JOPAT Vol 21(2), 846 - 858, 2022. December. 2022 Edition

All Stakeholders International Conference on COVID-19 (ASICC) Edition.

ISSN 2636–5448 *https*://dx.doi.org/10.4314/jopat.v21i2.7

Preparation and Characterization of Crosslinked Starch-Albumin Films for Coating of Prednisolone Tablets for use in Covid-19 Related Respiratory Disease

Philip F. Builders[1*], Judith E. John[1], John Alfa[2], B. B. Mohammed [3]

[1]Department of Pharmaceutical Technology and Raw Materials Development, National Institute for Pharmaceutical Research & Development, Idu, Abuja, Nigeria.

[2] Department of Pharmaceutics and Pharmaceutical Technology, Bingham University Karu, Nasarawa State, Nigeria.

[3]Department of Pharmaceutics & Industrial Pharmacy, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna, Nigeria.

ABSTRACT

Steroidal products have been found useful in inflammations associated with Covid 19. Prednisolone is one readily available steroid, which is often found as uncoated normal release tablets. Modified release prednisolone may be desirable in Covid 19 for sustained actions. This is expected to reduce dosing frequency and enhance compliance. This study is concerned with development of controlled release prednisolone using coating technology with bio-compatible, cross-linked starch-albumin films. Starches and proteins are excellent film formers with good flexibility, transparency, and bio-compatibility. The cross-linked starch-albumin films were prepared using glycerol as the plasticizer: starch (A), starch-albumin (B), starch-albumin cross-linked with formaldehyde at 1 % (C), 5 % (D) and 10 % (E). Equilibrium moisture sorption (EMS) at 100 % relative humidity, equilibrium swelling (ESC) in buffer solutions of pHs 2, 7 and 9, and DSC thermal properties were evaluated. I*n-vitro* drug release from the film coated prednisolone tablets were evaluated in 0.1N HCl, water and phosphate buffer 8.0 as dissolution media. Films showed ESC in the order A>D>E>B>C; D>C>A>E>B and A>D>C>B>E in acidic, neutral and alkali media respectively. EMS was in the order B>E>A>D>C; with slight shift in the melting temperatures. *In-vitro* release at 240 min varied from 78 to 117 % (E>D>C>A>B); 19 to 60 % (D>B>C>E>A) and 49 to 60 % (B>A>C>D>E) in 0.1N HCl, water and PBS respectively. Cross-linking improved the stability and swelling of films. The *in-vitro* release in alkaline medium suggests their usefulness for controlled drug delivery. New pH-responsive polymers, with improved physicochemical properties for coating prednisolone tablets were developed.

Keywords: Starch, serum-albumin, films, cross-linking, Prednisolone, COVID-19

*Corresponding author: philsonsky@yahoo.com, +234 8035874698

INTRODUCTION

Corona virus disease (COVID-19) is an infectious disease and a global public health crisis which originated as an epidemic in Wuhan, China, and has now become a pandemic as declared by the WHO on March 11th, 2020 [1]. It has affected every part of the globe including Nigeria with first recorded case in February, 2020. Although Covid-19 vaccines are now available, corticosteroids like prednisolone have been used in the management of respiratory and inflammatory symptoms [2-4].

Starches are renewable biopolymers that are widely available and can be obtained from different botanical sources [4]. They are excellent film formers as they are used as coatings for pharmaceutical formulations in drug delivery systems and as packaging materials. The advantages of starch-based films include biodegradability, renewability, ease of modification or combination with other materials to improve their functional and physicochemical properties. Composites prepared with starch and proteins are known to enhance the strength of the matrix and form good barriers to gasses [5].

Biopolymers are usually prepared into films with the use of plasticizers such as polyols (glycerol) in order to improve their handling (bending and stretching) [6]. Moreover, it is necessary to cross-link starch with multifunctional reagents so as to improve its physico-mechanical properties [4]. When cross-linked, films become impermeable and sturdy to withstand the pressures that come with handling, heat and acid treatments [7].

Proteins such as Serum albumin are natural and eco-friendly with good water solubility, little toxicity, and can be used to sustain the release of drugs [8]]. They have acid and amino groups that function as polyelectrolytes in aqueous solutions [9]. Albumin have been used extensively to prepare biodegradable films, however, they have to be cross-linked for better functionality [10]. The aims of this study therefore are to prepare and characterize cross-linked starch-albumin films as coating material for controlled release of prednisolone tablets.

MATERIALS AND METHODS

Materials:

Potato starch, serum albumin, glycerol, formaldehyde, buffer solutions (pH 2, 7 and 9), Prednisolone crystals (All were from Sigma-Aldrich, Germany), Prednisolone tablet (Hovid, Malaysia). All other reagents are of analytical grade

Methods:

Preparation of Starch and Starch-Albumin Films

Five gram of potato starch was dispersed in 70 ml of water in a beaker labeled A. Albumin (2 g) was dispersed in 30 ml of water in a beaker labeled B. Beaker A was placed in a water bath at 70 °C to obtain a gelatinized starch. The contents of beakers A and B were mixed and stirred. Glycerol (5 ml) was added to the mixture as a plasticizer and stirred. The mixture obtained was poured into Petri dishes (30 ml). The dishes and their content were dried in a drying oven (Biobase-Biodustry, Sharndong) and stored for 24 hours at 40 °C after which they were characterized.

Preparation of Cross-linked Starch-Albumin Films

A stock solution of 37 % Formaldehyde was used to prepare three concentrations of Formaldehyde (1%, 5% and 10%). The method for the preparation of starch-albumin were repeated, and Formaldehyde (5 ml) for each of the concentrations (C, D and E respectively) was added and stirred. The resulting mixture was poured into Petri dishes (30 ml), and the filled dishes placed in the drying oven. The dishes were stored at 40 °C for 24 hrs after which they were characterized.

CHARACTERIZATION

Equilibrium Moisture Sorption Capacity (EMS)

Square films (measuring 1 cm x 1 cm) were excised from the stock film samples (A, B, C, D and E) and weighed (Mettler Toledo). They were placed in pre-weighed thin aluminum foils and put in a desiccator containing distilled water (100 % RH) at room temperature (27 ºC). The moisture uptake was determined after seven days of standing. This was done in triplicates.

Swelling capacity

Excised films (as above) were weighed and placed on thin square glass slips (2 cm x 2 cm) and placed in Petri dishes containing 30 ml of the swelling media (pHs 4, 7 and 9). The weights of each sample (film + glass slip) were measured after every 6 hrs until there was no further increase in weight. This was performed in triplicates and the average % swelling for each sample were determined.

Differential Scanning Calorimetry (DSC)

The thermal properties of films were studied using a DSC machine (204 F1 Phoenix NETZSCH, Switzerland). The instrument was calibrated using Indium (MP 156.6 °C, Heat of fusion 28.5J/g) as internal standard and dry nitrogen was used as the purge gas (purge 20 ml/min). Seven milligram of each film sample were weighed into an aluminum pan and covered with a perforated lid. The probes were heated at a temperature of 25-300 ºC at a rate of 10 ºC/min. The various thermal transitions were evaluated using a computer Proteus software.

***In-vitro* release from film-coated tablets**

A brand of prednisolone tablet was purchased from the pharmacy. Each tablet was coated with the composite films to determine the *in-vitro* release of the drug from the coated tablets in different dissolution media (0.1 N HCl of pH 1.2; water and phosphate buffered saline (PBS) of pH 8.0). The test was conducted in 900 mL of the dissolution medium, at 37 ^{o}C under mild agitation (50 rpm). Aliquots of the samples were withdrawn from the medium and replaced with equal volume of the test medium to maintain sink conditions. This was carried out within a 6-hour period at regular time intervals of 0, 10, 20 30….to 240 min, and the samples were analyzed using the UV spectrophotometer (Cary-60, UV-Vis, Agilent Technologies, UK) at 245, 246 and 240 nm for 0.1 N HCl, water and PBS respectively. This was done for three tablets representing each composite films.

RESULTS

Equilibrium Moisture Content (EMC) of Starch-Albumin Films

The results for moisture content of composite films is in the order D>B>A>E>C as presented in figure 1. There is no significant difference in MC when albumin was added to starch, however, a decrease in moisture content was observed when cross-linked with formaldehyde.

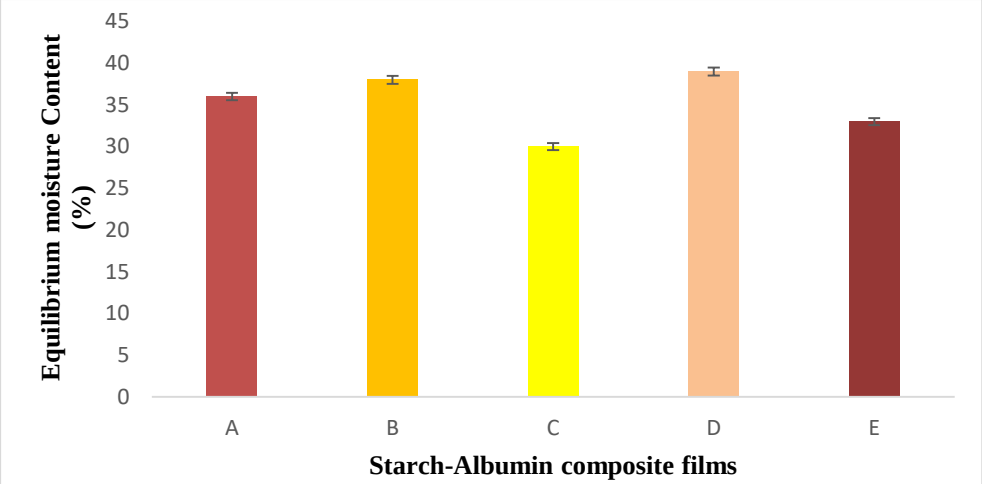

Figure 1: Equilibrium moisture content of composite films. Samples A = Starch Only; B = Starch +Albumin; C = Starch +Albumin (1 % cross-linked); D = Starch +Albumin (5 % cross-linked); E= Starch +Albumin (10 % cross-linked). Values are mean of triplicate readings ± standard deviation

Equilibrium Swelling Capacity (ESC) of Starch-Albumin Films

Figure 2 shows the ESC of composite films in different pH (2, 7 and 9) media. The ESC is in the order D>E>A>B>C in acidic medium, D>C>A>E>B in neutral medium and A>D>E>B>C in alkaline medium.

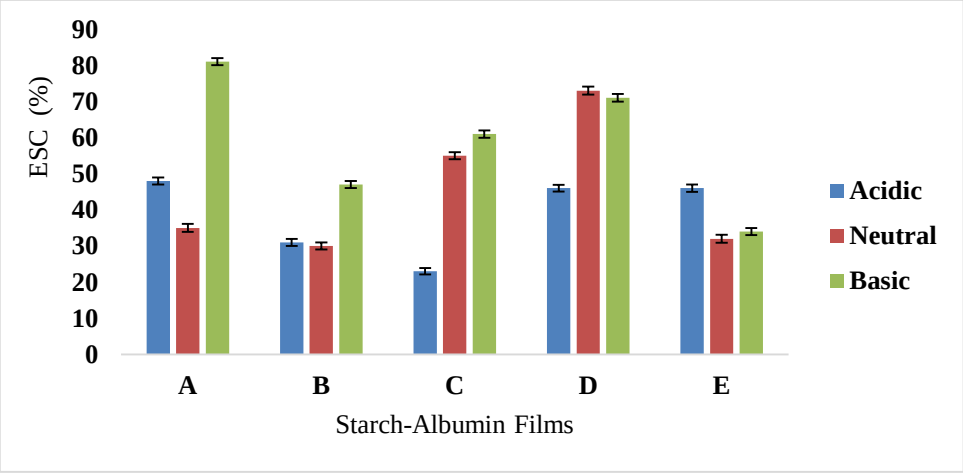

Figure 2: The equilibrium swelling capacity (ESC) of composite films in different pH media after 45 min: Samples A = Starch Only; B = Starch + Albumin; C = Starch + Albumin (1 % cross-linked); D = Starch + Albumin (5 % cross-linked); E= Starch + Albumin (10 % cross-linked). Values are means of triplicate readings ± standard deviation.

Equilibrium Moisture Sorption Capacity (EMS) of Starch-Albumin Films

The EMS (at 100 %) of starch-albumin composite films at day 5 is in the order B>E>A>D>C as shown in Figure 4. It was observed that the addition of albumin to starch film increased the moisture sorption by about 2.9 folds. However, there was a drastic reduction in moisture sorption when the films were cross-linked with formaldehyde, with an increase in moisture sorption as the concentration of formaldehyde increased.

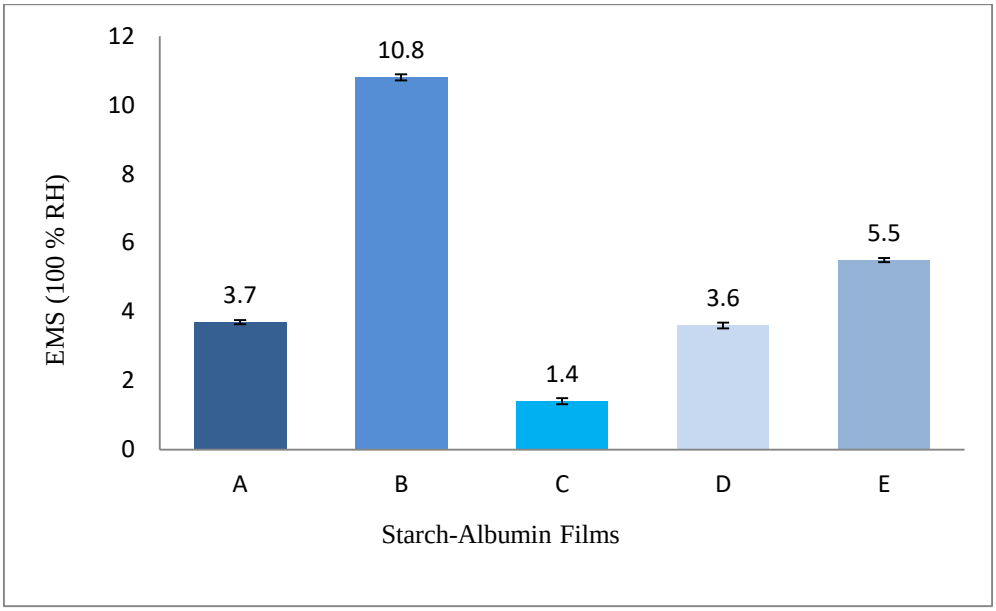

Figure 4: The equilibrium moisture sorption (at 100 % RH) of composite films at 5 days: Samples A = Starch Only; B = Starch + Albumin; C = Starch + Albumin (1 % cross-linked); D = Starch + Albumin (5 % cross-linked); E= Starch + Albumin (10 % cross-linked). Values are mean of three readings ± standard deviation.

DSC thermogram of Starch and Starch-Albumin Films

The thermal properties of the composite films (A-E) are presented in Figure 3. These thermograms are characterized by two endothermic transitions similar to that of starch. The first endothermic transition relates to the melting of the potato starch amylopectin crystals and the second a broadened peak relate to the melting of the amylose and albumin.

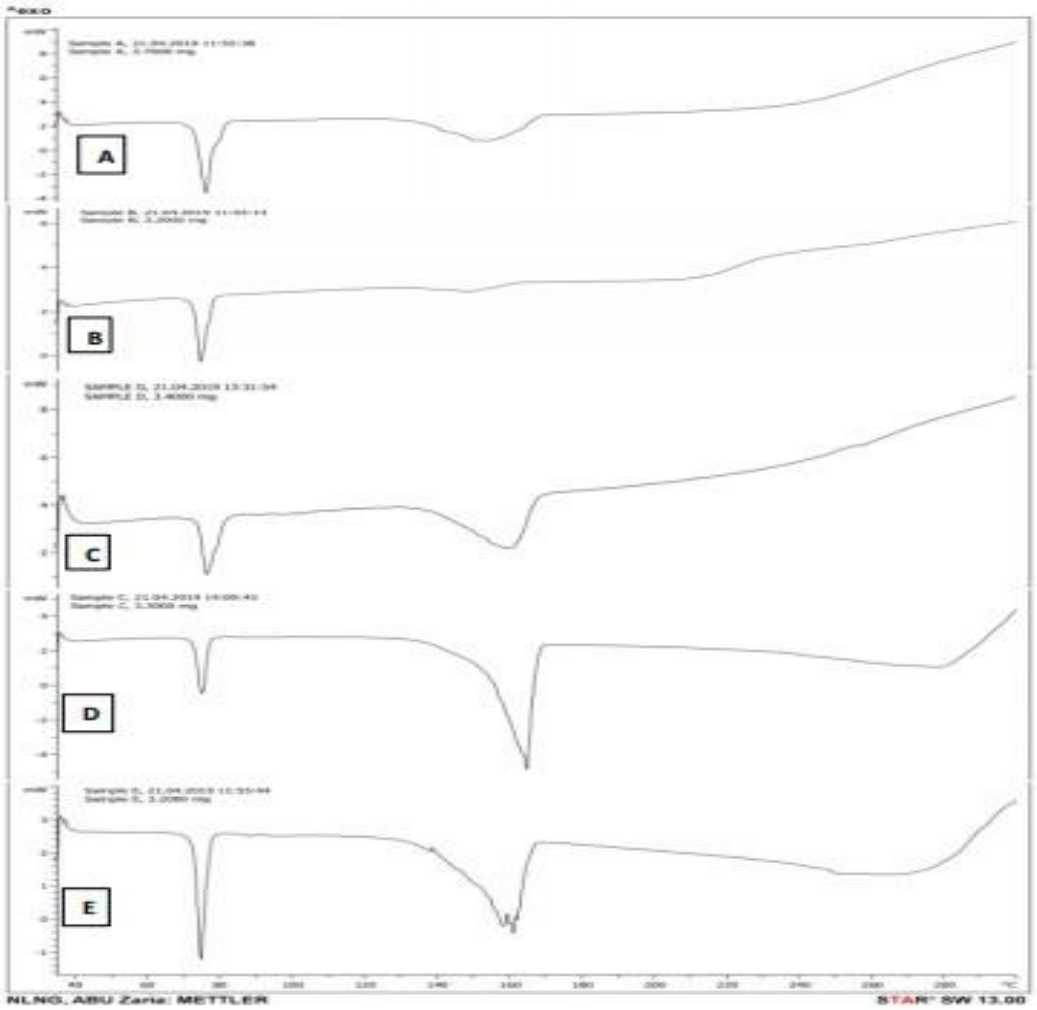

Figure 3: DSC thermograph of Starch and Starch-Albumin composite films: Samples A = Starch Only; B = Starch + Albumin; C = Starch + Albumin (1 % cross-linked); D = Starch + Albumin (5 % cross-linked); E= Starch + Albumin (10 % cross-linked). Values are means of triplicate readings ± standard deviation.

In-vitro Release profile of Prednisolone from Starch-Albumin Films

The *In-vitro* release profile of prednisolone from composite films were simulated in the gastric (0.1 N HCl), water and the intestine (phosphate buffer - 8.0), shown in figure 4a-c respectively. The *In-vitro* release of prednisolone from composite films at 240 min varied from 78 to 117 % (E>D>C>A>B); 19 to 60 % (D>B>C>E>A) and 49 to 60 % (B>A>C>D>E) in 0.1N HCl, water and PBS respectively.

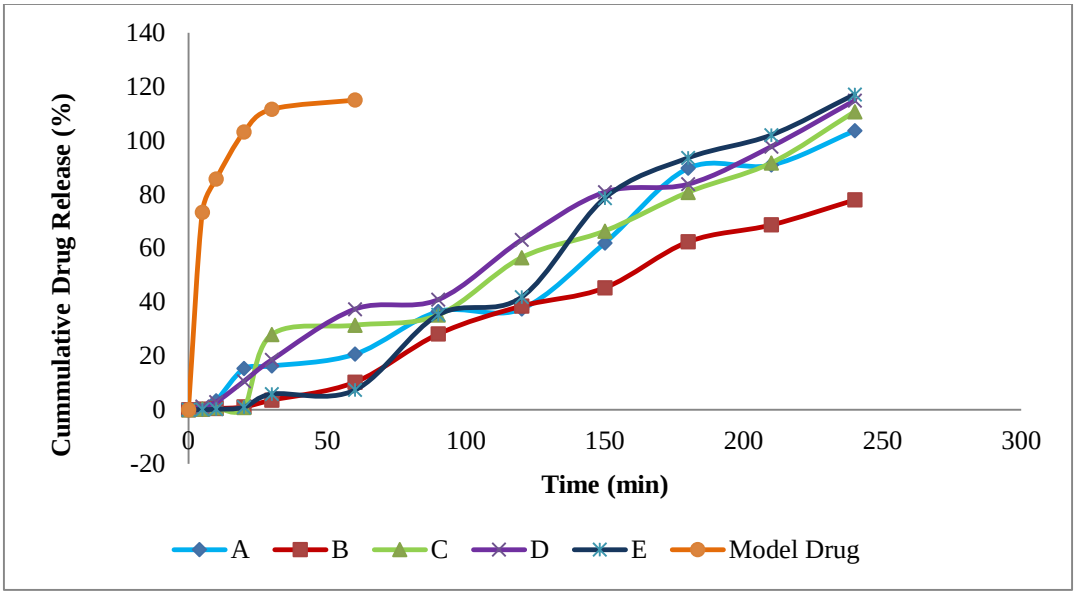

Figure 4a: Release profile of prednisolone from tablets coated with composite films in 0.1N HCl:

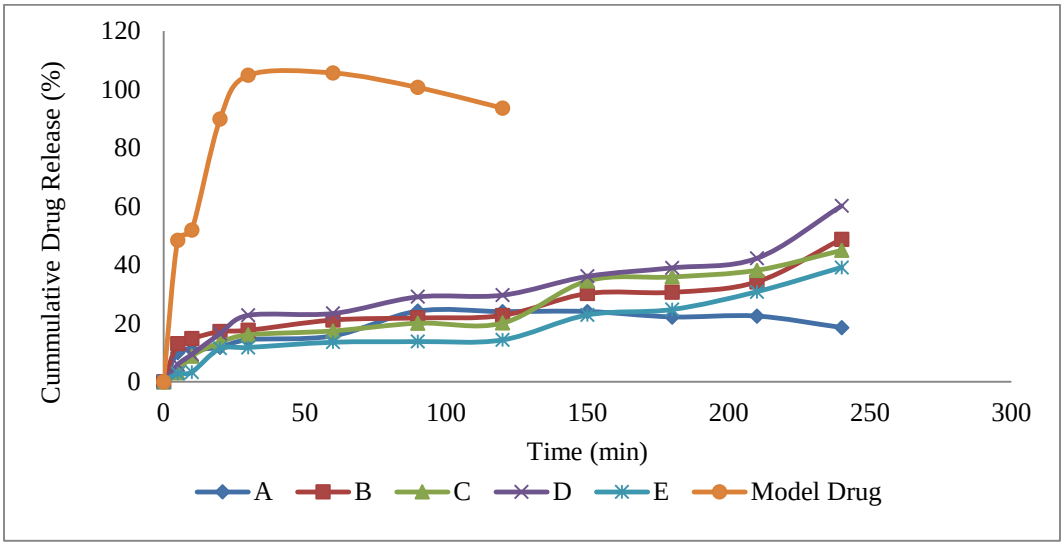

Figure 4b: Release profile of prednisolone from tablets coated with composite films in water,

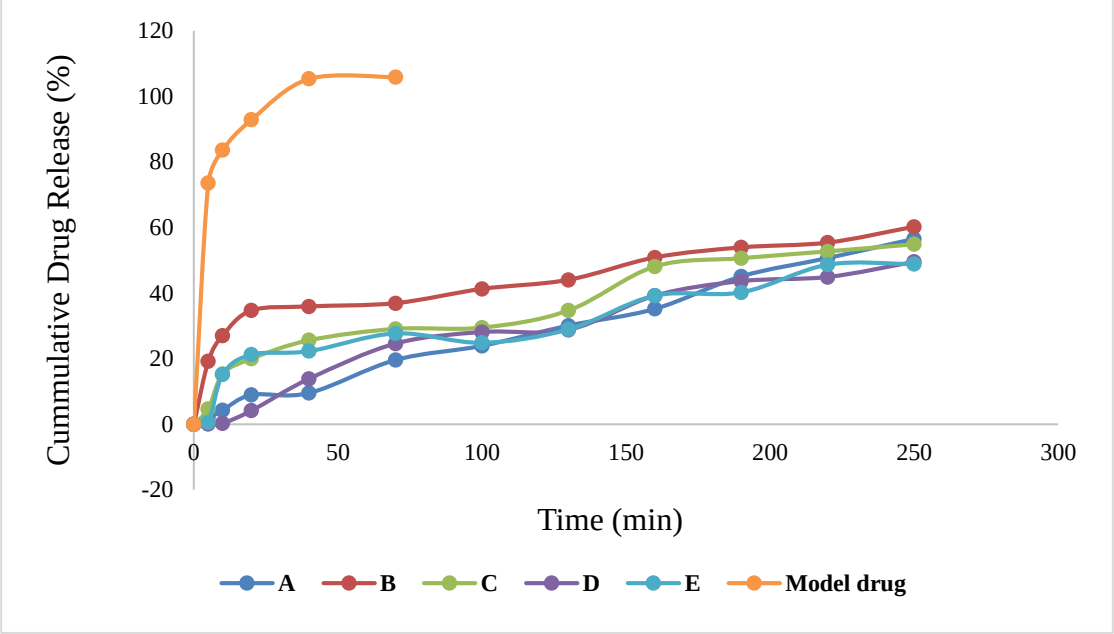

Figure 4c: Release profile of prednisolone from tablets coated with composite films in Phosphate buffer 8.0.

Samples A = Starch Only; B = Starch +Albumin; C = Starch + Albumin (1 % cross-linked); D = Starch + Albumin (5 % cross-linked); E= Starch + Albumin (10 % cross-linked); Model drug = Prednisolone

DISCUSSION

It was observed that the addition of albumin did not have any remarkable effect on the EMS of starch films, although, when cross-linked, a decrease in EMS was observed, with a further increase as the concentration of the cross-linker increased.

The formation of a more tight structure after cross-linking prevents the swelling of starch-albumin films and also restricts the movement of molecules, leading to a decrease in the moisture content [15]. The moisture content of a sample is an indication of its stability during storage. When the RH of the environment increases above the equilibrium RH, the sample tends to adsorb moisture from the atmosphere, leading to an increase in moisture content and instability [16]. Therefore, the film (C) show a better stability pattern than the uncross-linked starch-albumin films, with the 1 % cross-linked starch-albumin film showing more ability than the rest of the cross-linked composite films.

The conformational changes observed with the thermal properties of the films may be due to the presence of albumin [11]. A decrease in the melting peak and an increase in the second melting peak was observed in B; a slight shift in the melting peak for C was observed at 168 ºC; while D is characterized by two endothermic peaks, E is characterized by two bold and sharp

peaks. For starch and the starch-albumin composite films, the first transition which should correspond to the glass transition of the polymer(s) was characterized by a prolonged transition relating to the effect of heat on the plasticizer and moisture present in the film [12].

Glycerol increases the water permeability index of hydrophilic polymers, resulting in high moisture content [13]. Although, glass transition temperature is known to decrease in the presence of glycerol, the long transition may be due to the evaporation of moisture contained in the film (14). Thus, the actual glass transition of the starch and the starch-albumin complex merged with the plasticizer and prolonged water evaporation present in the films. With the addition of formaldehyde as a cross-linking agent, a sharp melting transition corresponding to increase in the crystallinity of the material was observed.

The *In-vitro* release of prednisolone from the different composite films in 0.1 N HCl at 240 min reveals that the addition of Albumin to starch films (B) lowered the capacity of the films to release the drug [17]. An increase in release was observed when the concentration of the cross-linker increased. The opposite effect was observed in PBS (8.0), with the highest release observed with B, but there was a reduction in the release as the concentration of cross-linker increased. Generally, however, the composite films demonstrated a delay in the release of prednisolone in all the media and therefore can be used in tablet coatings for controlled drug release [17].

The behavior of these polymers in different pH media suggests that they are pH responsive and so can be termed as "smart polymers" [18]. Albumin contains carboxylic and amino acid groups that display changes in ionization states to be protonated or deprotonated when placed in various pH media. When pH is decreased, the polymer exhibits a cleavage of its bonds to cause either a degradation of polymer chains or a dissociation of polymer aggregates [19]. As such, these changes in these polymers led to changes in their swelling behavior as well as release mechanisms that can find usefulness in tablet coatings for controlled/targeted drug delivery [20].

CONCLUSION

Starch and proteins are good film formers that may be used as coating materials for controlled drug release. The composite prepared with starch and albumin produced a polymer with new characteristic physicochemical properties. Also, treating the composite with formaldehyde as a cross-linking agent further improved some relevant functional properties of the new polymer such as film stability and pH-responsiveness in its swelling characteristics. The new cross-linked starch-albumin composite was effectively used for coating prednisolone tablets with remarkable pH responsive release profile.

CONFLICT OF INTEREST

The Authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors are grateful to Mr. Lidou Gilman, Mr. Hassan Chama and Mrs. Rakiya Akuboh of the Department of Pharmaceutical Technology and Raw Materials Development, National Institute for Pharmaceutical Research and Development, Idu, Abuja, Nigeria for their technical assistance.

REFERENCES

1. Njenga MK, Dawa J, Nanyingi M, Gachohi J, Ngere I, Letko M, et al. Why is there low morbidity and mortality of COVID-19 in Africa? Am J Trop Med Hyg. 2020;103(2):564–569.

2. Sarkar I, Sen A. In silico screening predicts common cold drug Dextromethorphan along with Prednisolone and Dexamethasone can be effective against novel Coronavirus disease (COVID-19). J Biomol Struct Dyn. 2020;0(0):1–5. doi.org/10.1080/07391102.2020.1850528

3. Caruso F, Rossi M, Pedersen JZ, Incerpi S. Computational studies reveal mechanism by which quinone derivatives can inhibit SARS-CoV-2. Study of embelin and two therapeutic compounds of interest, methyl prednisolone and dexamethasone. J Infect Public Health. 2020;13(12):1868–1877.

4. Grossmann MVE, Martino MN, Flores S, Zaritzky NE, Sobral P, Famá L. Innovations in Starch-Based Film Technology. In: Film Technology. 2005. p. 431–432.

5. Campos, C.A.; Gerschenson, L.N.; Flores S. Development of Edible Films and Coatings with Antimicrobial Activity. Food Bioprocess Technol. 2011;4(6):849–2875.

6. Lu DR, Xiao CM, Xu SJ. Starch-based completely biodegradable polymer materials. Express Polym Lett. 2009;3(6):366–375.

7. González-soto RA, Núñez-santiago MC, Bello-pérez LA. Preparation and partial characterization of films made with dual-modified (acetylation and crosslinking) potato starch. J Sci Food Agric. 2019;99:3134–3141.

8. Luo R, Lin M, Zhang C, Shi J, Zhang S, Chen Q, et al. Genipin-crosslinked human serum albumin coating using a tannic acid layer for enhanced oral administration of curcumin in the treatment of ulcerative colitis. Food Chem. 2020;330:127241. doi.org/10.1016/j.foodchem.2020.127241

9. Ma R, Zhitomirsky I. Electrophoretic deposition of chitosan – albumin and alginate – albumin films. Surf Eng. 2011;27(1):51–57.

- 10. Yamazoe H. Spectroscopic study on the conformation of serum albumin in fi lm state. J Biosci Bioeng. 2018; 127(4). doi:10.1016/j.jbiosc.2018.09.0.

11. Molodenskiy D, Shirshin E, Tikhonova T, Gruzinov A, Peters G, Spinozzi F. Thermally induced conformational changes and protein-protein interactions of bovine serum albumin in aqueous solution under different pH and ionic strengths as revealed by SAXS measurements. Phys Chem Chem Phys. 2017;19(26):17143–55.

12. Kaewtatip K, Tanrattanakul V, Szécsényi KM, Pavličević J, Budinski-Simendić J. Thermal properties and morphology of cassava starch grafted with different content of polystyrene. J Therm Anal Calorim. 2010;102(3):1035–1041.

13. Jouki M, Khazaei N, Ghasemlou M, Hadinezhad M. Effect of glycerol concentration on edible film production from cress seed carbohydrate gum. Carbohydr Polym. 2013;96(1):39–46.

14. Laohakunjit N, Noomhorm A. Effect of plasticizers on mechanical and barrier properties of rice starch film. Starch/Staerke. 2004;56(8):348–356.

15. Reddy N, Yang Y. Citric acid cross-linking of starch films. Food Chem. 2010;118(3):702–711.

16. Subramaniam P. The stability and shelf life of confectionery products. Food and beverage Stablity and Shelf Life. Woodhead Publishing Limited; 2011. 716–742 p.

17. Abbas Z, SACHIN S. Albumin Microspheres: New Approach for Sustained Drug Delivery. Indian J Nov Drug Deliv. 2012;4(1):2–16.

18. Marandi GB, Esfandiari K, Biranvand F, Babapour M, Sadeh S, Mahdavinia GR. pH Sensitivity and Swelling Behavior of Partially Hydrolyzed Formaldehyde-Crosslinked Poly (acrylamide) Superabsorbent Hydrogels. J Appl Polym Sci. 2018;109:1083–1092.

19. Tang H, Zhao W, Yu J, Li Y, Zhao C. Recent Development of pH-Responsive Polymers for. Molecules. 2019;24(4):1–24.

20. Schmaljohann D. Thermo- and pH-responsive polymers in drug delivery. Adv Drug Deliv Rev. 2006;58:1655–1670.