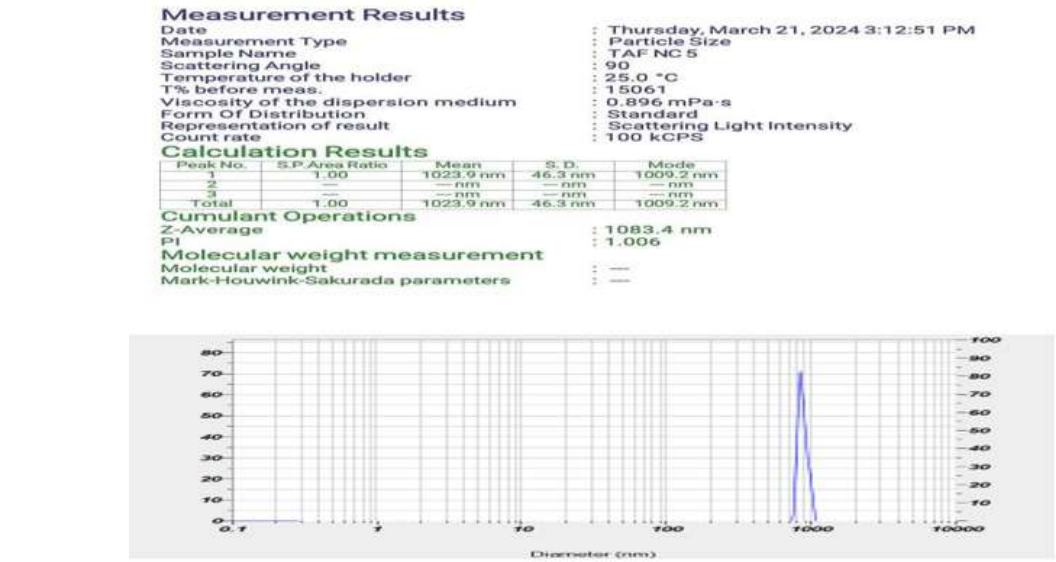
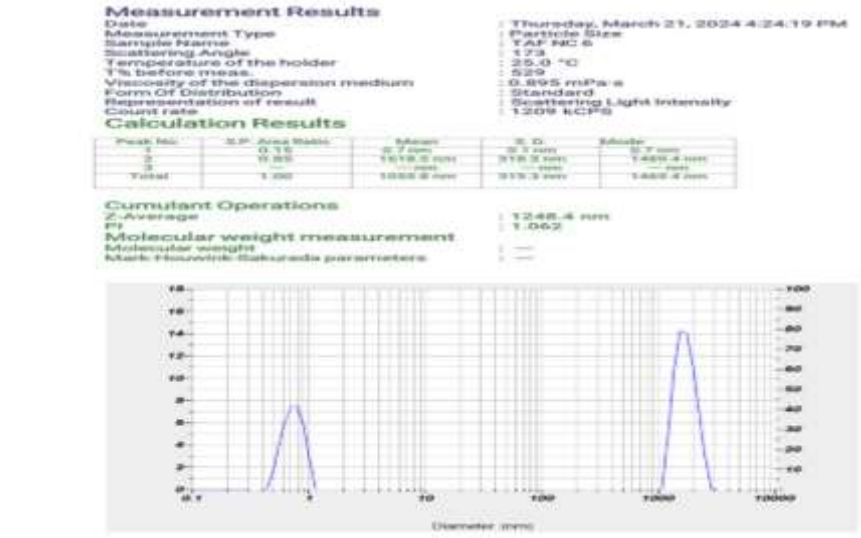


Figure 6: Particle size of TAF NC 6.



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Percentage yield of nanocapsules of TAF

Percentage yield of prepared nanocapsules TAF NC 1 to TAF NC 6 are shown in Table 4. Percentage yield for TAF NC 2, TAF NC 5 and TAF NC 6 has the good yield when compared with other formulations.

Table 4: Percentage yield of nanocapsules of TAF

S.NO	Formulation	% yield
1	TAF NC 1	85.2%
2	TAF NC 2	92.3%
3	TAF NC 3	87.6%
4	TAF NC 4	83.4%
5	TAF NC 5	95.8%
6	TAF NC 6	94.1%

Entrapment efficiency

The percentage entrapment efficiency of the nanocapsules of tenofovir alafenamide fumarate is given in the below Table 5. Data in the above table shows that TAF NC 5 and TAF NC 6 has highest entrapment comparing to others and TAF NC 3 has lowest entrapment

Table 5: Entrapment efficiency data of TAF nanocapsules

S.NO	Formulation	% entrapment
1	**TAF NC 1**	**90.2%**
2	**TAF NC 2**	**92.1%**
3	**TAF NC 3**	**82.3%**
4	**TAF NC 4**	**90.1%**
5	**TAF NC 5**	**97.24%**
6	**TAF NC 6**	**96.5%**

Flow property of TAF nanocapsules -Angle of repose

Angles of repose for prepared nanocapsules of TAF shown in Table 6 are in range of $11^0 \pm 0.81$ to$19^0 \pm 0.41$ indicates the free flowing characteristics of all formulations.

Table 6: The angle of repose data of TAF nanocapsules

S.NO	Formulation	Angle of repose
1	TAF NC 1	$12^0 \pm 0.45$
2	TAF NC 2	$11^0 \pm 0.81$
3	TAF NC 3	$19^0 \pm 0.41$
4	TAF NC 4	$16^0 \pm 0.65$
5	TAF NC 5	$15^0 \pm 0.24$
6	TAF NC 6	$13^0 \pm 0.57$

Protein binding studies

Protein binding of pure drug tenofovir alafenamide fumarate:

The data protein binding of values of pure TAF is shown in the Table 7 and it is evident that the pure drug tenofovir alafenamide fumarate shows protein binding up to 73.5% in duration of 60 mins.

Protein binding evaluation was carried out for TAF NC 5 and TAF NC 6 as these two has highest values of encapsulation efficiency and Percent yield values (Table 5 and 6).

The protein binding data in Table 8 of TAF NC 5 is up to 16.0% in duration of 60 min. indicating very reasonably reduced binding of the drug upon conversion to nanocapsules. Similarly TAF NC 6 shows a reduction in protein binding up to 22.4.0% in duration of 60 (Table 10)mins when compared with the pure drug. Therefore it is considered that the conversion of TAF into poloxamer coated nanocapsules is proved to reduce protein binding in bloddd circulation to enhance bioavailability of the drugs.

Table 7: Protein binding of pure drug

S.NO	TIME (min)	Absorbance (nm)	Concentration	Drug unbound	Drug bound	% of drug bound
1	10	0.154	5.1	0.096	0.371	79.4%
2	20	0.162	5.4	0.101	0.361	78.2%
3	30	0.176	5.8	0.109	0.357	76.4%
4	40	0.181	6.0	0.112	0.354	75.8%
5	50	0.192	6.4	0.119	0.347	74.3%
6	60	0.198	6.6	0.123	0.343	73.5%

Table 8: Protein binding values of TAF NC 5

S.NO	TIME (min)	Absorbance (nm)	Concentration	Drug unbound	Drug bound	% of drug bound
1	10	0.467	15.5	0.291	0.175	37.6%
2	20	0.502	16.7	0.313	0.153	32.9%
3	30	0.577	19.2	0.359	0.107	22.9%
4	40	0.586	19.5	0.365	0.101	21.7%
5	50	0.603	20.1	0.376	0.090	19.4%
6	60	0.629	20.9	0.392	0.074	16.0%

Table 9: Protein binding values of TAF NC 6

S.NO	TIME (min)	Absorbance (nm)	Concentration	Drug unbound	Drug bound	% of drug bound
1	10	0.442	14.7	0.275	0.191	40.97%
2	20	0.524	17.4	0.326	0.140	30.05%
3	30	0.536	17.8	0.334	0.132	28.45%
4	40	0.549	18.3	0.342	0.124	26.65%
5	50	0.563	18.7	0.351	0.115	24.85%
6	60	0.582	19.4	0.362	0.104	22.24%

Drug excipient interaction studies

FT-IR spectrum of pure drug tenofovir alafenamide fumarate and nanocapsules of TAF NC 5 are given in Fig 10. And their absorption peak valus are shown in Table 10. The pur drug Tenofovir alafenamide fumarate showed its absorption peaks at 1741.15 cm^{-1} due to C=O stretch, 1248.93cm^{-1} due to C-N stretch ,1146.68 cm^{-1}due to P=O and P-O stretch, 1598.28 cm^{-1}due to C=C stretch, C-H bending at 1471.71cm^{-1}, All these peaks are existing even in IR spectrum of nanocapsules TAF NC 5 with slight variation. The presence of new peaks at 1151.50 is the C-O-C absorption peak of Poloxamer. Hence it is considered that there is no interaction between the TAF and the polymer used for preparing nanocapsules.

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Figure 7: FTIR pattern for pure drug

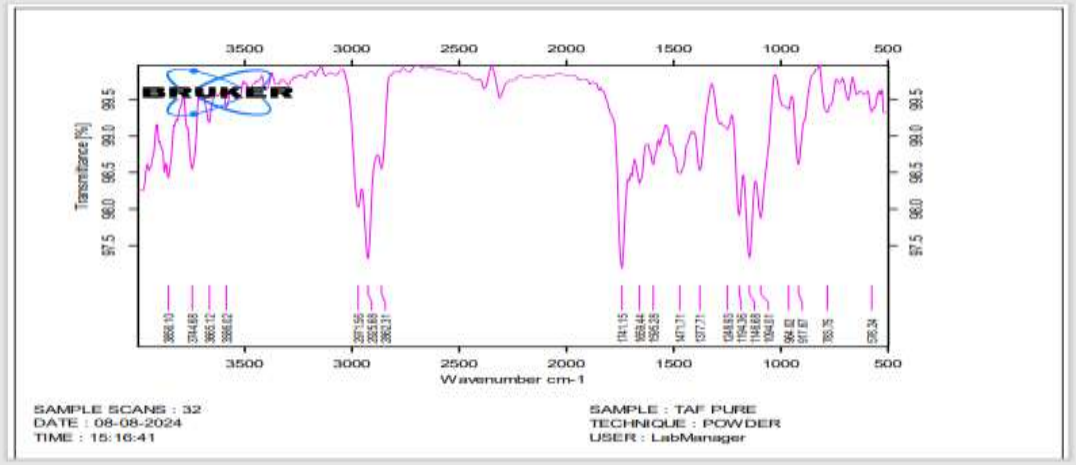

Figure 8: FTIR spectrum for TAF nanocapsules

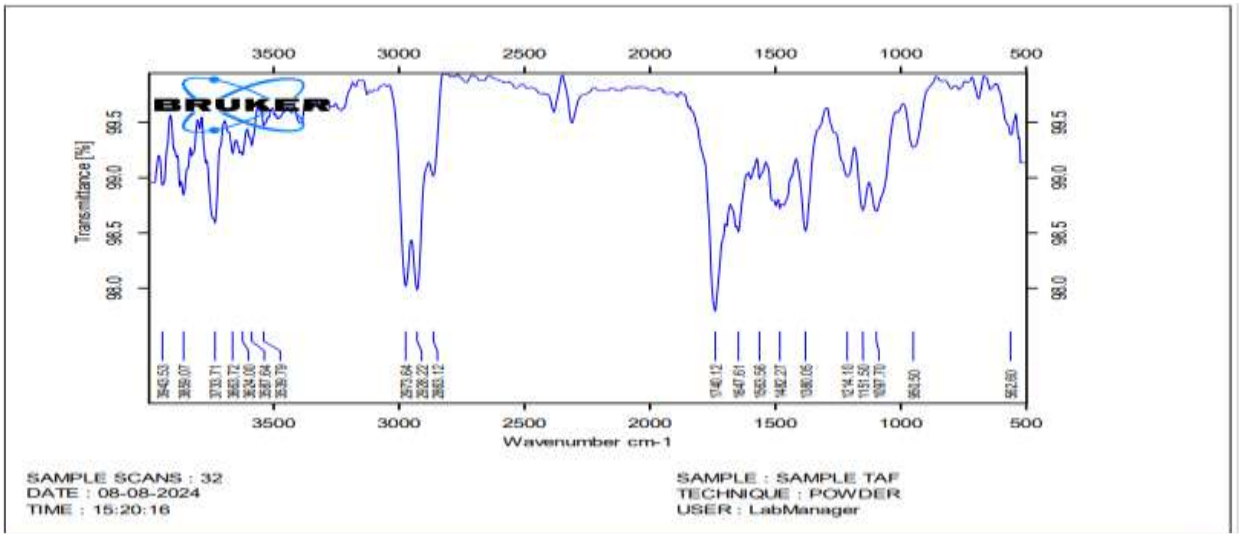

Table 10: FTIR absorption ranges for TAF nanocapsules

S. NO	Functional groups	Absorption peaks of TAF	Absorption peaks of TAF nanocapsules
1	C=O	The 1741.15	1740.12
2	C-N	1248.93	1214.10
3	C-H	2925.68	2928.22
4	C=C	1598.28	1563.56
5	C-H	1471.71	1482.27
6	C-O-C	-	1151.50

CONCLUSION

Poloxamer coated tenofovir alafenamide fumarate nanocapsules containing 25 mg of tenofovir alafenamide fumarate can be produced successfully by coacervation phase technique to possess the ideal nano

particle size range with highly reduced percent protein bound values to act promising product for enhanced bioavailability of tenofovir alafenamide fumarate.

Scope of the study

The present work can be extended for production of injection of presently prepared nanocapsules of TAF using all the precautions and production techniques required for production of long acting injection formulations. Since the drug is generally prescribed for long dosage regimens.

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