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Synthesis of Polymer Conjugates of Efavirenz, Tenofovir and Dolutegravir

Johnson O. Ogira[1,2*], P.O. Odumosu[2] and J. O. Onah[1,2]

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Science, Bingham University, Karu, Nasarawa State Nigeria.
[2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Science, University, of Jos, Jos. Plateau State, Nigeria.

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**Corresponding Author:*
Johnson. O Ogira.
Tel: +2347086242071
Email: oguche.ogira@binghamuni.edu.ng, ogirajohnson@gmail.com.

ABSTRACT

Background and purpose: Chitosan and alginate are examples of biodegradable polymers which have the advantage of controlled drug delivery, prevent overdose and toxicity. The aim of the study is to synthesize the three anti-retroviral drug-polymer conjugates of chitosan and alginate.
Method: The synthetic pathways involved the synthesis of three different conjugates labelled as, A, B and C through a Schiff process reaction, Diazotization reaction and esterification reaction, respectively. After synthesis, the products were purified by recrystallisation and preparative TLC. The calibration curve data and IR analyses were conducted.
Result: The synthetic yield for the drug conjugates was 85%, 75% and 82% for efavirenz-chitosan (A) Tenofovir-chitosan (B) and dolutegravir-alginate (C), respectively. The drug conjugates obeyed Beer–Lambert's law and showed linear response in the concentration range of 5–40 µg/mL with a correlation coefficient (R^2) of 0.999 for all three drug-polymer conjugates. The IR spectra of conjugate A with the functional group (C=N) appeared at 1997.96 cm^{-1}, B Conjugate with functional group (N=N) appeared at 2303.5 cm^{-1} and C conjugate with functional group (COO-) appeared at 1736.3 cm^{-1}, respectively.
Conclusion: The Synthesis of Polymer Conjugates of Efavirenz, Tenofovir and Dolutegravir was achieved through different synthetic pathways and monitored by Thin-layer chromatography, melting point and UV and then confirmed by Fourier Transform infra-red (FTRI) spectroscopy.

1.0 Introduction

1.1 Biodegradable polymers

Natural biodegradable polymers are called biopolymers. Polysaccharides such as starch and cellulose represent the most characteristic family of these natural polymers. Other natural polymers like proteins can be used to produce biodegradable materials. These are the two main renewable sources of biopolymers. The polymers employed in this study are Chitosan and alginate.

1.1.1 Chitosan

Chitosan and derivatives are continually receiving a great deal of interest as regards medical and pharmaceutical application because they have interesting properties that make them suitable for use in the biomedical field, such as biocompatibility, bio-degradability and non-toxicity. Chitosan has excellent bioavailability, biodegradability, and adsorption properties, as well as nontoxicity. These properties make it very useful in various types of applications in multiple fields like wound treatment, drug carrier, food packaging, dietary supplement, chelating agent, pharmaceutical and biomaterial purposes, etc. [1.] Chitin and chitosan can be extracted from the exoskeleton of crustaceans by chemical and biological methods [2,3.] The biological process of chitin production is more

environmentally friendly, cost-effective (depending on the microorganism used) with excellent decalcification efficacy (up to 86%), and high viscosity than the chemical method. The natural way also decreases the residual proteins in the shrimp shell. Moreover, studies have confirmed that biological processes of exoskeleton extraction of crustaceans produce chitosan with quality higher than the chemical method [4]. The Food and Drug Administration (FDA) of USA has approved chitosan as a feed additive in 1983. Chitosan is now widely applied in functional food, environmental protection, and biotechnology [5].

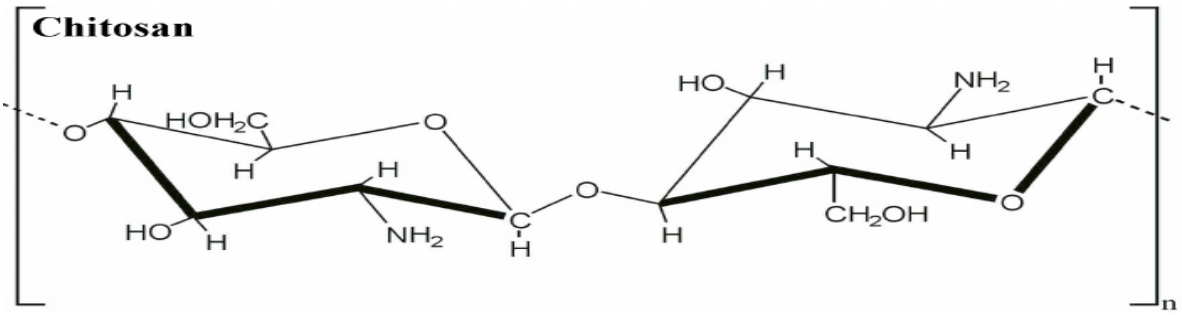


Fig. 1 Structure of Chitosan

1.1.2 Alginate

Alginates are mainly the scaffolding polysaccharides, extracted from brown seaweeds and alginate in the commercial form are extracted from the different species such as *Laminaria, Macrocystis*, *Sargassum*, *Ascophyllum*, *Lessonia*, *Eclonia* and *Durvillea* [6,7]. Alginate is the most abundant biopolymer available in the whole world and marine environment after cellulose [8,9]. It consists of (1–4)-bonded β-d-mannuronic acid with the blocks of α-l-guluronic acid [10,11]. The salt of Ca, Na and Ba alginate has the gelling capability because of the presence of divalent cations, highly viscous in the aqueous medium and hence extensively used in different biomedical fields mainly for cell transplantation, tissue regeneration and drug delivery. Now the most evolutionary concept of alginate for the biological systems is to treat, supplement of any organ, tissue or any part of the human body [12]. Alginate is also commonly used as a good therapeutic material for pain relieving along with anti-inflammatory and antibacterial activity. Alginate hydrogels are specifically attractive in drug delivery, wound healing and application in tissue engineering because alginate gel retain structural similarity to the extracellular matrices in tissues [13,14].

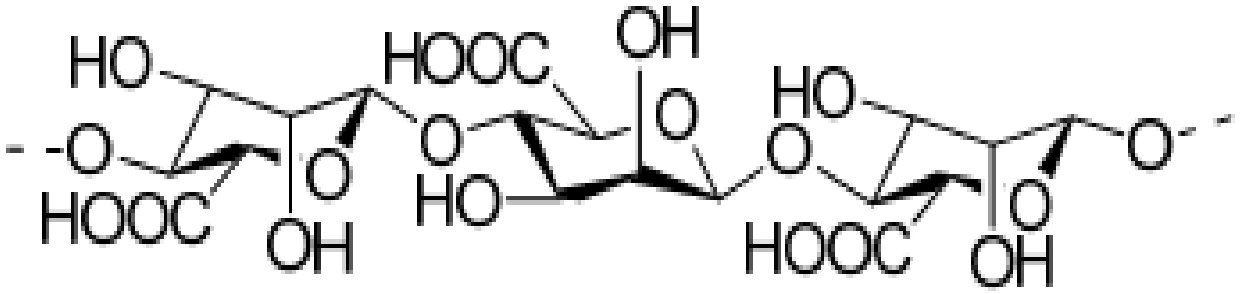


Fig. 2 Structure of Alginate

1.2 Antiretrovirals

1.2.1 Dolutegravir: Dolutegravir inhibits HIV integrase by binding to the integrase active site and blocking the strand transfer step of retroviral Deoxyribonucleic acid (DNA) integration which is essential for the HIV replication cycle.

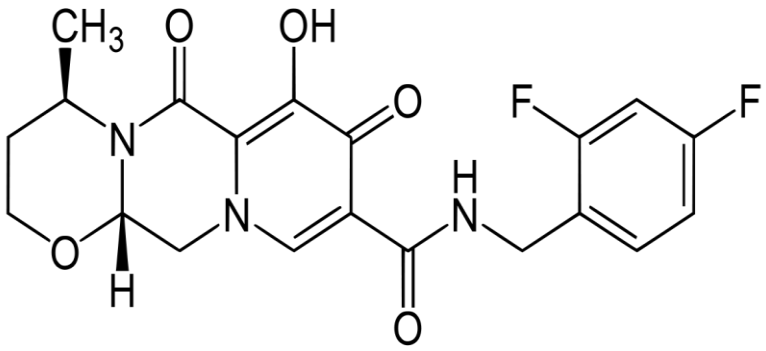


Fig. 3 Structure of Dolutegravir

1.2.2 Tenofovir: It belongs to a class of antiretroviral drugs known as nucleotide analog reverse transcriptase inhibitors (NtRTIs), which block reverse transcriptase, an enzyme necessary for viral production in HIV-infected individuals. This enables the management of HIV viral load through decreased viral replication. Tenofovir disoproxil fumarate is a prodrug that is absorbed and converted to its active form, *tenofovir*, a nucleoside monophosphate (nucleotide) analog and further converted to the active metabolite, *tenofovir diphosphate which is* a chain terminator.

Tenofovir diphosphate inhibits HIV-1 reverse transcriptase and the Hepatitis B polymerase by direct binding competition with the natural deoxyribonucleotide substrate (deoxyadenosine 5'-triphosphate) and, after integration into DNA, causes viral DNA chain termination.

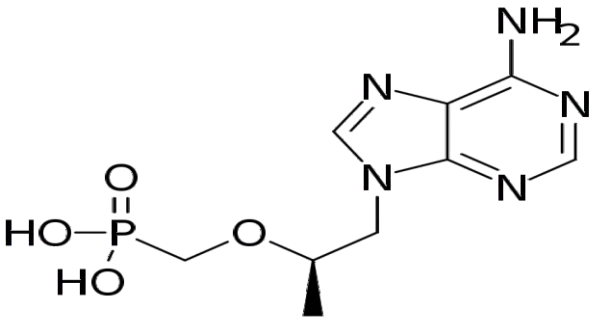
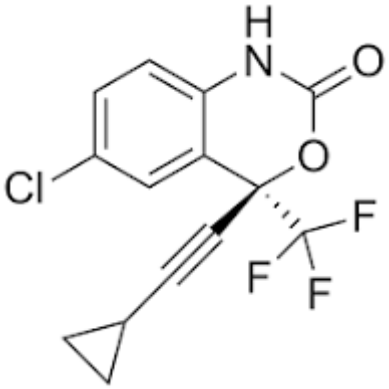


Fig. 4 Structure of Tenofovir

1.2.3 Efavirenz: It inhibits the activity of viral RNA-directed DNA polymerase. Antiviral activity of efavirenz is dependent on intracellular conversion to the active triphosphorylated form. The rate of efavirenz phosphorylation varies, depending on the cell type. It is believed that inhibition of reverse transcriptase interferes with the generation of DNA copies of viral RNA, which are necessary for synthesis of new virions. Even though human DNA polymerase is less susceptible to the pharmacologic effects of triphosphorylated efavirenz, this action may nevertheless account for some of the drug's toxicity.

Fig. 5 Structure of Efavirenz



Aim of the study: To synthesize and characterize the physicochemical properties of the three anti-retroviral drug-polymer conjugates,

2.0 MATERIALS AND METHOD

2.1 Materials

2.1.1 Reagents: Sulphuric acid (farm Italia, conloerba; Italy), chloroform (Iso Merck), glacial acetic acid (Guanghua Chemicals Factory Co. Ltd), sodium hydroxide, ethanol, and Acetic acid (Guanghua Chemicals Factory Co. Ltd) and distilled water, all reagents are of analytical grade and so were not purified further. Other reagents used include methanol (BDH Chemicals Ltd England), tetraoxosulphate VI acid (H_2SO_4, 90%, (BDH Chemicals Ltd England); ethyl acetate (Guanghua Chemicals Factory Co. Ltd), ammonia solution (Farm Italia, Conloerba, Italy), toluene (BDH Chemicals Ltd England), chloroform (Guanghua Chemicals Factory Co. Ltd), Sodium hydroxide (Sigma Aldrich Iceland) and sodium bicarbonate (Sigma Aldrich Iceland).

2.1.2 Drugs/Chemicals: Dolutegravir Tablet was kindly provided by Pharm. Bawo Michael of the Department of Pharmacy, Rivers State Teaching Hospital, Rivers State Nigeria. Tenofovir, Dolutegravir and Efavirenz were purchased from pharmacy stores in the city of Karu, Nasarawa State, and the active drug were extracted with ethanol as secondary standard and purified by chromatography. Alginate and Chitosan polymer were purchased from Sigma Aldrich, Germany.

2.2 METHODOLOGY

2.2.1 Synthesis of Conjugate

2.2.1.1 Synthesis A: Schiff process

Extracted Efavirenz of 0.1 mole was added to 0.2 mole of chitosan in a round bottom flask and triturated for 2 minutes to obtain a homogenous mixture. 100 ml of 0.1 M sulphuric acid was added into the mixture of efavirenz and chitosan then stirred vigorously for another two minutes; the pH of the mixture was maintained at 4-5 by monitoring the reaction with a pH meter for maximum yield. The mixture was introduced into the round bottom flask and mounted onto the reflux apparatus condenser set up with the heating mantle and made to reflux over three hours for proper condensation reaction to be completed. The end product was an imine which came off with an oily product. The product was washed with sodium bicarbonate to remove any excess sulphuric acid by neutralization reaction; the residue of the product was filtered and dried.

2.2.1.2 Synthesis B: Diazotization Reaction

Extracted Tenofovir of 0.5 M was weighed and added to 0.125 mole of chitosan in a round bottom flask and triturated for 2 minutes, then 100 ml of 0.1 M hydrochloric acid was added into the mixture of extracted Tenofovir and chitosan for protonation of the nitrite ion, after which it was stirred vigorously for another two minutes; the temperature was maintained at 0-5°C using ice pack during refluxing for maximum yield.

The mixture was introduced into the round bottom flask and mounted onto the reflux apparatus condenser set up with the heating mantle and made to reflux over three hours for proper condensation reaction to be completed. The end product was an azo compound which came off with a choking smell, and water. The product was washed with sodium bicarbonate to remove any excess HCl acid by neutralization reaction; the residue of the product was filtered and dried.

2.2.1.3 Synthesis C: Esterification Reaction

Extracted dolutegravir of 0.85 M was added to 0.1 mole of Alginate in a round bottom flask and triturated for 2 minutes to obtain a homogenous mixture. 100 ml of 0.1M sulphuric acid was added into the mixture of the extracted dolutegravir and alginate then stirred vigorously for another two minutes.

The mixture was introduced into the round bottom flask mounted over reflux apparatus condenser set up with the

heating mantle and made to reflux over three hours for proper condensation reaction to be completed. The end product was an ester which came off with a sweet smell, and water. The product was washed with sodium bicarbonate to remove any excess sulphuric acid by neutralization reaction; the residue of the product was filtered and dried. All the reaction pathways were monitored by TLC.

2.2.2 Melting Point Determination

Efavirenz-chitosan conjugate, Tenofovir-chitosan conjugate and Dolutegravir-alginate conjugates were packed into a capillary tube alongside with controls and placed in a melting point apparatus and set at temperature 25°C and the transition temperature noted.

2.2.3 Determination of Calibration and Validation Curve

Accurate weight of 0.2 mg each of Efavirenz, Tenofovir, Dolutegravir drugs alone and Efavirenz-chitosan, Tenofovir-chitosan and Dolutegravir-alginate conjugates were weighed and dissolved in glacial acetic acid. Serial dilution of the drugs was prepared with water ranging from 0.2 mg/ml to 0.00125 mg/ml concentration and the absorbance for the drugs alone and their conjugates are obtained and plots of absorbances against concentration were obtained.

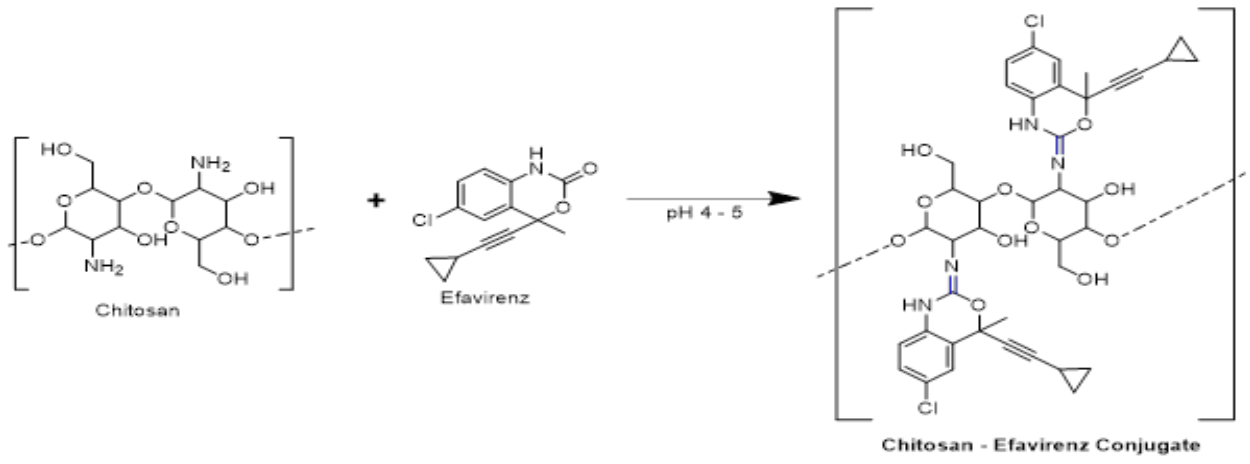
2.2.4 Infra-Red spectrometry

The infrared spectra [KBR] were recorded using FTIR spectrophotometer with attenuated total reflection technique for investigation. The drug samples (Efavirenz, Tenofovir, and Dolutegravir), polymer (chitosan and alginate) and conjugates (Efavirenz-chitosan, Tenofovir-chitosan and Dolutegravir-alginate) film were taken and infrared data were collected between 600 – 4000 cm^{-1}.

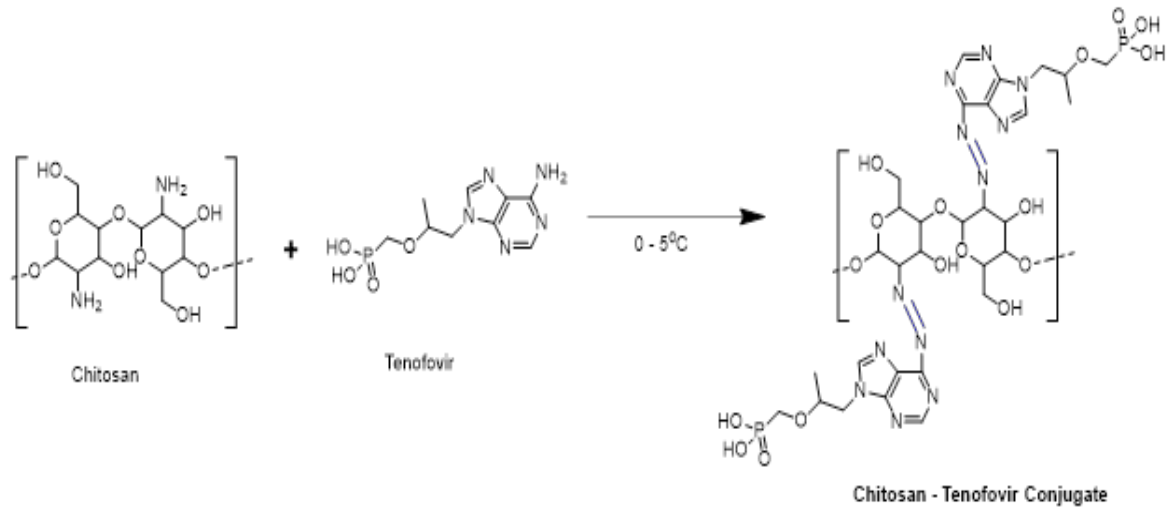
RESULT

3.1 Synthesis

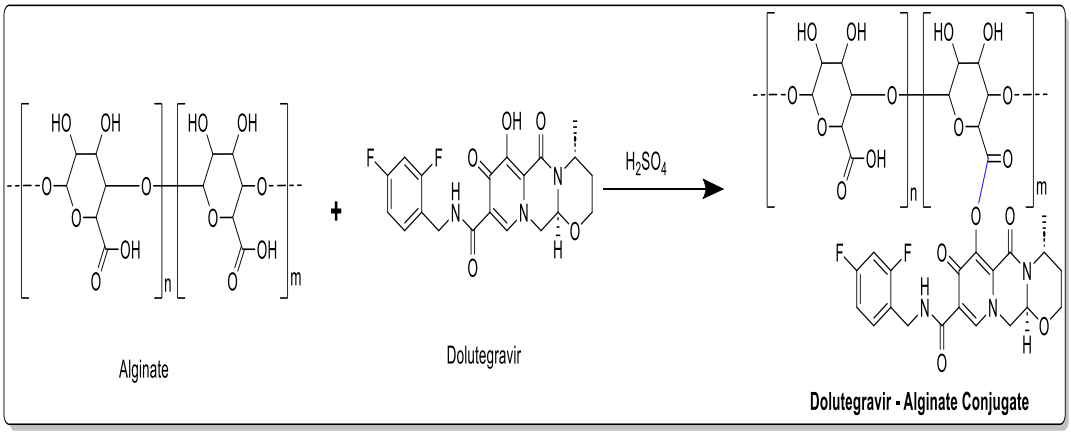
The reaction between the carbonyl (C=O) functional group of the efavirenz and hydrolysis primary amine group of chitosan is a Schiff reaction that has been established. Synthesis was successful and valid since there is good percentage yield. Equation for the reaction is illustrated in scheme I, relatively low molarity of H_2SO_4 was used for the reaction to avoid destruction of ether bonds present in Chitosan with the pH maintained at 4-5. In the reaction between chitosan and tenofovir, the amines group of both the reactants are targeted through the process of diazotization reaction at a regulated temperature of 0-5°C. The reaction between dolutegravir and Alginate is an esterification where the carboxylic acid functional groups of dolutegravir were made to react with the hydroxyl groups of the Alginate producing an ester through the process of esterification reaction. Percentage yield of Efavirenz-chitosan (85%), Tenofovir-chitosan (75%), Dolutegravir-Alginate (82%), gives a good yield and shows complete reaction. The equation of the reaction between Efavirenz-chitosan, Tenofovir-chitosan and Dolutegravir-Alginate is as follows:



Scheme 1: Schiff synthetic pathway of Efavirenz-chitosan conjugate.



Scheme 2: Diazotization Synthetic pathway of Tenofovir-chitosan conjugate



Scheme 3: Esterification pathway of Dolutegravir-alginate conjugate.

3.2 Physical Properties

3.2.1 Melting Points in Degrees of Temperature (°C)

The melting point of the different conjugates varies depending the size of the polymers involved

Table 1: Melting Point of Drugs alone and Polymers Conjugate

Drugs, Polymer and Drug Conjugates	Melting Point (°C)
Efarvirenz	**134 -136**
Tenofovir	**117 -121**
Dolutegravir	**190 -193**
Chitosan	**102 -105**
Alginate	**229 -234**
Efarvirenz -Chitosan	**121 -125**
Tenofovir -Chitosan	**109 -115**
Dolutegravir -Alginate	**221 -228**

3.2.2 UV Reading

From the beer Lambert plot, the entire graphs obeyed Beer-Lambert's law since they all passed through the origin at absorption maximum at 348 nm (Figures 1a, 1c and 1e). The regression analyses (R^2) obtained for Tenofovir-Chitosan, Efavirenz-Chitosan and Dolutegravir were0.9998, 0.9999 and 0.99989, respectively (Figures 1b, 1d and 1f).

The beer Lambert equation and regression coefficient are as follows:

Y = mx+c.

Tenofovir-Chitosan: $y = 0.9923x + 0$; R^2=0.9998

Efavirenz-Chitosan: $y = 0.9916x + 0$; R^2=0.9999

Dolutegravir-Alginate: $y = 0.9988x + 0$; R^2=0.99989

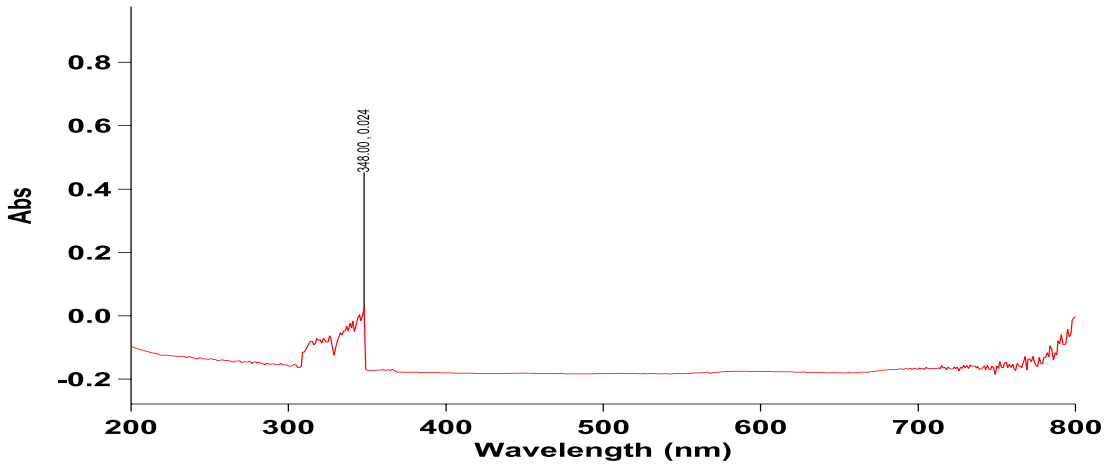


Fig. 6a Absorption maximum for Efavirenz-Chitosan at 348 nm

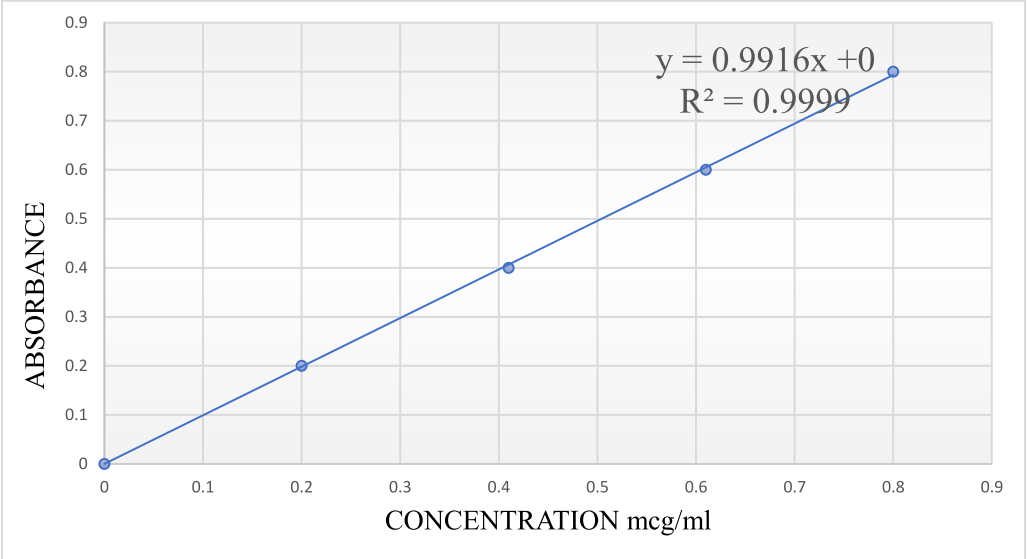


Fig. 6b Calibration Curve for Efavirenz-Chitosan

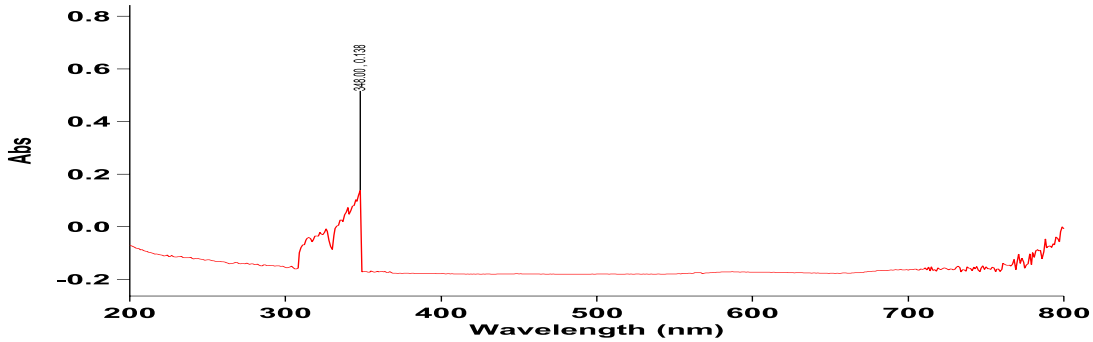


Figure 6c. Absorption maximum for Tenofovir-Chitosan at 348 nm

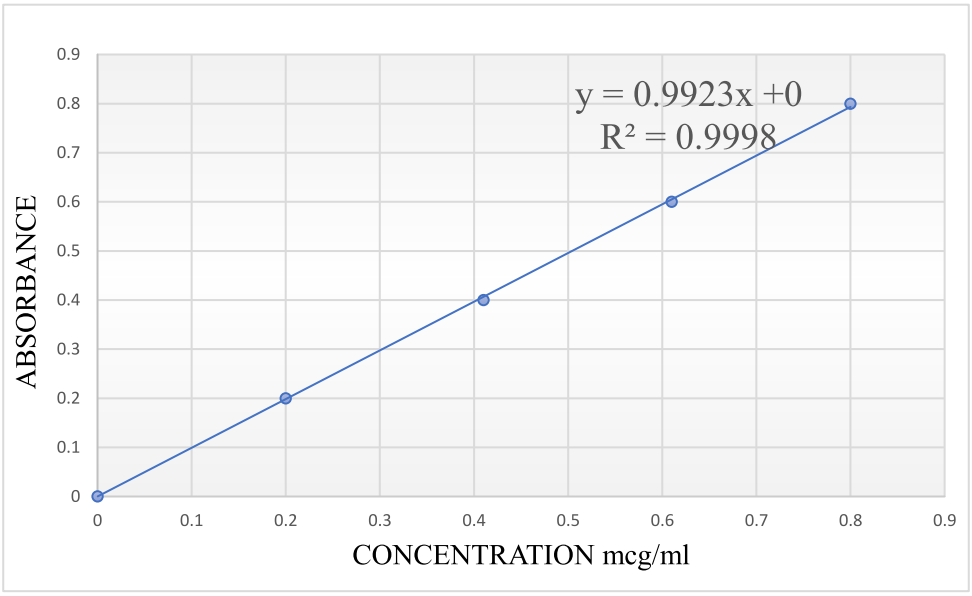


Figure 6d. Calibration Curve for Tenofovir-Chitosan

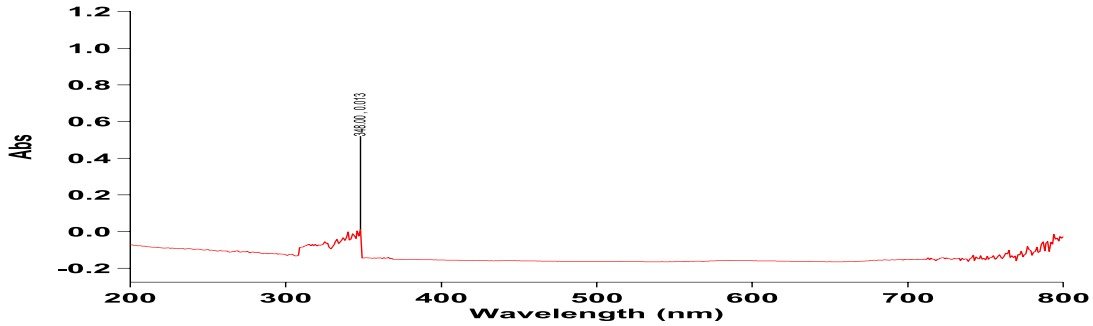


Fig. 6e Absorption maximum for Dolutegravir-Alginate at 348 nm

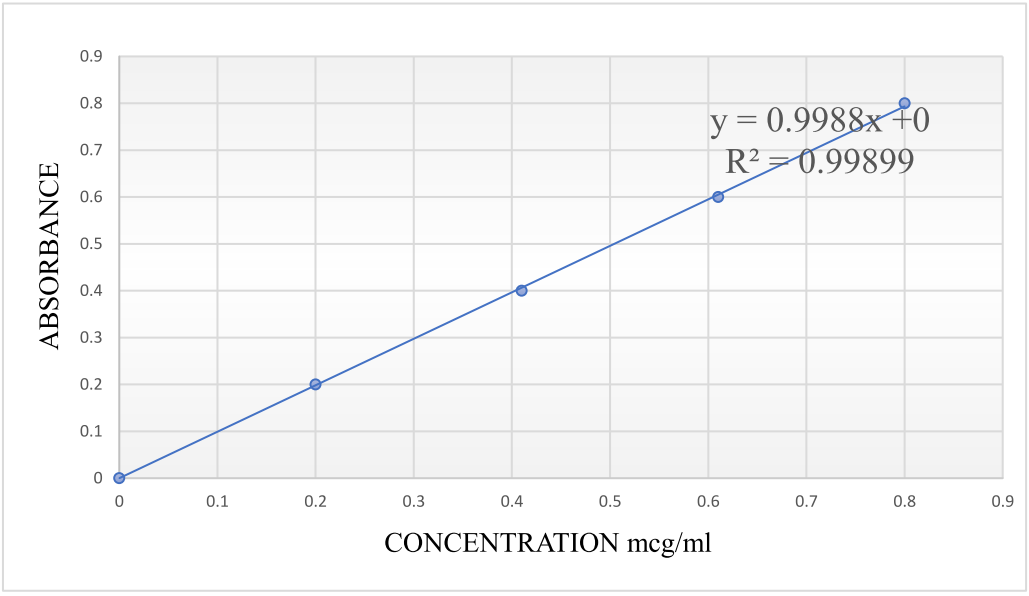


Fig. 6f. Calibration Curve for Dolutegravir-Alginate.

3. 4 IR SPECTRA AND INTERPRETATIONS OF THE CONJUGATES

The IR Spectra of the three conjugates were carried out, with the spectra of the polymers alone and conjugates conducted separately. The disappearance of the functional groups involved in the conjugation from the polymers and appearance of new function groups in the conjugates confirm the formation of the drug-conjugates Figs. 2a, 2b and 2c.

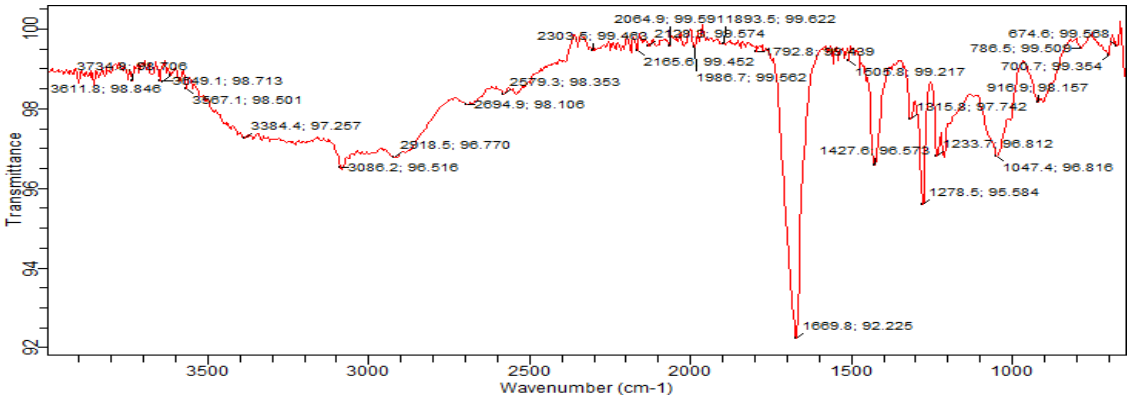


Figure 7a. IR Spectra of Tenofovir-Chitosan conjugate

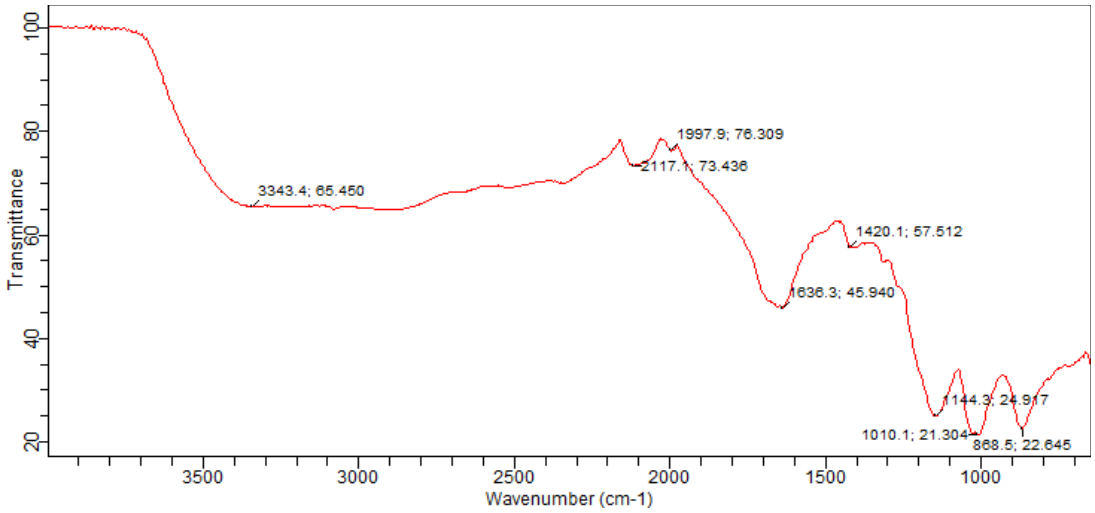


Figure 7b. IR Spectra of Efavirenz-Chitosan conjugate

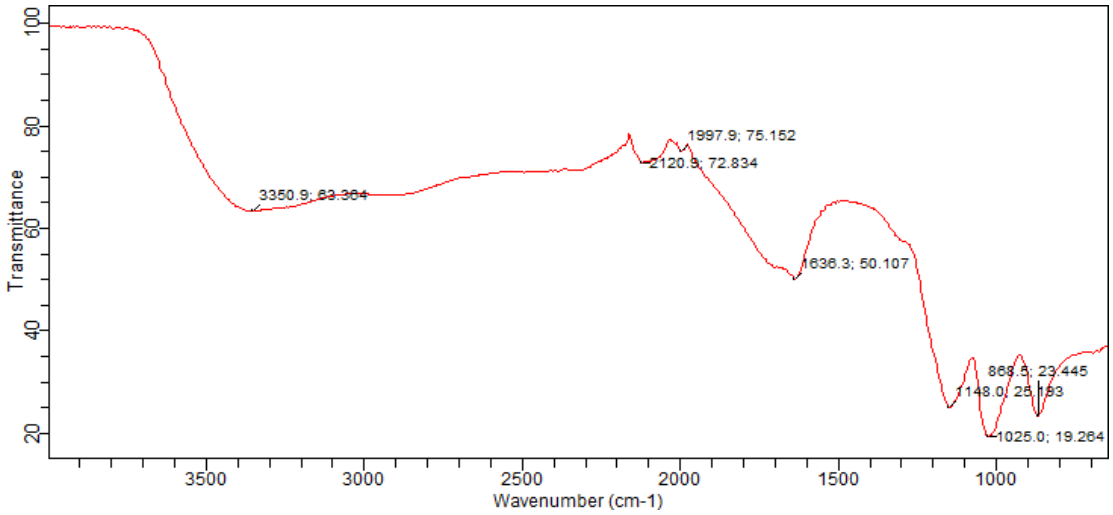


Figure 7c. IR Spectra of Dolutegravir-Alginate

4.0 Discussion

4.1 Melting Points of drugs and conjugates

The variations in the melting point of the drug conjugate confirms that the conjugates were actually formed suggesting that the increase in size of the polymer is responsible for the lower melting point. The melting point of Efavirenz is 134-136°C and chitosan is 102-105°C, while the melting point of the conjugate (Efavirenz-chitosan) is 121-125°C signifying a reduction from 134-136°C (melting point of Efavirenz alone) and increase in the melting point of chitosan from 102-105°C. This explanation applies to Tenofovir-Chitosan, and Dolutegravir-Alginate. The melting points are shown in the Table 1.

4.2 UV Reading

The absorption maximum for Tenofovir-Chitosan at 348 nm differs from the drug alone (Tenofovir at 260 nm) due to the formation of hydrazine (N=N) which caused a bathochromic shift from the wave length of 260 nm to 348 nm (Figure 6c). The absorption maximum for Efavirenz-Chitosan is at 348 nm which also differs from the drug alone (Efavirenz) which absorbed maximally at wavelength of 246nm causing a bathochromic shift from 246 nm to 348 nm due to the formation of an imine (N=C) group (Fig 6a).

The absorption maximum for Dolutegravir-Alginate at 350 nm which differs from the drug alone (Dolutegravir) which absorbs at 258 nm. The bathochromic shift from 258 nm to 350 nm is due to the formation of an ester ($RCOOR^1$) functional group (Figure 6e). From the beer Lambert plot, the entire graph obeys the Beer-Lambert's law because all passed through the origin. The regression analyses obtained are good for Tenofovir-Chitosan, Efavirenz-Chitosan and Dolutegravir alginate (R^2 = 0.9998, 0.9999 and 0.99989 respectively) (Figures 6b,6d and 6f).

4.3 I R SPECTROSCOPY OF THE CONJUGATES

For chitosan spectrum alone: A weak peak at 3671.419 cm^{-1} is that of free O – H bond stretch of glucopyranose units; 3356.88 cm^{-1} (which is assigned to the N – H and hydrogen bonded O – H stretch vibration frequencies); 2870.19 cm^{-1} (C – H stretch), 2195.496 cm^{-1} (C – N asymmetric band stretching), 1651.295 cm^{-1} (amide II band N-H stretch) 1319.594 cm^{-1} (asymmetric C-H Bending of CH_2 group); and 1056.0839 (skeletal vibration involving the bridge C-O stretch) of glucosamine residue. Tenofovir-chitosan conjugate spectra appear at 2303.5 (medium and strong). a new peak corresponding to diazonium salt N=N; 2918.5 (wide and stretch) assigned to C – H stretch from alkyl group C-H; 3384.4 (wide and stretch) (broad and very weak) intermolecular and weaken bonded O – H; and 3080.2 (sharp) assigned to phenyl group Ar-CH (Figure 7a)**.** For efavirenz-chitosan spectra, peaks appear at 1997.96 cm^{-1} (medium and sharp) which is assigned to C=N a new peak corresponding to Imine functional group, 3343.4 (broad and strong) assigned to hydroxyl group O-H; 1636.3 cm^{-1} (broad) assigned to unsaturated carbon-carbon double bond C=C and 1420 (medium and wide) Aromatic amines (Figure 7b).

For alginate alone, a peak at 3257.79 cm^{-1} (medium and sharp) hydroxyl group O-H, 2922.2 cm^{-1} (medium and sharp) assigned to stretch C-H and 1794.4 cm^{-1} (sharp) C=O (from carboxylic acid). For dolutegravir alone, 3242.8 cm^{-1} (broad) is assigned to hydroxyl group O-H (from alcohol), 2937.1 cm^{-1} (sharp) correspond to C-H; and 1654.9 cm^{-1} (sharp) assigned to C=O (from amide). For dolutegravir-alginate conjugate a peak at 1636.3 cm^{-1} (broad and wide) is assigned to C=O (from ester); new peak corresponding to carbonyl functional group from the Ester formed**;** 1148.0 cm^{-1} (sharp) assigned to Ar-F (Figures 7a, 7b and 7c).

5.0 Conclusion

The Synthesis of Polymer Conjugates of Efavirenz, Tenofovir and Dolutegravir was achieved through different synthetic pathways like the Schiff process, diazotization reaction and esterification reaction which was monitored by thin layer chromatography, melting point and UV spectroscopy and finally confirmed by Fourier Transform infra-red (FIT); spectroscopy.

Recommendation: The research work here majorly is synthesis of the conjugates of the three antiretroviral drugs (Efavirenz, Tenofovir and Dolutegravir), monitoring and confirmation. We recommend physicochemical characterisation and biological screening to be carried on the synthesised products.

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