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PHYSICOCHEMICAL CHARACTERISATION OF POLYMER CONJUGATES OF EFAVIRENZ-CHITOSAN, TENOFOVIR-CHITOSAN AND DOLUTEGRAVIR-ALGINATE

Ogira Johnson Ogwuche*[1,2], Onah Johnson O.[1,2], Odumosu Patricia O.[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.

***Corresponding Author: Ogira Johnson Ogwuche**

Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Science, Bingham University, Karu Nasarawa State Nigeria.

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ABSTRACT

Background and purpose: Chitosan nanofibrils are promising scaffold materials for biomedical applications while alginate is mixed with other polymers to improve the biological properties as they are good for molding with a suitable biological nature. The aim of the study is to characterise the physicochemical properties of the three anti-retroviral drug-polymer conjugates of chitosan and alginate. **Method:** Conjugates of Efavirenz, Tenofovir and Dolutegravir were analysed by determining the stability profile using Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD) and Buffer hydrolysis. **Result:** The DSC Profile of Efavirenz-chitosan showed an endothermic peak at 138°c indicating conjugation and retention of the integrity of the polymer while that of Tenofovir-chitosan and Dolutegravir-Alginate showed endothermic peaks at 180°c and 258.12°c respectively. Buffer hydrolysis studies gave a drug release rate at half-lives of 4.1 hr, 5.1 hr and 7.5 h respectively and XRD results indicated the Stability of the polymer when conjugated with the drug with broad and sharp peak at 2θ = 22.5°, 20° &24° and 19.5°&23.5° for Efavirenz-chitosan, Tenofovir-chitosan and Dolutegravir-Alginate respectively. **Conclusion:** The three antiretroviral drugs were successfully conjugated to the polymers and the stability profile showed that the polymers were intact after conjugation.

KEYWORDS: Efavirenz, Tenofovir, Dolutegravir, Chitosan, Alginate, DSC, XRD and Buffer hydrolysis.

INTRODUCTION

Chitosan is a derivative of chitin obtained by deacetylation of chitin found in the shells of crustaceans and insects, cell walls in fungi, and among other sources.[1] Native chitin nanofibers are highly crystalline but undergo deacetylation by hot alkali treatment to yield soft chitosan networks with low crystallinity.[2,3] The accessibility of reactive surface-exposed amine groups of chitosan makes them easy to functionalize with additives.[3] This is an interesting way to introduce attractive properties, such as electrical conductivity, into biodegradable polymers.[4] Chitosan nanofibrils are promising scaffold materials for biomedical applications.[5,6,7] The high porosity, large surface area, large interface, and soft network lead chitosan biopolymer to host π-conjugated polymers beneficial for enhanced performance of the electrical conductivity and mechanical strength.[8,9,10] Over the past few decades, extensive studies on the combination of π-conjugated polymers with chitosan into conducting composites have been reported.[11,12,13] However, the inspiration of polythiophene/chitosan assemblies in a photonic Bouligand membrane on the macroscopic scale for electrochemical sensors is underdeveloped.

Alginate, is obtained from bacteria and brown algae. It is mixed with other polymers to improve the biological properties as they are good for molding with a suitable biological nature.[14] Furthermore, lower alginate concentration solutions have lower mechanical properties

though they have properties to promote cell proliferation and viability. A homogenous pre-crosslinking technique was developed by Hazur et al.[15], which is widely used for all materials based on alginate.

AIM OF THE STUDY: To characterise the physicochemical properties of Efavirenz-chitosan, Tenofovir-chitosan and Dolutegravir-alginate polymer conjugates

2.0 MATERIALS AND METHOD

2.1 Materials

Apparatus: Filter paper (Whatman Int. Ltd, Sprink field England 4cm); litmus paper (D2 Labtech. England), pre-coated TLC plates (GF254 Kieselgel 60 Merck TLC silica plates), measuring cylinder, funnels, separating funnels;250ml beakers; conical flasks; flat bottom flasks; Volumetric flasks; reagent bottles; sample bottles; TLC development tank and capillary tubes.

2.1.1 Equipment: Sensitive weighing balance (Mettler Toledo Balance AB204, Switzerland) rotary evaporator (RE100, BIBBY Stirilin Ltd. England) UV Lamp (Eagle scientific Ltd, 254-365nm) water bath (Gallen Kamp Amps 2.5, England); Vaccum pump (fisones, PYB 580 0011J) thermometer, hot plate (Clifton, nelson-s-mare Avon, England); oven (Memmert, West Germany); differential scanning calorimeter, X-ray diffractometer UV Spectrophotometer (spectronic 20, ES eagle scientific Ltd Nottingham, England.

2.1.2 Reagents: Sulphuric acid (farm Italia, conloerba; Italy), chloroform (Iso Merck), sodium hydroxide, ethanol, and Acetic acid (Wardle chemicals Ltd, USA) and distilled water, all reagents are of analytical grade and so were not purified further.

Methanol, tetraoxosulphate VI acid (H_2SO_4 90%, farm Italia, conloerba, Italy) Ethyl acetate, ammonia solution; Toluene; glacial acetic acid (Wardle chemicals Ltd, USA); chloroform (Iso Merck).

2.2 PHYSICOCHEMICAL PROPERTIES

2.2.1 Stability profiling

2.2.2 Differential scanning calorimetry (DSC)

Procedure: 8.8000mg, 7.000mg, 5.5000mg, 6.5000mg, 7.2000mg, 5.9000mg, 4.8000mg and 5.3000mg of Alginate, chitosan, Efavirenz, tenofovir, dolutegravir tenofovir-chitosan, Efavirenz-chitosan and dolutegravir-alginate samples respectively, were heated in an aluminium crucible at a temperature range of 34°C to 300°C with Mettler Toledo DSC using STAR SW 13.00 software, after which the signals are read out as thermogram on the Personal Computer.

2.2.3 X-Rays Diffraction (XRD) Techniques

The phenomena by which X-rays are reflected from the atoms in a crystalline solid is called diffraction. The diffracted X-rays generate a pattern that reveals structural orientation of each atom in a given compound. X-ray diffraction is extensively used in chemistry for the characterization of organic and inorganic compounds that are made for pharmaceutical companies.

XRD finds the geometry or shape of a molecule using X-rays. XRD techniques are based on the elastic scattering of X-rays from structures that have long range order. The X-rays get diffracted by a crystal because the wavelength of X-rays is similar to the inter-atomic spacing in the crystals.

Procedure

The sample was grinded into a talc-like powder (<0.062 mm), and a small amount of the sample is placed in the center of a quartz disk and 2–3 drops of distilled water is added to the sample. The sample was spread on a thin layer with a glass rod that is rolling it over the sample and finally placed in a desiccator to dry. After drying the sample on the desiccator, it is then transferred to sample holder for XRD analysis.

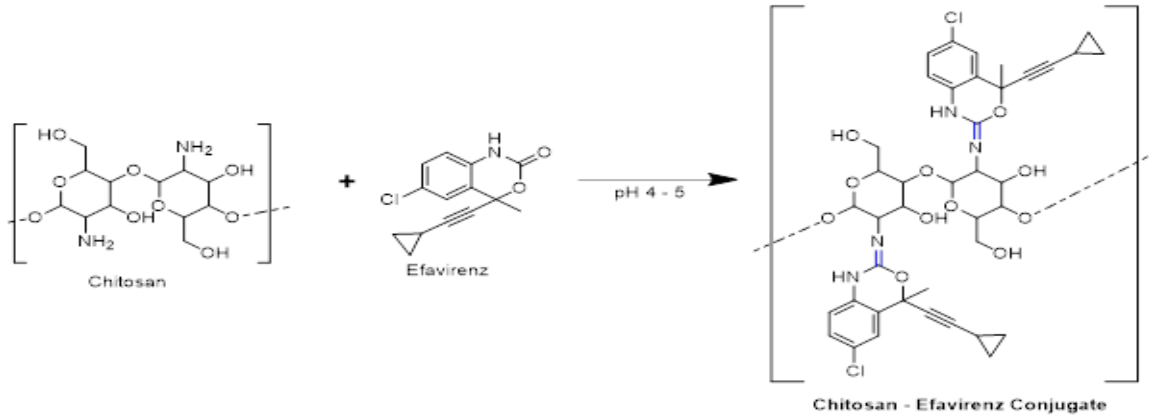
2.2.4 Determination of Quantitative Solubility Test for Efavirenz-chitosan, Tenofovir-chitosan, Dolutegravir-alginate Conjugates using Gravimetric Method

An increasing amount of 40g of Efavirenz-chitosan, Tenofovir-chitosan and Dolutegravir-alginate is added to about 100ml water in a conical flask with shaking until the solution is saturated and a part of solid is left undissolved. The solution was filtered and 20ml of filtrate is pipetted out into a pre-weighed evaporating dish. The dish containing 10ml filtrate is weighed then the filtrate is evaporated to dryness and further dried at 100°c in an oven, then it was cooled and weighed. The drying continued until a constant weighed is obtained.

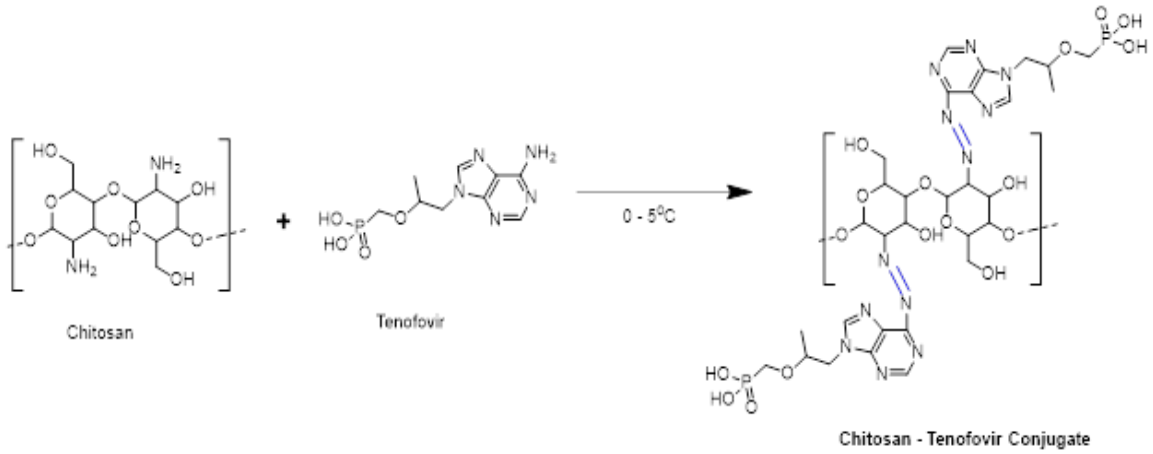
2.3.5 Buffer Hydrolysis of the Conjugates (Stability Studies)

Hydrolysis profiles were obtained in phosphate buffer at pH 6.1, 7.4 and 8.1 as follows. Efavirenz-chitosan, Tenofovir-chitosan, Dolutegravir-alginate conjugates (20 mg/mL) were dissolved in phosphate buffer and maintained at 37°c throughout the course of the hydrolysis study. Aliquots of 1.0 mL was removed at different time points (0, 2, 4, 6 and 8 hours) and centrifuged at high speed to get a supernatant which was then analyzed by UV at absorption maximum for the particular conjugate. The procedure was repeated six more times for each conjugate.

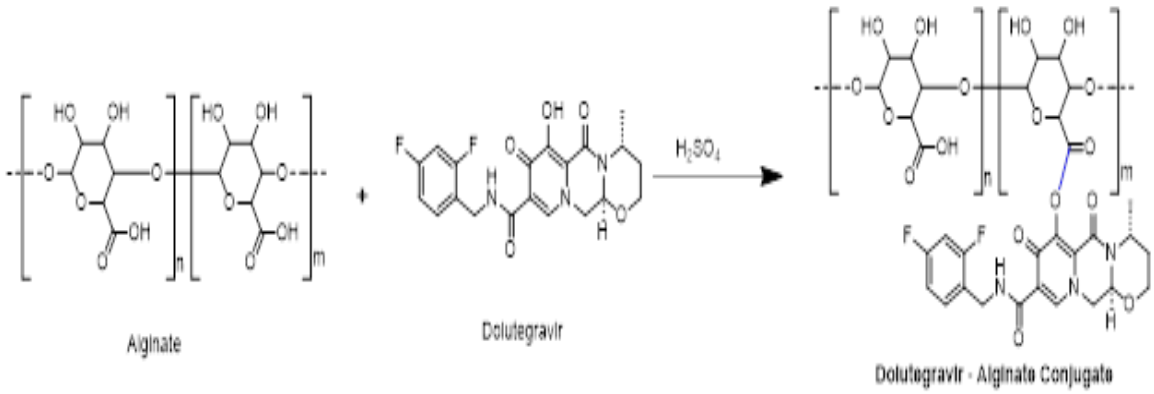
RESULT



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.

3.1 Physicochemical profiles of the antiretroviral drug-polymer conjugates

3.1.1 Stability profiling

Differential Scanning colorimetry (DSC):

DSC thermogram of Tenofovir (TE) alone showed endothermic peak at 80°c with a broader peak area while chitosan (CH) showed endothermic peak at 181°c and 204°c.

DSC thermogram of Tenofovir-chitosan (TEN-CH) conjugate showed endothermic peak at 65°c and 218°c respectively which was slightly different from the drug alone and the polymer (chitosan).

DSC thermogram of Efavirenz (EF) alone showed two endothermic peaks at 80°c and 223°c respectively while that of Efavirenz-chitosan (EF-CH) conjugate showed endothermic peak at 2°c.

DSC thermogram of Dolutegravir (Do) alone had a remarkable melting peak at147°c

DSC thermogram of Alginate (ALG) polymer did not show any appreciable endothermic peak except for Thermogram temperature at 80°c and 233°c while the thermogram of Dolutegravir-Alginate conjugate: This showed endothermic peak at 147°c and 183°c.

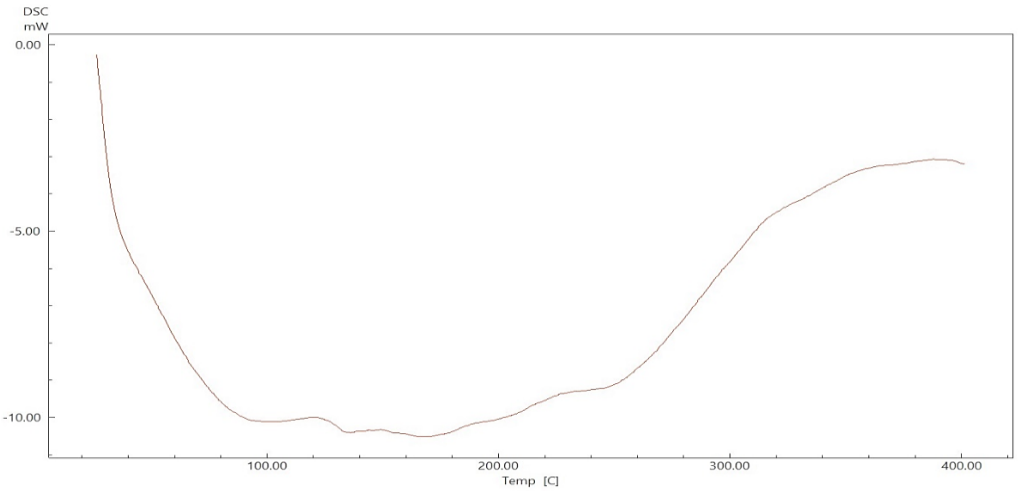


Figure 1a: DSC thermogram of Tenofovir-chitosan (TEN-CH) conjugate: TCEN-CH showed endothermic peak at 130°c.

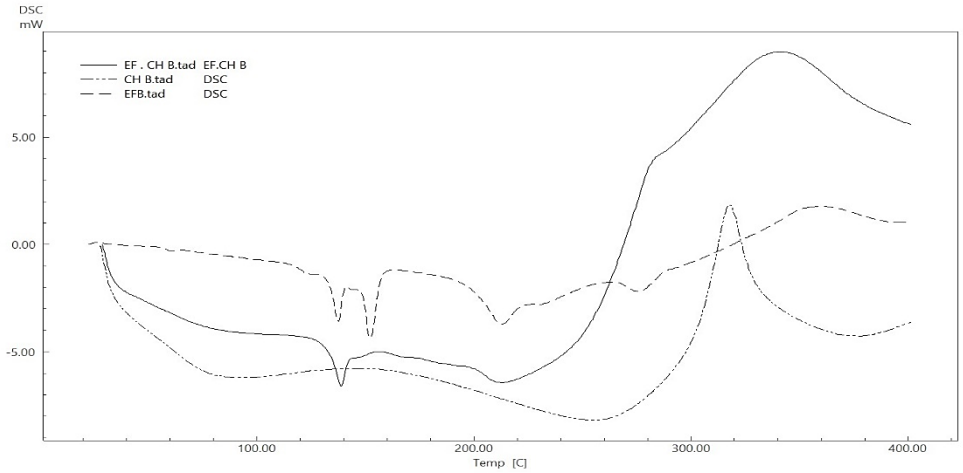


Figure 1b. DSC thermogram of Efavirenz-chitosan (EF-CH) conjugate superimposed with efavirenz and chitosan: EF-CH Showed endothermic peak at 138°C.

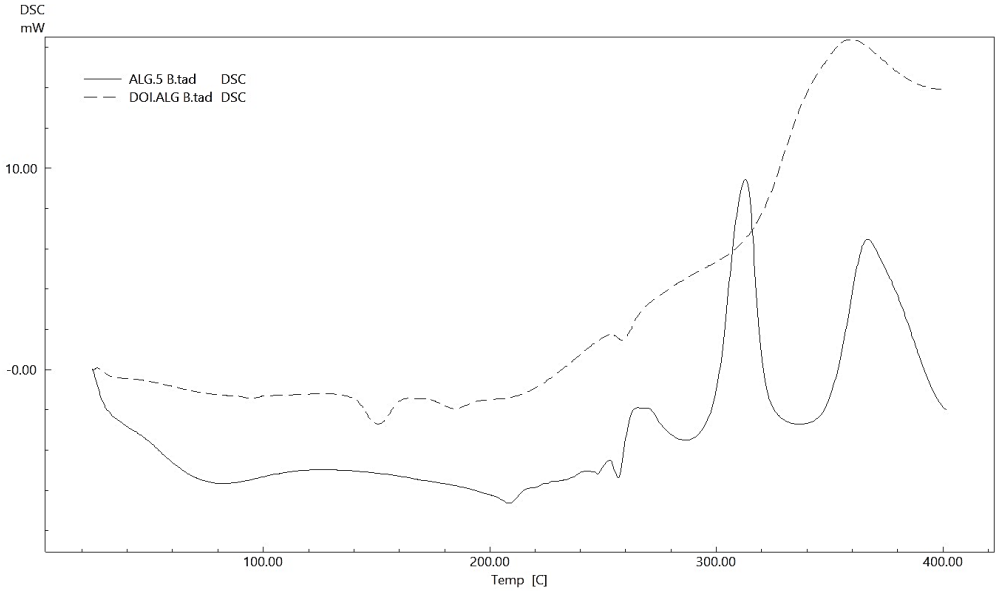


Figure 1c. DSC thermogram of Dolutegravir-Alginate conjugate superimposed with dolutegravir: This showed endothermic peak at 220°C

3.1.2 X-RAY DIFFRACTION (XRD) ANALYSIS

The width of the peaks is inversely proportional to the crystal size. A thinner peak corresponds to a bigger crystal. A broader peak means that there may be a smaller crystal, defect in the crystalline structure, or that the sample might be amorphous in nature, a solid lacking perfect crystallinity. The peak shows considerably moderate size of peaks for the drug alone and it conjugates, showing a good level of crystal formation and purity.

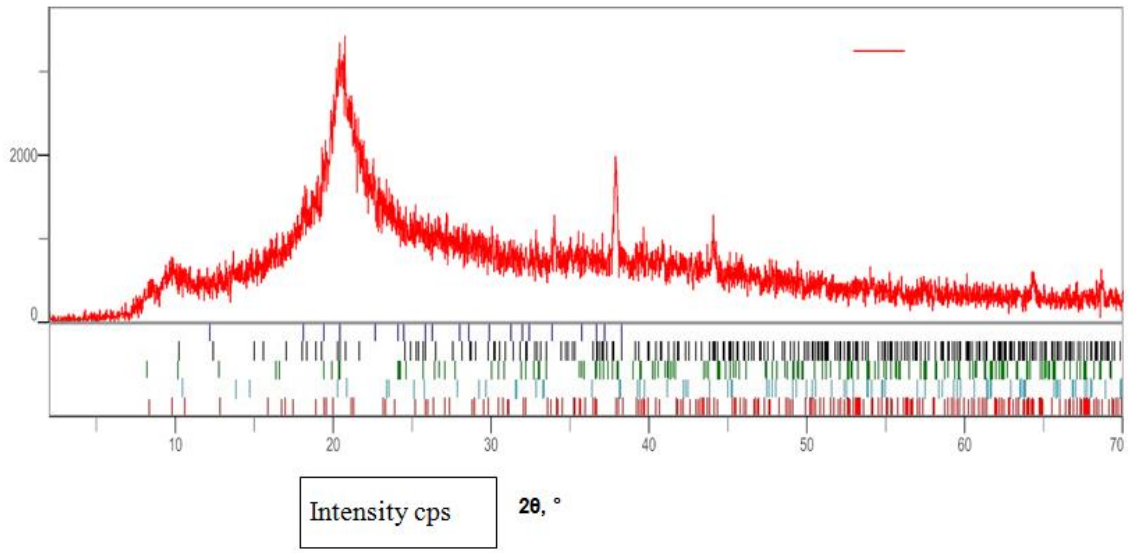


Figure 2a: XRD of Efavirenz-Chitosan.

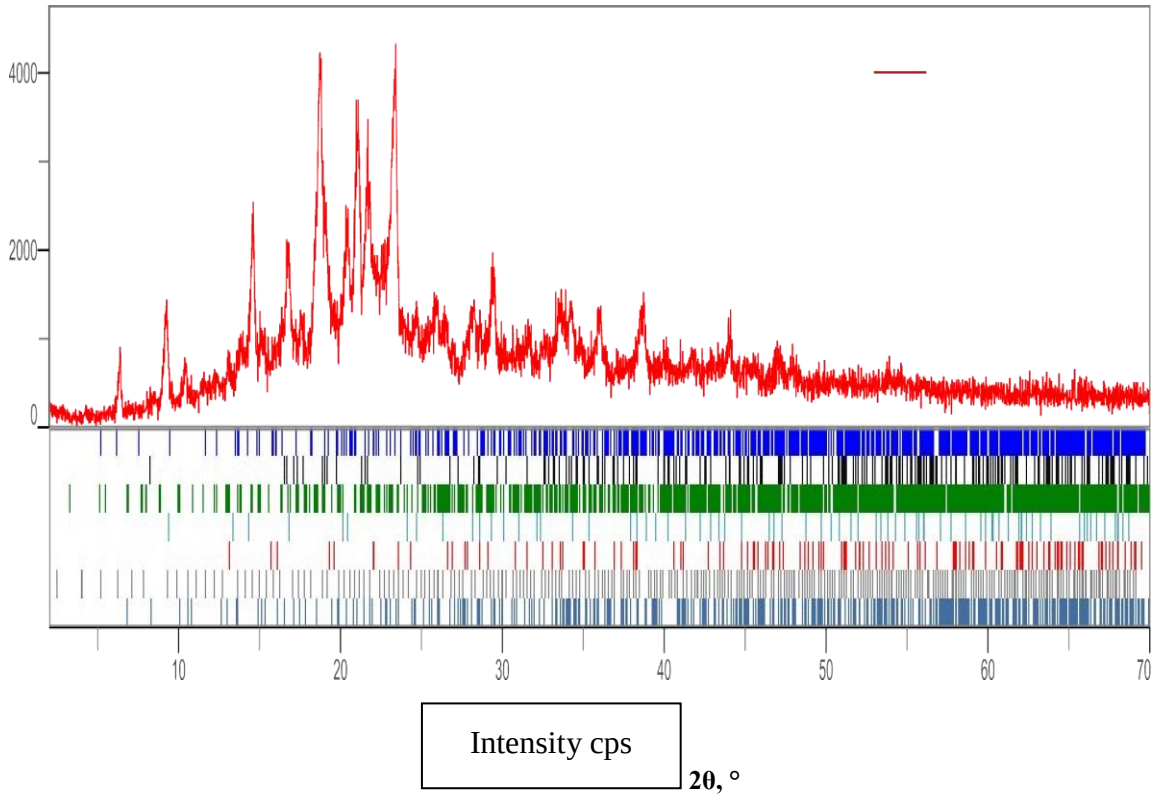


Figure 2b: XRD of Tenofovir-Chitosan.

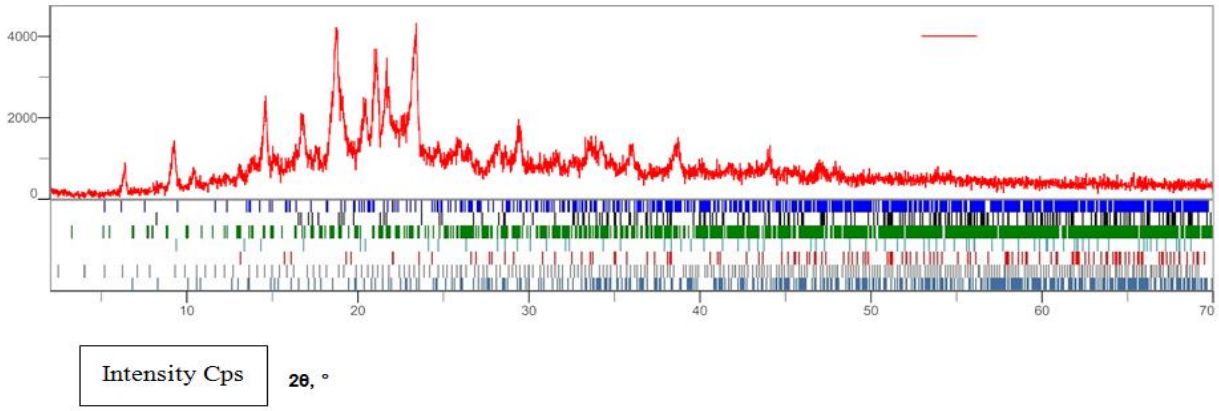


Figure 2c: XRD of Dolutegravir-Alginate.

3.1.3 Solubility

The solubility of the conjugates was obtained by gravimetric method using the density of each solvent involved. The density of water is 1g/cm^3 (997kg/m^3); ethanol is 0.789g/ cm^3(789 kg/m^3) and Acetic acid is 1.049 g/cm^3 (1049kg/m^3). Solubility in each of the solvent is given as 1gm of solute required solvent = weight of solvent in 20ml solution (W5)/weight of solute in 20ml solution (W4) X Density of solvent.

Table 1: Quantitative solubility profile of the three conjugates.

S/N	Drug Conjugates	g/L in water	Ppm	g/L in ethanol	ppm	g/L in acetic acid	Ppm	Descriptive Term
1	Efavirenz-Chitosan	75.0	7.5	39.0	3.9	31.4	3.14	Soluble
2	Tenofovir-Chitosan	29.0	2.9	54.0	5.4	21.0	2.1	Soluble
3	Dolutegravir-Alginate	20.0	2.0	15.6	1.56	12.0	1.2	Soluble

3.1.4 BUFFER HYDROLYSIS OF DRUG CONJUGATE AT DIFFERENT pH OVER 8 HOURS

C=Coe-^{kt}------1

Log= log Co-kt/2.303------2

Relating equation (2) to regression equation

Y=mx+C

The hydrolysis of the conjugates in buffer under the (pH 6.1 to 8.1) are shown in 5a to 5c.

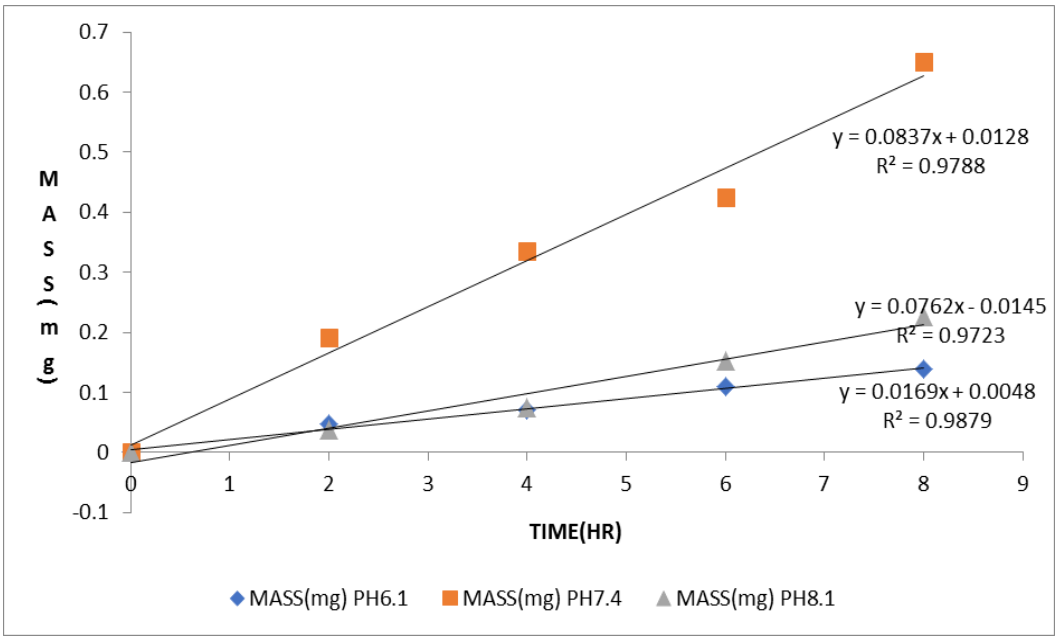


Figure 3a: Showing Efavirenz-Chitosan hydrolysis at pH 6.1; 7.4 and 8.1 over 8hrs respectively.

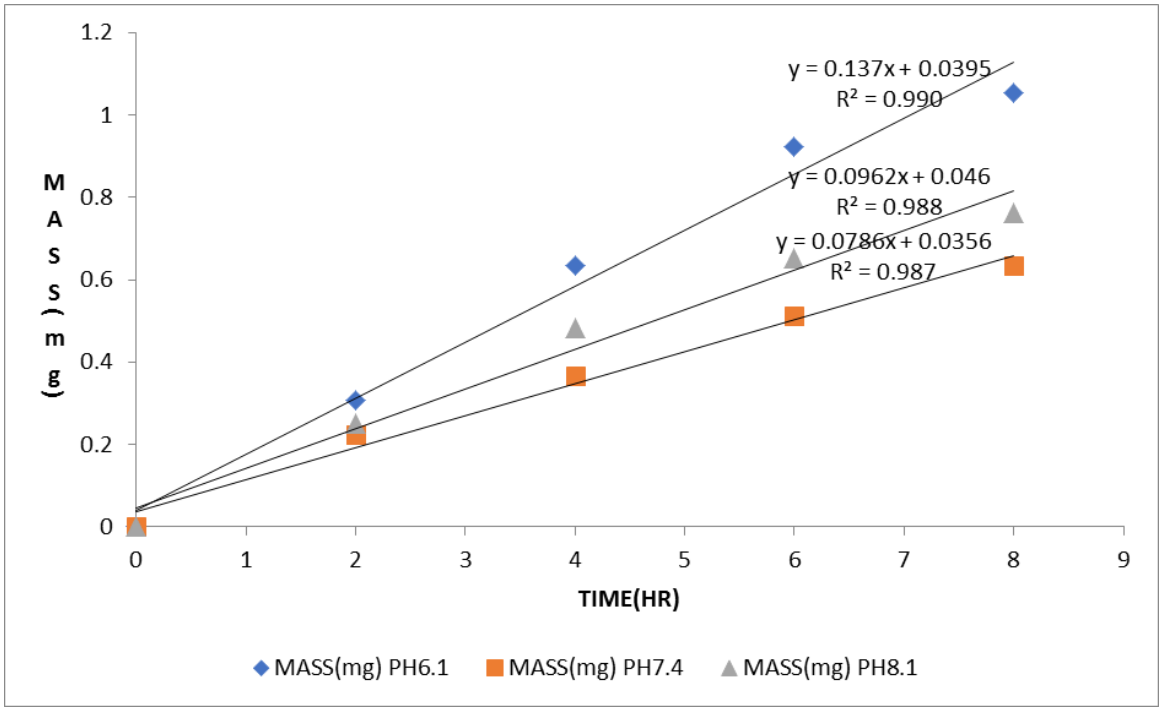


Figure 3b: Tenofovir-Chitosan Hydrolysis at pH 6.1; 7.4 and 8.1 over 8hrs.

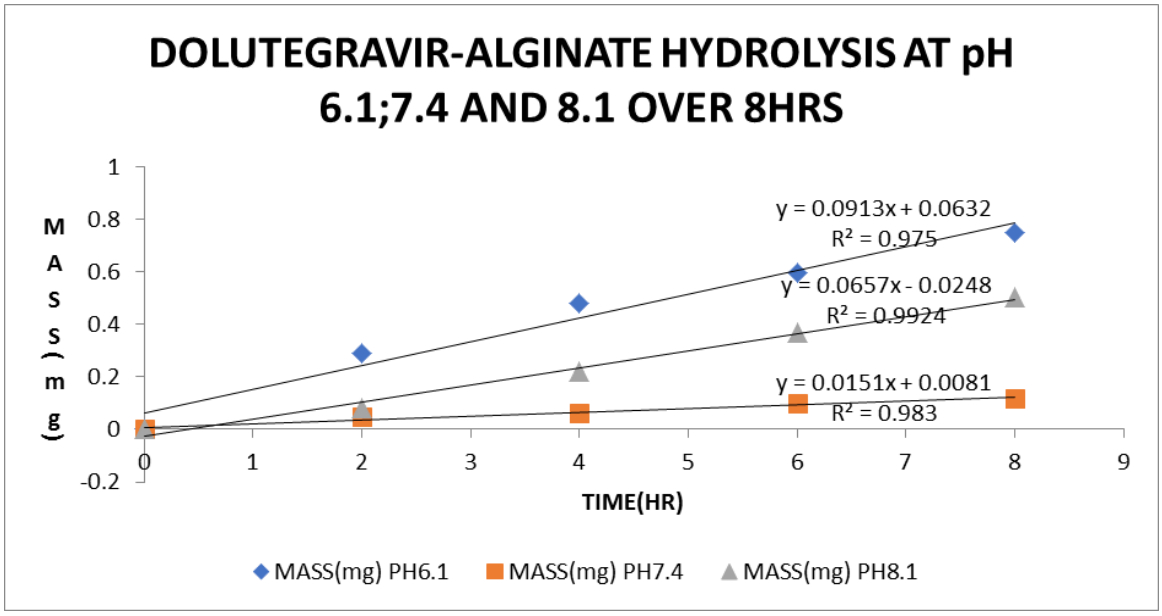


Figure 3c: Dolutegravir-Alginate Hydrolysis at pH 6.1, 7.4 and 8.1 over 8 hr.

4.0 DISCUSSION

4.1 DIFFERENTIAL SCANNING CALORIMETERY ANALYSIS OF THE POLYMERS AND THE CONJUGATES

DSC thermogram of Tenofovir (TE) alone showed endothermic peak at 1161.17°c with a broader peak area while chitosan (CH) showed endothermic peak at 181°c and 204°c while the DSC thermogram of the conjugated Tenofovir-chitosan (TEN-CH) conjugate showed endothermic peak at 130°c and 180°c respectively which was slightly different from the drug alone and the polymer (chitosan), confirming the purity of the drug and the polymer and suggesting conjugation between the drug and the polymer and also indicating that the polymer remain intact before and after the conjugation (See fig. 3a).

DSC thermogram of Efavirenz (EF) alone showed two endothermic peaks also at 137°c, 152.3°c and 213°c respectively while the thermogram of the conjugated drug (Efavirenz-chitosan EF-CH) showed endothermic peak at 138°c indicating conjugation and maintaining the integrity of the polymer in the conjugate (see fig. 3b respectively).

DSC thermogram of Dolutegravir (Do) alone had a remarkable melting peak at147°c while the DSC thermogram of Alginate (ALG) polymer did not show

any appreciable endothermic peak except for Thermogram temperature at 81.72°c and 208°c while the thermogram of Dolutegravir-Alginate conjugate shows endothermic peak at 150.91°c and 184.4°c and a sharp peak at 258.12°c showing effective conjugation (see fig.3c).

4.2 X-RAY DIFFRACTION (XRD) ANALYSIS OF THE DRUG ALONE AND THE CONJUGATE

XRD of efavirenz exhibits weak reflection in the regions of 2ɵ = 65°, 20°, 23° corresponding to the intensity at 2200, 4300 and 3000 respectively while that of efavirenz-chitosan conjugate exhibits a weak and broad reflection of 2ɵ at 22° and a slight peak at 35° corresponding to the intensity at 3200 and 2000 respectively, indicating conjugation of the polymer to the drug, see fig. 4a. The XRD of tenofovir alone exhibits weak and sharp with shoulder peaks of reflection at 2ɵ =20°, 22° and 26° corresponding to 4400, 3800 and 2200 intensity respectively while the XRD of tenofovir-chitosan exhibits reflection at 2ɵ = 15°, 19° and 24° corresponding to 2300, 4200 and 4400 intensity suggesting conjugation of tenofovir and chitosan. See fig. 4b.

The XRD of dolutegravir exhibits a sharp peak reflection at 32° corresponding to the intensity of 5500 while that of the dolutegravir-alginate exhibits a reflection at 2ɵ at 20°, with shoulder at 22° and 24° corresponding to intensity of 4300, 3800 and 4300 respectively, confirming successful conjugation of dolutegravir-alginate and purity of the conjugate, see fig. 4c.

The XRD analysis of the drug alone and the dolutegravir-alginate carried out gave a distinctive pattern in terms of the block copolymers of the drug alone and the conjugate showing the degree of rotation of the crystals in the conjugate and its intensities of rotation.

4.3 SOLUBILITY ANALYSIS

The solubility tests carried out was a qualitative and quantitative test, qualitative test to show if the drug conjugates can dissolve in the different solvent media and quantitative test to know the amount that actually dissolved in the different solvent media.

Efavirenz-chitosan, Tenofovir-chitosan and Dolutegravir-alginate dissolves in polar solvents because of the polarity polymers and its ability to form salts easily. As a result of this, it was not surprising that the conjugates are insoluble in non-polar solvents. The fact that the conjugates of alginate (dolutegravir-alginate) are soluble in aqueous medium suggests that these conjugates can be administered through the systemic route where plasma esterases can easily release the drugs into the system, thus enhancing the rapid increases in plasma concentration of these drugs. As regards chitosan derivatives, systemic administration might lead to sustained release of the drugs since the release will be equilibrium dependent. These results further confirm the justification for the use of these polymers as biocompatible polymers in drug delivery and sustained release vehicles. The summary of the solubility profile is shown in table 1.

4.4 BUFFER HYDROLYSIS OF CONJUGATES

The buffer (pH 6.1 to 8.1) hydrolysis of the polymer conjugates are shown in fig.5a to 5c and summary of the rate of rate reaction and half-life in table 2. It was observed that over the 8hrs duration of the experiment, the rate of hydrolysis proceeds uniformly producing a linear plot (see fig 5a, 5b and 5c respectively). In the first 8hrs of the hydrolysis kinetics produced linear plots, in which the rates were calculated to be: 1.69×10^{-2} mg/hr, for Efavirenz-chitosan at pH 6.1; 8.3×10^{-2} mg/hr; for Efavirenz-chitosan at pH 7.4; 7.62×10^{-2} mg/h; for Efavirenz-chitosan at pH 8.1; Tenofovir-chitosan at pH 6.1; 1.37×10^{-1} mg/hr, Tenofovir-chitosan at pH 7.4; 7.86×10^{-2} mg/hr.; Tenofovir-chitosan at pH 8.1; 9.62×10^{-2} mg/hr, then Dolutegravir-alginate at pH 6.1; 9.13×10^{-2} mg/hr., Dolutegravir-alginate at pH 7.4; 2.37×10^{-2} mg/hr.; Dolutegravir-alginate at pH 8.15; 6.57×10^{-2} mg/hr.; respectively. Similarly, the calculated half-lives were 41hrs, 8.3hrs and 9hrs for Efavirenz-chitosan at pH 6.1, 7.4 and 8.1 respectively.

Then the half-lives for Tenofovir-chitosan were 5.1hrs, 8.8hrs and 7.2hrs at the pH of 6.1, 7.4 and 8.1 respectively. Finally, the half-lives for Dolutegravir-alginate ranges from 7.5hrs, 45.9hrs and 10.5hrs at the pH of 6.1, 7.4 and 8.1 respectively. The kinetics order of reaction in both polymers was determined to be first order reaction for the first 8hrs. The observation that the rate of hydrolysis at pH 8.1 is fastest than the rate at pH 7.4 suggests that the hydrolysis is based catalysed. The buffer hydrolysis is based on enzymatic action of esterase enzyme on the ester (polymer conjugates) where by releasing the drug and polymer for therapeutical activity. The activity of the esterase enzyme varies in different buffer pH, but it was far better in buffer pH of 7.4 which is the normal physiological pH of the human blood like wisely the Schiff conjugate that was synthesis that is efavirenz-chitosan conjugate varies in different buffer pH but was also better in 7.4 pH which is also the normal physiological pH of the human blood. The diazonium salt formed from the conjugate of Tenofovir-chitosan can easily be dissociated in the presence of violet light nearby hence releasing the drug itself.

5.0 CONCLUSION

The Polymer Conjugates of Efavirenz-chitosan, Tenofovir-chitosan and Dolutegravir-alginate were successfully characterised physiochemically through buffer hydrolysis, X- ray diffractometry (XRD) and differential colorimetry (DSC)**.** The three antiretroviral drug conjugates show good stability profile and both the drug and the polymer are intact after conjugation.

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