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Mucoadhesive Potentials of a Natural Polymer Obtained from the Seeds of *Dioclea reflexa* in Aminophylline Tablet Formulations

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Authors' contributions

This work was carried out in collaboration between all authors. Author CCM designed, wrote protocol, planned, sourced materials, carried out some of the evalution tests, data analysis, discussed results, prepared initial draft and revised the manuscript. Author CU literature search, tablet preparation, carried out some evaluation tests. Author PFB planning and supervision. Author MAM managed the literature search and discussion of results. Author OOK criticized the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The mucoadhesive potentials of a natural gummy polysaccharide obtained from the seeds of *Dioclea reflexa* Hook F., (Fam.: Papilionaceae) (DR) was evaluated under varying pH conditions using aminophylline tablet formulations.

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Study Design: The work was designed to experimentally evaluate the mucoadhesive effects of DR in comparison with that of a synthetic polymer, hydroxypropyl methylcellulose (HPMC).
Place and Duration of Study: The study was carried out in the Department of PT & RMD of NIPRD, Idu, Abuja, Nigeria between May 2011 and June 2012.
Methodology: The tablets were prepared by the wet granulation method using 1% w/w of the polymers as binders and 2.5% w/w as coating solutions, respectively. Some basic tablet characteristics were evaluated following standard procedures. *In vitro* drug release was also assessed after tablet coating. The mucoadhesive potentials at various pH levels: 1.2, 4.0, 6.5, 7.4 and 9.2, corresponding to those of the different gastrointestinal tract compartments, were evaluated by the wash-off method. The results were analyzed by analysis of variance (ANOVA) and *t*-test at $P = .05$.
Results: The release profiles showed that coating caused prolongation up to 8 h for both DR- and HPMC-coated tablets with the latter having significantly ($P < .05$) higher rates from 0.25 to 6 h (86.23±0.50: 44.10±0.31% and 101.71±0.20: 94.15±0.33% in 1 and 6 h for HPMC and DR tablets, respectively). The mucoadhesive potentials of DR were generally less than that of HPMC over the range of pH tested, except at 6.5 where it performed significantly ($P < .05$) better.
Conclusion: DR is useful as a mucoadhesive pharmaceutical excipient in aminophylline tablet formulations. It had optimum and better mucoadhesive strength than HPMC at the pH of 6.5 but less at 1.2, 4.0, 7.4 and 9.2.

Keywords: Mucoadhesion; pH; wash-off method; Dioclea reflexa; aminophylline.

1. INTRODUCTION

The discovery of potentials for new uses of materials is a major driving force for research into naturally occurring polymers which abound in our environments. Drug delivery systems based on adhesive properties of materials have been developed for both systemic and topically applied drugs, including nanomedicines [1]. Mucoadhesion is a phenomenon of interfacial molecular attractive forces between surfaces of a polymeric material and a mucosal layer. The term bioadhesion generally refers to adhesion between surfaces of a polymer and that of any biological substrate. The interactive forces allow the polymer to adhere to the biological surfaces thereby creating intimate contact for an extended period of time [2,3].

The use of mucoadhesive polymers for the development of pharmaceutical formulations dates back to 1947, when attempts were made to formulate a penicillin drug delivery system for delivering the bioactive agent to the oral mucosa using gum tragacanth and dental adhesive powders [4]. Research in this area has continued since then leading to the development of various bioadhesive polymeric systems and products for various biomedical applications [5]. The importance of mucoadhesive drug delivery systems includes increased drug availability, protection of labile molecules and the ability to provide controlled/prolonged drug release, properties which have been shown to be directly or indirectly related to increased *in situ* residence and intimate contact of these systems with mucosal surfaces [6,1]. With increase in contact time, release of the active ingredient can be extended or controlled therefore, dose/dosing frequency can be reduced.

A number of theories have been proposed to explain the mechanism of mucoadhesion [7-9]. Also, certain properties of a polymer including crosslinking, molecular weight, chain length, spatial arrangement and the presence and type of functional groups, have been noted to influence mucoadhesion [8]. Anionic polyelectrolytes have been found to form stronger adhesion when compared with neutral polymers [10,11]. It has been reported that chitosan (a cationic polyelectrolyte) exhibit excellent mucoadhesive property in neutral or alkaline medium [12]. Polymer concentration, contact time and environmental factors such as pH also affect mucoadhesion. Polymer concentration in the range of 1-2.5 wt % may exhibit sufficient mucoadhesive property for biomedical applications [8,13].

Various means have been identified for evaluating the mucoadhesive properties of polymers [8,14]. The wash-off method involves measurement of the time for the complete detachment of the attached delivery system from the mucosal layer [15]. Wash-off time, that is, the time taken for the tablet to detach from the mucosal surface, reflects the extent of its

retention and sustenance of release of the active ingredient in that region of the gastrointestinal tract (GIT). It actually represents the time taken to overcome the interactive forces of adhesion between the dosage form and the biological substrate.

Cellulose derivatives are among the polymers commonly used for mucoadhesive drug formulations [16]. *Dioclea reflexa* Hook F., (Fam.: Papilionaceae), is a tropical leguminous climbing/twining plant seeds of which are used locally, especially in eastern Nigeria, as thickener in soups and other food preparations [17]. Gum obtained from the seeds of *Dioclea reflexa* (DR) is believed to be composed of polysaccharides, mainly cellulose [Mbah et al. NIPRD, Nigeria, Unpublished report]. DR was selected in the present study because it is obtained from edible plant part and is already being used as food material (soup thickener) hence, the decision to further evaluate other possible pharmaceutical uses. The use of the polymer from edible DR seeds and other natural polymers for mucoadhesive purposes may be more advantageous because they are easily biodegradable unlike synthetic polymers of which biodegradability is uncertain [18].

Aminophylline [2 parts theophylline (3,7-Dihydro-1,3-dimethylpurine-2,6(1*H*)-dione or 1,3-Dimethylxanthine) plus 1 part ethylenediamine] is a xanthine phosphodiesterase inhibitor which causes bronchial smooth muscle relaxation, stimulates the CNS and myocardium and causes diuresis. It is used in the management of reversible airway obstructions such as asthma and in chronic obstructive pulmonary diseases like emphysema and chronic bronchitis. It is indicated in the dosage of about 800 to a maximum of 1500 mg in three to four divided doses daily or at twice daily for modified-release tablet formulations [19,20]. Although aminophylline is now less prominently used as first-line therapy due to the narrow therapeutic range and the requirement for monitoring of blood levels [21], however, nocturnal asthma can be improved with slow-release formulations [22]. The choice of aminophylline for this study was based on the fact that it is a prototype very soluble and orally administered drug which makes it a candidate for sustained release formulation. The selection was in the hope that a mucoadhesive tablet formulation of aminophylline and similar soluble drugs may offer sustained delivery and overcome the inconvenience of multiple dosing. Moreover, its use as a slow-release sustained delivery form may be apt in the management of nocturnal asthma.

This work was designed to formulate mucoadhesive controlled release tablets of aminophylline using the natural polymer (DR) extracted from the edible seeds of *Dioclea reflexa* and evaluate its *ex vivo* adhesion on pig intestinal mucosa in comparison with hydroxypropylmethyl cellulose (HPMC), a synthetic cellulose derivative.

2. MATERIALS AND METHODS

2.1 Materials

Pharmaceutical/food grade HPMC powder, aminophylline powder, maize starch, magnesium stearate (Sigma-Aldreich), commercial aminophylline tablet (Erica Niramaya EthicalsPvt Ltd., India) purchased from a pharmacy in Abuja, pig intestine obtained from a local abattoir in Abuja, DR powder obtained by extraction. Other analytical grade reagents were used without further purification.

2.2 Methods

2.2.1 Collection and identification of plant material

Seeds of *D. reflexa* were obtained from Karmo, a local market in Abuja, Nigeria and identified by Mrs. Ugbabe Grace at the herbarium of NIPRD (Herbarium Number: NIPRD/H/6413).

2.2.1.1 Extraction of polymer

The gummy polymer (DR) was extracted from the seeds of *D. reflexa* by the method previously described [26] and the dried powder used for this study. Some physicochemical characteristics of the polymer had been reported [23].

2.2.2 Preparation of tablets

Aminophylline tablets were prepared by the wet granulation technique [24] using the formula in Table 1. Homogenous dispersions of 1.0% w/v DR and HPMC powders in distilled water were employed as granulating fluids, respectively. The granules were dried in oven at 50ºC for 1 h before mixing with the lubricant, magnesium stearate powder. The granules were then compressed using a tableting machine (THP

Tianxiangb Chentai Pharmaceutical Machinery Co. Ltd., China) fitted with 11.5 mm flat-faced single punch and die at a compression force of 11.00 Kgf. Prior to compression, the punch and die were cleaned and lubricated with a 10.0% w/v dispersion of magnesium stearate in ethanol. The tablets were stored in air-tight screw-capped containers and kept into desiccators for 24 h before evaluation of tablet parameters. This was to enable the tablets equilibrate thereby allowing for elastic recovery, hardening and prevention of falsely low yield values [25].

2.2.3 Coating of tablets

A 2.5% w/w dispersion of the polymer (DR or HPMC powder) in distilled water was separately prepared. Equal volumes of the dispersions were carefully applied as thin coats to the face of each tablet by means of a syringe such that it dripped to cover the sides. The coated tablets were then dried in an oven at 50ºC for 3 h after which the reverse sides were treated the same way before being used for the mucoadhesive study.

2.2.4 Evaluation of tablets

The formulated and the commercial aminophylline tablets were evaluated for hardness, uniformity of weight, friability, thickness, disintegration time and *in vitro* drug release before coating, following standard test procedures.

2.2.4.1 Hardness test

The resistance of the tablets to crushing was tested by the diametric compression test using a hardness tester (ERWEKA HT, Germany) [26]. The tablets were individually placed in between the clamps of the device in such a way that each tablet was lightly gripped on two opposite points along its circumference and gradually increasing forces applied until it split along its diameter or crushed. The force required to split or crush the tablet was recorded as the hardness. Ten tablets per batch were used for the test.

2.2.4.2 *Uniformity of weight*

This was done following the British Pharmacopeia (BP) method [26]. Twenty randomly selected tablets were first weighed together, then individually, using a high precision analytical weighing balance (AB54 Mettler Toledo, Switzerland). The difference in weight was then obtained by comparing the individual weights with the average and per cent variation calculated.

2.2.4.3 Friability

The method prescribed for friability of uncoated tablets was followed [26]. The friability test was done to determine the ability of the tablets to withstand the stress of handling and transportation. Ten randomly selected tablets were carefully dusted, weighed together and placed in a Roche Friabilator (Erweka TA220, Germany). The instrument was then run at 25 rpm for 4 min. Then the tablets were removed, dusted and weighed again. The percentage of weight loss was determined as the friability thus:

$$\text{Friability} = [(\text{initial weight} - \text{final weight})/\text{initial weight}] \times 100\% \quad \ldots\ldots (1)$$

2.2.4.4 Disintegration time test

The disintegration time test was carried out on the tablets before and after coating in order to find out the effect of coating. The United States Pharmacopoeia (USP) method was adopted [27] using the Erweka® disintegration tester. This test was carried out using 6 tablets from each batch and 900mL of distilled water as medium. The equipment was maintained at a temperature of 37±2°C throughout the test. One tablet was placed on the mesh screen at the bottom of each of the 6 glass baskets and the equipment run with the tubes maintaining an up and down movement in and out of the medium at a frequency of about 28 to 32 cycles per min. until all the tablet particles passed through the screen (for the uncoated tablets). The disintegration time was noted by means of a stopwatch. The test was performed in triplicates for each batch.

2.2.4.5 Thickness of coat

To determine the thickness of coat on the tablets, the diameter and thickness were measured before and after coating using a Vernier caliper (Mitutoyo, Japan).

2.2.4.6 Swelling characteristics

The swelling characteristics of the DR- and HPMC-coated aminophylline tablets were studied in potassium phosphate buffer solutions of pH 1.2, 4.0, 6.5, 7.4 and 9.2, respectively. The tablets were first stored in desiccators overnight to remove any residual moisture and the procedure earlier described [28] adopted with

slight modifications. The dry tablets were separately placed on tarred glass plates measuring about 2 × 2 cm and weighed (W_d). These were then transferred into Petri dishes containing 60 ml of buffer solutions of pH 1.2, 4.0, 6.5, 7.4, and 9.2, respectively maintained at the laboratory temperature, 30°C. The test was also done using distilled water. At intervals, the glass plates with the hydrated tablets were removed, dried by carefully blotting with tissue paper and then weighed (W_t), until no further increase in weight. The swelling ratio (*SR*) was calculated using Eqn. 2:

$$SR = W_t/W_d \quad \text{……..} \quad (2)$$

2.2.4.7 In vitro drug release study

In vitro drug release was carried out on the tablets before and after coating. The test was performed using an Erweka dissolution apparatus following the paddle method at 50 revolutions per minute (rpm) [27]. The dissolution medium was 900mL distilled water maintained at 37±0.5°C. A standard solution of aminophylline in distilled water was prepared and scanned to obtain the wavelength of maximum absorption (λ_{max}) at 270 nm for analysis using a UV-Visible double beam spectrophotometer (UV-160A, Shimadzu Corporation, Japan) with 1 cm quartz cell. At specified time intervals, 5 mL samples were withdrawn from the medium and immediately replaced with equal volumes of distilled water maintained at the same temperature. The samples were analyzed spectrophotometrically at 270 nm. The *in vitro* drug release test was carried out in triplicates for both the uncoated DR- and HPMC-containing as well as the commercial aminophylline tablets over the period of 1 h and 8 h for the coated tablets.

2.2.4.8 Drug release kinetics

In order to find out the mechanism of drug release, the data obtained from *in vitro* study of the DR- and HPMC-coated tablets were fitted to kinetic models representing zero order, first order, Higuchi, and Korsmeyer-Peppas. The cumulative per cent of drug released against time, log percentage of drug remaining against time, cumulative per cent of drug released against square root of time and log cumulative per cent of drug released against log time were plotted and the regression analysis noted for zero order, first order, Higuchi and Korsmeyer-Peppas models, respectively [29].

2.2.4.9 Drug content test

The drug content test was carried out before coating of the tablets. Five tablets were powdered in a mortar and an equivalent of mean tablet weight was extracted with distilled water. The solution was filtered using a filter paper (Whatman No.1). The filtrate was suitably diluted with distilled water and analyzed for aminophylline (theophylline) spectrophotometrically at 270 nm.

2.2.5 *Ex vivo* Mucoadhesive test

2.2.5.1 Mucoadhesive test of DR

The mucoadhesive potential of the DR polymer was assessed by the weight method [30] with some modifications, using HPMC as reference. The *ex vivo* study complied with the requirements of NIPRD's Ethical Committee in accordance with the NIH Publication No. 85, revised in 1985. A piece of male pig intestinal mucosa freshly obtained from a local abattoir and stored in potassium phosphate buffer solution (pH 7.4) and equilibrated at 37±1°C to maintain viability, was glued to a glass slide, dorsal side down. Few drops of the 2.5% polymer dispersion was uniformly applied/spread on the underside of a glass slide to which strings of equal size and length with 2.0 g standard weights (Ohaus) attached at both ends, and carefully placed on the mucosal surface. The set up was allowed to stay in contact for 1.0 min to allow adhesion between the polymer and mucosa. Additional weights were then added to one side of the balanced slide until it slid over. The weight difference that caused the sliding movement was noted and recorded as the force of adhesion.

2.2.5.2 Mucoadhesive test of the DR- and HPMC-coated tablets

Ex vivo mucoadhesion of the polymer-coated aminophylline tablets was carried out by an improvised lever balance method. A freshly excised male pig intestinal mucosa stored and hydrated in potassium phosphate buffer solution (pH 7.4) was prepared as above. The coated tablet was glued to the underside of a weighed aluminum foil packing and suspended on one arm of the lever system by a cotton thread measuring 11.0 cm long. A fabricated pan of equal weight was also suspended on the opposite arm of the lever with a thread of equal length (Fig. 1). The mucosa was then positioned to ensure contact with the tablet under a 20.0 g

weight for 2.0 min. After 2.0 min, the 20.0 g weight was removed and standard weights added gradually on the pan until the tablet detached from the mucosa contact. The total weight that caused detachment was recorded as the mucoadhesive strength of the polymer coating. The test was performed in triplicates.

2.2.5.3 Wash off Mucoadhesive test

The wash off method previously described [15] was adopted with slight modifications. A piece of pig intestinal mucosa measuring about 7 x 2.5 cm, was freshly cut with a scalpel and adhering fecal material carefully removed before being attached to the surface of a microscope slide using a double sided adhesive tape. The set up was then suspended on the arm of a disintegration tester using a string and a clip. Phosphate buffer saline solutions of varying pH: 1.2, 4.0, 6.5, 7.4 and 9.2 were prepared [26] and used as the media for the wash off experiment. The polymer-coated tablets were then attached to the mucosal surface for a contact period of 60 s and the apparatus switched on, making an in and out movement into the medium until the tablets detached. The medium was maintained at 37±1ºC throughout the test. The time taken to detach was noted and the procedure repeated for each media. The test was performed in triplicates for both the DR- and HPMC-coated tablets.

2.2.6 Data analysis

All the tests were carried out in triplicates. Statistical analysis of the results was performed by one way analysis of variance (ANOVA and paired *t*-test using Excel Microsoft Office, 2007. Statistical significance was taken at (P = .05), with P values less than .05 considered significant.

Table 1. Formula for preparation of tablets

Ingredient	Amount (mg)
Aminophylline (Sigma)	100.00*
DR or HPMC (1%w/w))	3.00*
Maize starch (10%w/w)	30.00*
Magnesium stearate (1%w/w)	3.00*
Lactose *qs*	300.00

**per tablet weighing 300 mg"*

Fig. 1. Photograph of *Ex vivo* mucoadhesive test set up for the DR- and HPMC-coated tablets

3. RESULTS AND DISCUSSION

Table 2 shows some results obtained from the tablet evaluation, namely: hardness, uniformity of weight, friability, disintegration time and thickness of coat tests.

The results showed that the aminophylline tablets formulated with DR and HPMC as binders had mean hardness of 6.88±0.85 and 6.75±0.65 kgf, respectively, while the commercial brand had a value of 6.8±3.6 Kgf, all of which complied with specifications. It had been stated that well compacted tablets would give hardness of up to 6 Kgf or more [31]. Also a range of 4-8 Kgf had been given as values obtainable for crushing strength of tablets [32].

All the tablets had their friability values between 0.30±0.05 and 0.33±0.20%. This means that all the tablets passed the friability test as the results lay below the official requirement of not more than 1% w/w, implying they are firm enough to withstand friction due to shock and abrasive forces in the course of handling and transportation [33].

The weight variation studies showed that the tablets had average weights of 299.48±5.4 mg, 299.53±3.5 and 300.00±1.0 for the formulated DR, HPMC and the commercial brand, respectively. The variations in weight of 0.17±0.52, 0.16±3.5 and 0.00±1.0% were obtained for the DR, HPMC and commercial tablets, respectively. The commercial brand however had least or nearly no variation from the average weight. The results were in accordance with the BP requirement of not more than two (2) individual masses deviating by over 5% from the average mass, for oral tablets weighing 250 mg or more. This indicated that each of the tablets would most likely contain the labelled amount of active ingredient.

Results (Table 2) showed that both the HPMC and DR tablet formulations disintegrated in 9.0 min before coating while the disintegration time of the commercial brand was 8.33±0.52 min. After coating, the disintegration time increased by 100.00 and 233.33% for the HPMC and DR tablets, respectively. The results indicated that coating significantly increased disintegration time. This increase would likely lead to delayed release of the active ingredient.

The results of swelling characteristics of the coated tablets are shown in Figs. 2-4. Figs. 2 and 3 show that the two coated tablets exhibited similar pattern of swelling in the buffer media tested. The SR increased with time up to certain maximum then decreased to zero, indicating the point of total removal/erosion of the polymer coats. However, while total removal of coat occurred in about 3 h in the HPMC-coated, it took over 4 h for the DR-coated tablets. The similar swelling pattern might indicate that DR is also a hydrophilic polymer like HPMC. SR in the buffer media tested were of the order: pH 9.2 > 1.2 > 6.5 > 7.4 > 4.0 and 1.2 > 6.5 > 9.2 >4.0 > 7.4 for the DR-coated and HPMC-coated tablets, respectively.

Swelling of hydrophilic polymers in aqueous media is diffusion-controlled [34]. As water wetted and diffused into the coat, swelling of the polymer network occurred forming a gel layer around the tablet. Subsequently, the gel layer dissolved or eroded into the medium. From the swelling behaviors exhibited (Figs. 2-4), the two polymer coats might not be said to have formed very stable long-lasting gels around the tablets considering the extent and time. This might be attributed to thinness of the coats. Maintenance of swollen state of hydrogels over time is a result of balance between cohesive and hydration forces on the polymer chain network, which arises from the interaction of water molecules and the polar groups, mainly –OH and –COOH, on the polar backbone or side chains [35]. It had been found that the degree of swelling in different solvents varies with the type of polymer and solvent [36]. The swelling patterns of both DR- and HPMC-coated tablets in distilled water, (Fig. 4) showed increase up to certain maximum then decrease to below zero level. However, DR had higher SR than HPMC over the period of test. Zero SR indicated a point when all the gels formed around the tablet must have been completely eroded into solution. HPMC is a synthetic water soluble polymer [37]. This might have accounted for faster erosion of the HPMC gel than that of the natural DR polymer. Furthermore, the higher SR of DR than HPMC might be attributed to the relative numbers of polar groups in the polymers.

It has been argued that hydration of HPMC is not affected by natural variation in pH throughout the gastrointestinal tract (GIT). However, pH of the medium may affect dissolution rate of the active constituent primarily due to its effect on solubility of the drug [38]. Polymers with characteristic swelling profiles may offer versatile pharmaceutical applications including mucoadhesion.

The results obtained from the drug content test of the tablets were 85.60±0.57, 85.29±0.46 and 86.60±0.11%, for the DR, HPMC and commercial brand tablets, respectively, which complied with the BP requirement of 80.6 to 90.8% of theophylline in the stated amount of aminophylline [26].

Fig. 5 shows the release profiles of the aminophylline tablets before coating. The graph shows that all the tablets generally showed good release profiles. The dissolution rate profiles showed that all the tablets released their contents steadily with time up to about the first 15 min, after which it decreased slightly to a nearly stable rate for the rest of the test period. However, the rate of release differed slightly in the order: commercial brand > HPMC > DR. The differences observed might be attributed to formulation variables, such as influence of other excipients and drug: polymer ratio.

Table 2. Results of some tablet evaluations

Parameter	HPMC tablets	DR tablets	Commercial tablets
Hardness (kgf)*	6.75±0.65	6.88±0.85	6.8±3.6
Weight Variation (%)**	0.16±3.5	0.17±0.52	0.00±1.0
Drug Content (%)	85.29±0.46	85.60±0.57	86.60±0.11
Friability*	0.30±0.05	0.33±0.04	0.33±0.20
Disintegration time (min)*:			
Before coating:	9.0±0.50	9.0±0.97	8.33±0.52
After coating:	18.0±0.50	30.0±0.97	
Thickness of coat (mm)*:			
Diameter:	0.03±0.03	0.03±0.06	
Thickness:	0.42±0.26	0.41±0.14	

**(n=3); **(n=20)*

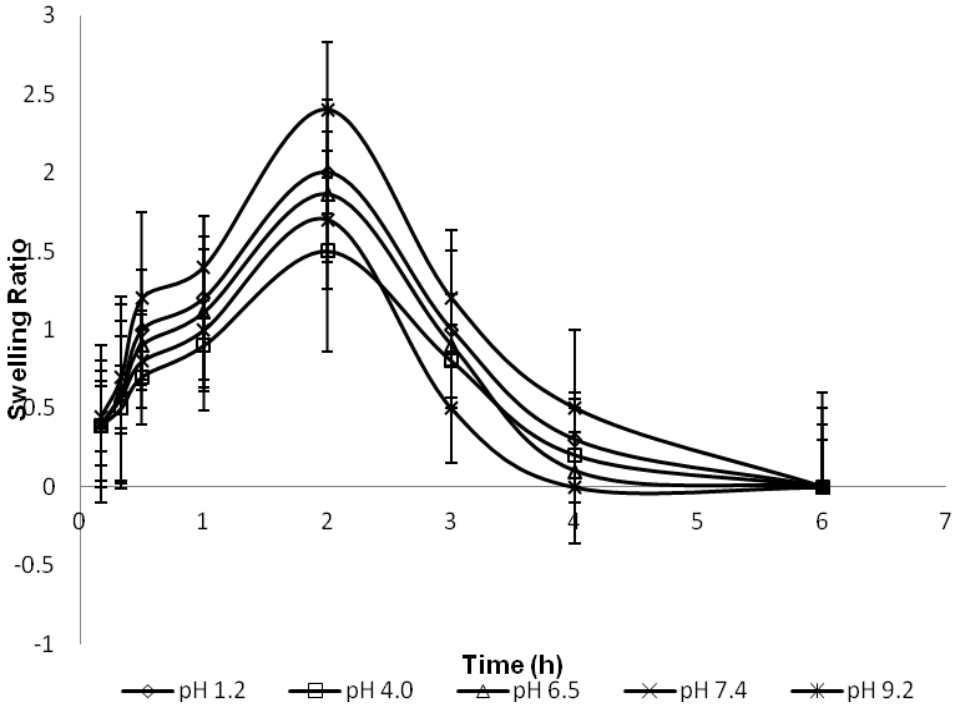

Fig. 2. Swelling profile of the DR-coated tablets in media of varying pH

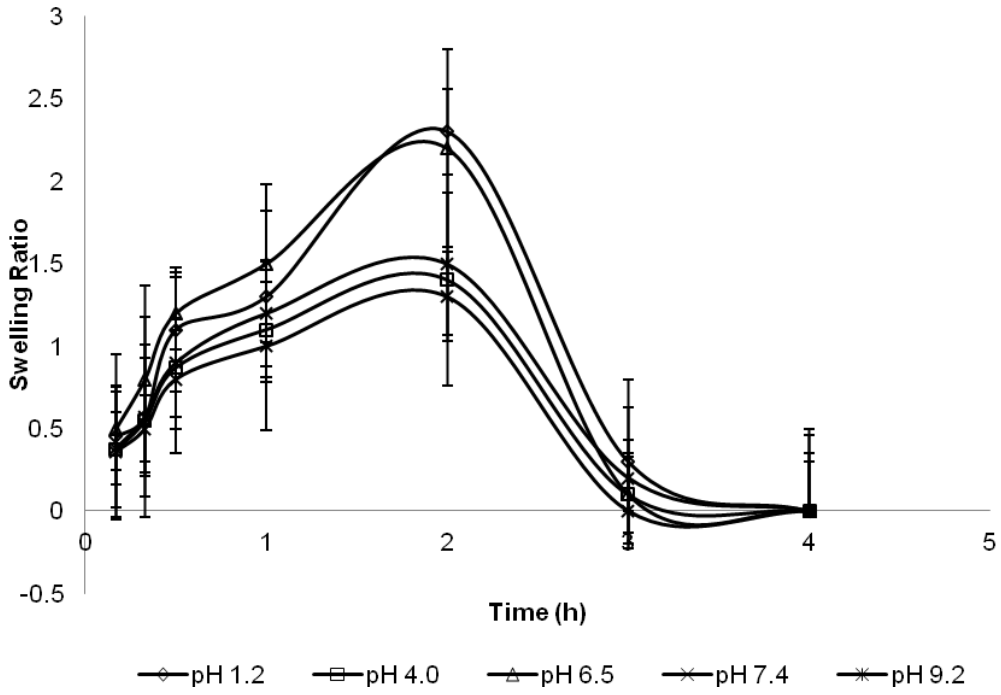

Fig. 3. Swelling profile of the HPMC-coated tablets in media of varying pH

However, all the tablets tested complied with the official specification of at least 75% release of the active ingredient within the first 45 min [27]. As the graph shows (Fig. 5), the control, pure aminophylline, freely and completely went into solution reaching the peak/steady level before 10 min.

After coating, the release profiles extended over 8 h (Fig. 6). The HPMC- coated tablet showed a higher release rate compared to that of DR, with significant (P < .05) differences between 0.25 and 6.0 h. The cumulative amounts released in 1 h were 86.23±0.50 and 44.10±0.31% for the HPMC- and DR-coated tablets, respectively. In 5 h the rates increased to 101.53±0.30 and 49.87±0.23% for the HPMC- and DR-coated tablets, respectively. The rate for the HPMC-coated tablet increased steadily up to the first 2 h then gradually till completion in 8 h. On the other hand, the release rate of the DR-coated tablet increased gradually up to the first 0.583 h (35 min) then slowed down, maintaining only slight increases up to 49.87±0.23% by the 5th h. Beyond this point, the release rate increased markedly to 94.15±0.33 and 100.45±0.66% in the 6th and 8th h, respectively. The sharp increase in rate might be due to a burst effect attributable to sudden erosion or dissolution of the coating. By the 6th and 8th h, the differences in rates of release narrowed to 101.71±0.20 : 94.15±0.33 and 101.53±0.30 : 100.45±0.66% for the HPMC- and DR-coated tablets, respectively. The observations showed that coating of the aminophylline tablets with the synthetic HPMC and natural DR polymers influenced (slowed) drug release. The controlled drug release was only limited to a few hours probably because of the thin size of coat. When hydrogels such as HPMC come in contact with aqueous media, they hydrate rapidly forming gelatinous layer round the tablet core. As water diffuses into the gel layer and the core, swelling and drug release occurs [34]. Release of very soluble drugs, such as aminophylline, from the core occurs largely by diffusion through or erosion of the gel layer. Therefore, the higher rate of release observed for the uncoated tablets reflected the delayed-release caused by presence of mucoadhesive coats on the DR- and HPMC-coated tablet formulations.

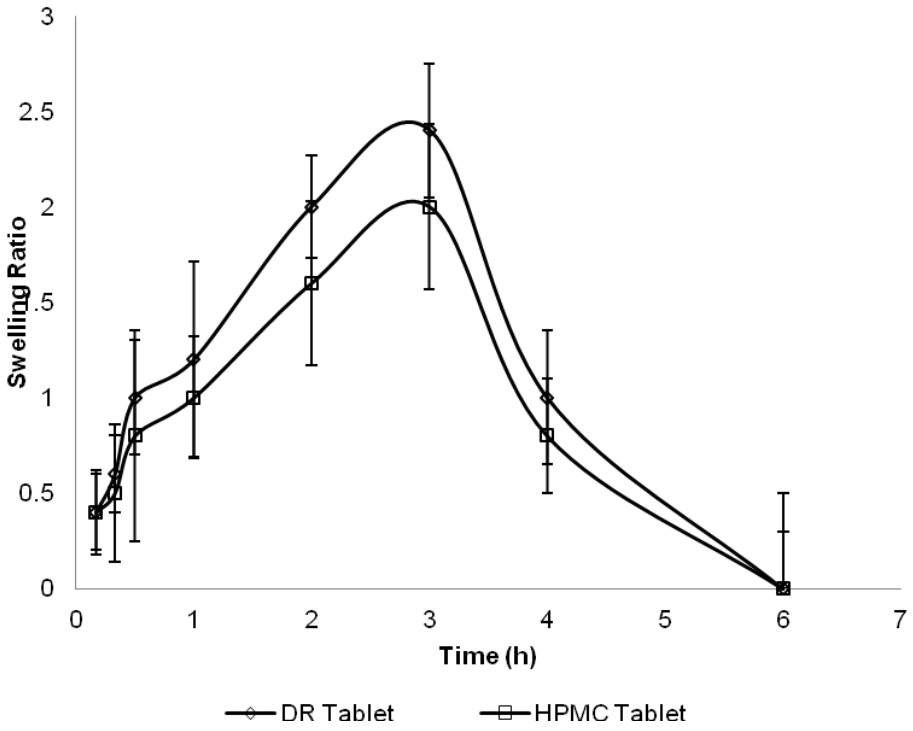

Fig. 4. Swelling profile of the DR- and HPMC-coated tablets in distilled water

Results of the drug release kinetics analysis indicated diffusion mechanism, as the plots showed high linearity (R^2 values in the range: 0.9469 to 0.9963 (Table 3). The Korsmeyer-Peppas plot further revealed involvement of anomalous release, with the release exponent (slope 'n') being 1.0500 and 1.4100 for the DR- and HPMC-coated tablets, respectively. Diffusion exponent, n equal to 0.45 is said to indicate Fickian diffusion, 0.45 < n < 0.89 anomalous (non-Fickian diffusion and erosion), 0.89 case II transport, > 0.89 super case II transport [29]. Anomalous release indicates occurrence of more than one mechanism. While Fickian release mechanism follows the usual molecular diffusion of the drug from the tablet, case II is associated with stresses in the polymer coating, including disentanglement and erosion. It appeared therefore, that both diffusion and case II transport, which describes release from swollen polymer, occurred independently in the release processes.

Results of the mucoadhesive test of the polymers showed mucoadhesive strengths of 1,400.00±0.00 mg and 1,100.00±0.00 mg, under the experimental condition for the DR and HPMC, respectively. For the coated tablets, the mucoadhesive strengths were 1,300.00±0.00 mg and 1,000.00±0.00 mg for the DR- and HPMC-coated. The results showed that DR had higher mucoadhesive strength than the HPMC.

Fig. 7 shows the mucoadhesive profiles of the HPMC- and DR-coated tablets at varying pH in terms of wash off (retention) time. The results showed that HPMC generally had higher mucoadhesive activity than DR gum at most of the pH levels, except at pH 6.5. At pH 6.5, the mucoadhesive effect of DR was higher than that of HPMC. HPMC showed its peak mucoadhesive activities at both slightly acidic and slightly alkaline pHs (pH 4.0 and 7.4, respectively). On the other hand, DR gum showed its peak mucoadhesiveactivity at only slightly acidic pH 4.0. It is not certain whether the high mucoadhesive effect of DR at pH 4.0 was probably due to possession of cationic functional groups which ionized under that condition and bound to mucin polymer strands by electrostatic interactions, hydrogen bonding, other electrochemical forces or if some different factor(s) were responsible. This, however, requires further investigation since the chemical nature of DR polymer is still under study.

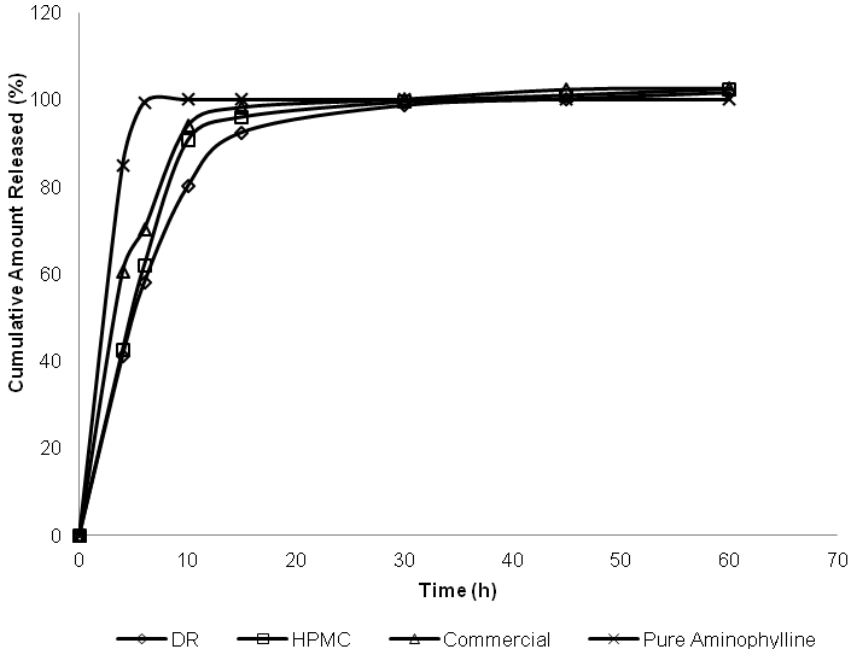

Fig. 5. *In vitro* release profiles of the aminophylline tablets before coating

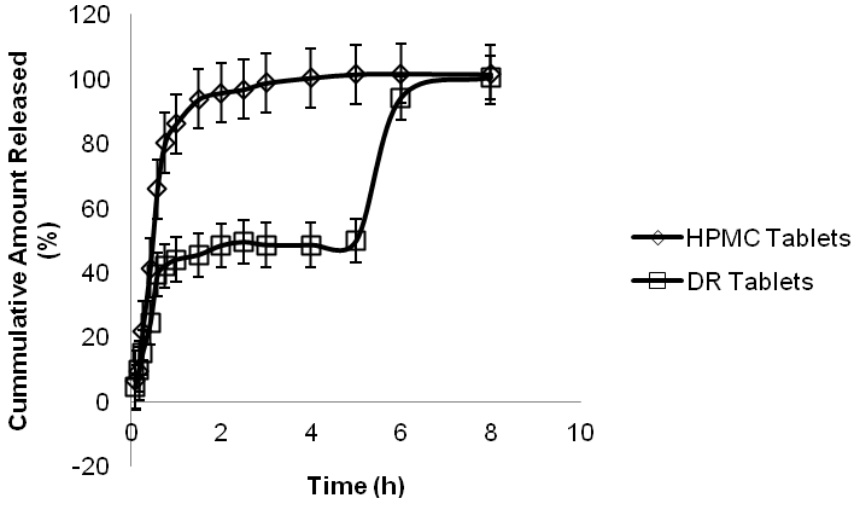

Fig. 6. *In vitro* drug release from the coated HPMC and DR tablets

Table 3. Results of the drug release kinetics analysis for the DR- and HPMC-coated tablet formulations

Formulation	Drug release kinetics (R^2 value)				
	Zero order plot	First order plot	Higuchi plot	Korsmeyer-peppas plot	
DR-coated tablet	0.9874	0.9469	0.9707	0.9963	Slope (n) = 1.05
HPMC-coated tablet	0.9923	0.9753	0.9736	0.9784	Slope (n) = 1.41

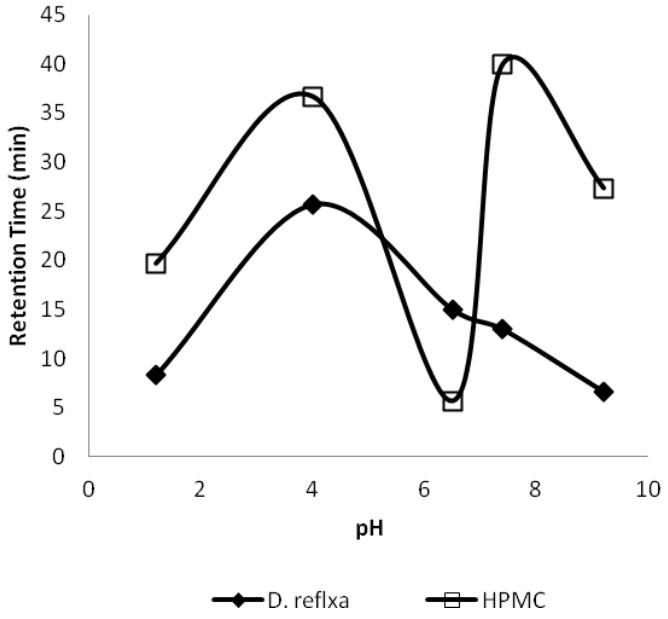

Fig. 7. Mucoadhesive profiles of HPMC and DR at varying pH. Wash-off time (min) of the polymer-coated tablets from pig intestinal mucosa, as measured with a stop clock in various pH media, was taken as an index of extent of mucoadhesion. DR was only significantly higher than HPMC at pH 6.5 ($P < .05$), (n=3). Measurements were carried out in phosphate buffer solutions maintained at 37±2ºC

The property of mucoadhesion on biological surfaces is largely due to the mucin content. Mucin is an anionic polyelectrolyte, which together with water (> 95%) constitutes over 99% of mucus [8]. Adhesion between polymer and mucus membrane involves three stages, namely, wetting, penetration and mechanical interlocking [16].

It has been reported that polymers with both cationic and anionic polyelectrolyte functional groups ionize at high and low pH values due to their amphoteric nature and this aids their interaction with mucin chains hence, improving their mucoadhesion [8]. This may account for the mucoadhesive properties exhibited by the DR and HPMC in this study. Both polymers exhibited relatively lower mucoadhesion around neutral pH with DR being significantly ($P < .05$) stronger than HPMC at pH 6.5. The fact that the DR-coated tablet was specifically good in mucoadhesion at pH 6.5 might indicate that the tablet may adhere more in parts of the GIT having similar hydrogen ion concentration. Therefore, more of the active content may likely be released in a sustained manner in such regions of the GIT. Although non-ionic, HPMC is a long chain polymer [37] and possesses large numbers of OH groups which form physical (including hydrogen) bonds that are responsible for mucoadhesion [16]. The observation of least mucoadhesive strength around neutral pH might indicate minimal or no ionization of the component molecular moieties of the polymers under that condition. This thought is in line with a previous report which said that at neutral or close to neutral pH values, the ionization potentials of polymers with polyelectrolyte functional groups decrease and other factors such as polymer molecular weight, chain length and spatial arrangement start to play a stronger role in mucoadhesion [39].

The 'wash off' test model used to test for the mucoadhesive potential of DR is based on the principle that as a constant washing effect is applied to a polymer attached to a piece of mucosal tissue, a point is reached at which the strength of adhesion becomes unable to sustain contact. The polymer then disengages from the mucosal surface [8]. Mucoadhesive property is dependent on many factors. For instance, the presence or absence of functional groups such as hydroxyl, carboxyl and amino groups can

influence mucoadhesion [40]. Such functional groups may ionize and bring about charge distribution on the polymer chains which would cause interactions with the mucosal linings and have remarkable effects on bioadhesion. Mucoadhesive polymers may also form physical and chemical entanglements with mucous glycoprotein followed by hydrogen bonds with sugar residues on oligosaccharide chains that result in the formation of a strengthened mucous gel network which allows the formulations to remain adhesive for an extended period of time [41]. Also changes such as shrinkage or swelling of the polymer chain can be observed when environmental conditions change, namely ionic strength, pH and osmotic pressure [42], or when additional materials are added [43]. The ionization of the functional group is dependent on the pH of the external medium. Hence, change in the pH of the external environment may play an important role in tailoring mucoadhesive properties of polymers. Furthermore, increasing amount of mucoadhesive polymer, such as HPMC, in a formulation increases its bioadhesive strength [16]. Therefore, the mucoadhesive effects of HPMC and DR polymers observed in this study would likely improve if the thickness of coating were increased.

Study on structural elucidation of DR gum polymer is to be undertaken in order to understand its molecular constitution and properly explain the mechanism of its mucoadhesion. The discovery of DR gum as a novel polymer with better mucoadhesion than HPMC at pH 6.5 may present it as a more suitable excipient for the formulation of mucoadhesive aminophylline tablets and perhaps other drugs for controlled and targeted release in such media.

4. CONCLUSION

The experiment showed that DR gum could be a suitable excipient for use in the formulation of mucoadhesive controlled release aminophylline tablets by the wet granulation technique. The DR gum showed generally less mucoadhesion at pH 1.2, 4.0, 7.4 and 9.2 than HPMC. However, it performed better at pH 6.5 as assessed by the wash off method. Therefore, DR may only be more suitable than HPMC as a mucoadhesive controlled release excipient in formulations intended for release to body regions having pH around 6.5 such as from the duodenum down the upper part of small intestine. Further investigation is needed to establish the functionality of DR as a mucoadhesive excipient.

CONSENT

This is not applicable.

ETHICAL APPROVAL

The authors declare that the *ex vivo* study complied with the requirements of NIPRD's Ethical Committee in accordance with the NIH Publication No. 85, revised in 1985.

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COMPETING INTERESTS

The authors state that there is no conflict of interest. This work was carried out by the authors without external support.

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