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Research Article

Evaluation of Mucoadhesive Properties of Native and Modified Starches of the Root Tubers of Cocoyam (*Xanthosoma sagittifolium*)

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ABSTRACT: The purpose of this study was to evaluate the bioadhesive properties of native and modified cocoyam (*Xanthosoma sagittifolium*) starches. The methods of modification were by pregelatinisation and acetylation. The starch particles were evaluated for characteristics like particle size, swelling ability, viscosity, and mucoadhesion. The mucoadhesive evaluation of the starches were done using the rotating cylinder methods in 0.1M HCl and Phosphate buffer (pH 6.8) to simulate the stomach and small intestine respectively. The mechanical properties of the compacted starches were determined using friability and crushing strength.The particles so prepared had irregular shape size ranged from 9.38 to 10.67mm. Mucoadhesion time was in the order Acetylated>Native>Pregelatinised starch in 0.1M HCl and Pregelatinised>Native>Acetylated starch in pH 6.8 Phosphate buffer. None of the severe signs such as appearance of epithelial necrosis, sloughing of epithelial cells were observed in ileum sections. The work concludes that modified cocoyam starches could be useful in targeted mucoadhesive drug delivery.

Keywords: Cocoyam starch, Mucoadhesion, starch modifications.

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INTRODUCTION

Over the last decade, bioadhesive polymers have been widely used in designing drug delivery systems to improve buccal, nasal, oral, ocular and vaginal administration of drugs, and considerable attention has been paid to the development of novel bioadhesive materials (Lee *et al*, 2000; Edsman *et al*, 2005; Dubolazov *et al*, 206). The process of bioadhesion involves the formation of a tight and intimate contact between the mucosal surface and the polymeric chains of the bioadhesive system. Bioadhesives utilise the ability of water-soluble polymers that become adhesive on hydration and hence can be used for targeting a drug to a particular region of the body for extended periods of time and releasing their drug content in a slow and gradual manner (Chowdary *et al*, 2004).

Bioadhesive polymers are synthetic or natural macromolecules which are capable of attaching to mucosal surfaces. The concept of bioadhesive polymers involve a promising strategy to prolong the residence time and to improve the specific localization of drug delivery systems on various membranes (Grabovac *et al*, 2005).

Bioadhesive polymers usually contain hyroxyl or carboxyl groups which help with the formation of hydrogen bonds with the mucosal surfaces. Typical polymers that have been used as bioadhesive drug carriers are poly(acrylic acid) (PAA) (Singla *et al*, 2000), poly(methacrylic acid) (Quintanar-Guerrero *et al*, 2001), cellulose derivatives (Suzuki *et al*, 1999), poly(ethylene oxide) (Tiwari *et al*,1999; Di Colo, 2001), lectin (Lehr, 2000) and chitosan. Of these, PAA

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and its crosslinked commercial forms, Carbopol® and Polycarbophil, exhibit strong bioadhesive properties (Chun *et al*, 2002). However, their severe mucosal irritation potential limits their use as bioadhesive drug carriers (Adriaens, 2003). Hence there have been attempts in recent times to develop natural polymers as bioadhesive drug delivery systems (Ameye *et al*, 2005). Starch is the second most abundant renewable polymer in nature that is inexpensive, fully biodegradable and widely studied for many years in the field of materials (Doane, 1992; Shogren *et al*,1994). It is a glucose polymer $(C_6H_{10}O_5)n$ abundant in the seeds of cereal plants and in bulbs and tubers. Starch grains are composed of two kinds of molecules— amylose and amylopectin. Chemical modification of starch is often required to better suit its properties to specific applications. Modification of the starch hydroxyl groups to form appropriate degree of substitution (DS) (1.5–3.0 DS) and high % DS (50–95%) imparts thermoplasticity and water resistance to the modified starch over the unmodified one.

Starch modification, which involves the alteration of the physical and chemical characteristics of the native starch to improve its functional characteristics, can be used to tailor starch to specific food applications. Starch modification is generally achieved through derivatization such as etherification, esterification, cross-linking and grafting of starch; decomposition (acid or enzymatic hydrolysis and oxidization of starch) or physical treatment of starch using heat or moisture. Chemical modification involves the introduction of functional groups into the starch molecule, resulting in markedly altered physico-chemical properties. Such modification of native granular starches profoundly alters their gelatinization, pasting and retrogradation behaviour (Singh *et al*, 2007).

The aim of the present work is thus to evaluate the biadhesive propereties of native and modified starches obtained from the root tuber of Cocoyams (*Xanthosoma sagittifolium*) for potential use in drug delivery systems.

MATERIALS AND METHODS:

Native starch was extracted from the tubers of cocoyam starch according to Alves *et al*. (1999). The starch was further modified physically by pregelatinisation (Adedokun *et al*, 2010) and chemically by acetylation (Singh *et al*, 2004). Briefly for pregelatinisation, 200g of native starch sample (*Xanthosoma sagittifolium*) was dispersed in 200mls of distilled water and heated with slow mixing until a paste was obtained. The pregelatinized starch obtained was laid on ceramic tiles as a thin film and dried at 100°C for 24 hours. It was then milled into smaller bits and sieved through a mesh with size 0.315mm and the resulting powder was stored in an air tight container. While for acetylation, 200g of the sample of the native starch was weighed into a wide mouth container. 60mls of sodium hydroxide (50%) was mixed into the powder. 100mls of acetic anhydride was then added gradually into the mix with thorough mixing. After it had been thoroughly mixed, the mixture was then spread on ceramic tiles and dried in the oven for 24 hours at 100°C. The dried lumps were blended, sieved through a sieve of mesh size 0.315mm and stored in an air tight container.

Determination of the physicochemical properties of starch:

Identification test: One gram each of the native, pregelatinized and acetylated starch were weighed for the cocoyam sample and mixed with 50ml of water. This was then boiled for one minute and cooled. To 1ml of the mucilage obtained, iodine solution was added and the resultant colour observed.

Swelling capacity: The experiment was carried out in a 100-mL graduated cylinder. The initial bulk volume of 1 g of each starch sample was noted and water was added in sufficient quantity to yield a 100-mL uniform dispersion. The sediment volume of the swollen mass was noted after 24 h at room temp. The swelling ratio was calculated by taking the ratio of the swollen volume to the initial bulk volume (Bowen *et al*, 1984).

Particle size determination: The mean particle size of samples of the native, pregelatinized and acetylated starch of cocoyam were determined microscopically with the aid of a calibrated eyepiece. The particle size of each sample dispersed in glycerol was calculated.

Angle of repose: The fixed-funnel and free-standing method was used to measure the angle of repose. The measurements were made in triplicate and the mean angle of repose was calculated.

Bulk density determination: The bulk density of the powder bed is simply the weight of the powder divided by the whole volume of the bed. Ten grammes each of the powders (native, pregelatinized and acetylated) were poured inside a measuring cylinder made of glass through a funnel at zero pressure. The bulk volume was recorded and the density determined in triplicates.

Determination of particle density: The particle density of the starch powders were determined by the

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pycnometer method using xylene as the displacement fluid.

pH of 0.1% solution
0.1% of the three starches were made and their pH taken. The results were then recorded.

Disintegration time: The disintegration time of the tablets were determined in distilled water at $37^0 \pm 0.5^0$C using the Apex disintegration testing Apparatus (Apex Construction Ltd; Northflect Gravescent and Darford, Kent, U.K).

Hardness and friability test.: The load required to break the tablet was determined at room temperature using atablet hardness tester, 400060, Model:EHHO1 (DBK Instrument, Mumbai, India). Five tablets, randomly selected, were used from each sample for the test. Friability was evaluated as the percentage weight loss of 20 tablets tumbled in a friabilator (Veego Scientific Devices, Mumbai, India) for 4 min at 25 rpm.

Determination of the mucoadhesive properties of cocoyam starch using the rotating cylinder method.
The rotating cylinder method is a method which can be carried out with a slightly modified dissolution apparatus described in the USP (Werle *et al*, 2007). An intestinal segment is fixed on a stainless-steel cylinder, the basolateral side facing the cylinder. Then the tablets containing the mucoadhesive polymer are pressed on the apical side and the cylinder is put into a medium containing about 500ml of buffer medium (pH 6.8) and 0.1N HCl. The rotation speed was about 50rpm. The time the tablets detach from the mucosa is observed.

Histopathological Evaluation of Mucosa: The histopathological evaluation of tissues immersed in phosphate buffer (pH 6.8) and that in 0.1N HCl were compared with tissue before adhesion. Tissue was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Sections were cut on glass slides and stained with hematoxylin and eosin. Sections were examined under a light microscope to detect any damage to the tissue.

Statistical analysis: Statistical analysis was done on the results obtained using Students' t-test and ANOVA. At 95% confidence interval, *p* value lower or equal to 0.05 was considered the limit of significance.

RESULTS

Swelling capacity is a measure of the ability of the starch absorb and to retain fluid. This is of importance as the first stage in mucoadhesion is characterized by the contact between the mucoadhesive and the mucous membrane, with spreading and swelling of the formulation, initiating its deep contact with the mucus layer (Hagerstrom, 2003). The native starch had the highest swelling capacity, while the pregelatinised starch had the lowest (Barimah *et al*, 2009) . The swelling capacity of the starches was in the rank order Native>Acetylated>Pregelatinized. While the pH of the starches was generally acidic with the pregelatinized starch the most acidic (Table 1). Flow properties of the powder are determined from the value of the angle of repose. Powder flowability have been found to depend on three general characteristics: the physical properties of the particle (e.g., shape, size, compressibility), the bulk powder properties (e.g., size distribution, compaction), and the processing environment (e.g., storage, humidity) (Rios, 2006). The results in Table 1 show the rank order of angle of repose native cocoyam starch > pregelatinized starch > acetylated starch. The results also indicate that the particle density of the pregelatinized cocoyam starch was the highest, while the pH values show that the pregelatinized cocoyam starch was the most acidic of the three. Compacts obtained from the starches showed the pregelatinized starches with the highest friability and acetylated non-friable. However, the native starches exhibited the highest crushing strength values with the acetylated being the least (Table 2).

TABLE 1:
Physical characteristics of native, pregelatinized and acetylated cocoyam starch

Starch	Swelling capacity	Angle of repose (θ)	Bulk density (g/cm^3)	Particle density (g/cm^3)	pH
Native	0.76 ± 0.01	67.55 ± 0.11°	0.416 ± 0.03	1.81 ± 0.04	5.44 ± 1.01
Pregelatinized	0.58 ± 0.12	64.32 ± 1.17°	0.437 ± 0.01	2.33 ± 0.12	4.63 ± 0.09
Acetylated	0.62 ± 0.05	59.04 ± 0.16°	0.546 ± 0.12	1.91 ± 0.04	6.13 ± 1.41

n=5; standard deviation for five determinations

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The crushing strength of tablets the native cocoyam starch had the highest value of 31.98N, the tablets of the pregelatinized cocoyam starch then followed with 31.06N and the tablets of the acetylated cocoyam starch was the least with 24.88N. This implies that the native cocoyam starch made the hardest tablets since it took more force to break it than the other two modifications of the starch; where the pregelatinized cocoyam starch made harder tablets than the acetylated cocoyam starch.

TABLE 2:
Tablet properties of native, pregelatinized and acetylated cocoyam

Starch	Friability (%)	Crushing strength(N)
Native	1.55 ± 0.02	31.98 ± 6.04
Pregelatinized	5.41 ± 0.01	31.06 ± 3.32
Acetylated	0.00 ± 0.00	24.88 ± 7.74

n=4; standard deviation for four determinations

TABLE 3:
Viscosity parameters for native, pregelatinized and acetylated cocoyam starches.

Starch	Peak Viscosity (RVU)	Trough (RVU)	Final Viscosity (RVU)
Native	372.75	205.58	340.92
Pregelatinized	313.25	183.67	280.83
Acetylated	285.67	169.25	230.64

Friability describes the ease to which the tablets can be reduced to tiny particles. The tablets of the pregelatinized cocoyam starch were the most friable of them with a percent friability of 5.41; the tablets of the native cocoyam starch followed with a percent friability of 1.55, while the tablets of the acetylated cocoyam starch had a zero percent friability.

The peak viscosity of the native starch (372 RVU) was much higher than that of the pregelatinized (313RVU) and acetylated (285RVU) starches. These changes by acid modification and chemical modification will cause the modified starches to be much more soluble than the native starch (Table 3).

The results show that the native starch had the highest value of viscosity . This was closely followed by the pregelatinized cocoyam starch while the acetylated cocoyam starch had the least values. The peak viscosity is also a measure of the stability of the starch showing resistance to breakdown of their granules. The mucoadhesion time for the compacts of the various starches on excised pig's ileum in 0.1M HCl and Phosphate buffer pH 6.8 is given in Fig 1. While these times were observed to be relatively short, the starches when blended with other polymers could be used to enhance the specificity of these polymers in acidic media. This rate of detachment may have been due to the fact that the acetylated cocoyam starch has a pH of 6.13, indicating that the pH is moving close to alkaline pH therefore making it more stable in the acidic medium as to be able to attach for a longer period of time than the other tablets. The tablets of the native cocoyam starch also had the tendency to adhere for a longer period than the pregelatinized cocoyam since its pH of 5.44 was higher and closer to alkaline pH than the pregelatinized cocoyam starch with pH of 4.63.

Whereas in the phosphate buffer (pH 6.8), the tablets of the pregelatinized cocoyam starch had the longest time detachment (22.0 secs), followed by the tablets of the native cocoyam starch (7.00 secs) and the tablets of the acetylated cocoyam starch had the least time of detachment of 3.54secs (p <0.05) . This trend may also have been due to the pH of the starch used in relation to the pH of the medium i.e. the tablets of the pregelatinized cocoyam starch adhere longest because the pregelatinized starch has the pH of 4.63 which was quite acidic when compared with the medium and so adhered longer while the acetylated cocoyam starch, having a pH of 6.13 is close to alkaline pH and also close to the pH of the medium and detached fastest. The mechanism of starch adhesion could therefore be primarily due to van der Walls interactions, hydrogen bonds or electrostatic attractions.

The light micrograph was taken of mucosa adhesion in the two media. Examination of tissue showed epithelium and normal goblet cell appearance. None of the severe signs such as appearance of epithelial necrosis, sloughing of epithelial cells were detected (Fig. 2). After getting contact with the intestinal mucosa, the starch tablets are believed to swell to form a viscous gel by withdrawing water from the mucosa and interaction with ions present intestinal secretions. The resultant gel formation decreases the clearance rate and as a consequence the residence time of the formulation at the mucosa is prolonged. The native and starches were observed to be biocompatible polymers, cause no deleterious effect or toxic response in the intestinal mucosal lining as evaluated by histopathological studies.

From the results, it is observed that the acetylated cocoyam starch adhered longest to the pig ileum in the 0.1M HCl medium. This medium which is an in-vitro representation of the stomach, shows that drugs can be incorporated into acetylated cocoyam starch when targeting the stomach for the targeting and controlled release of such drugs in the stomach.

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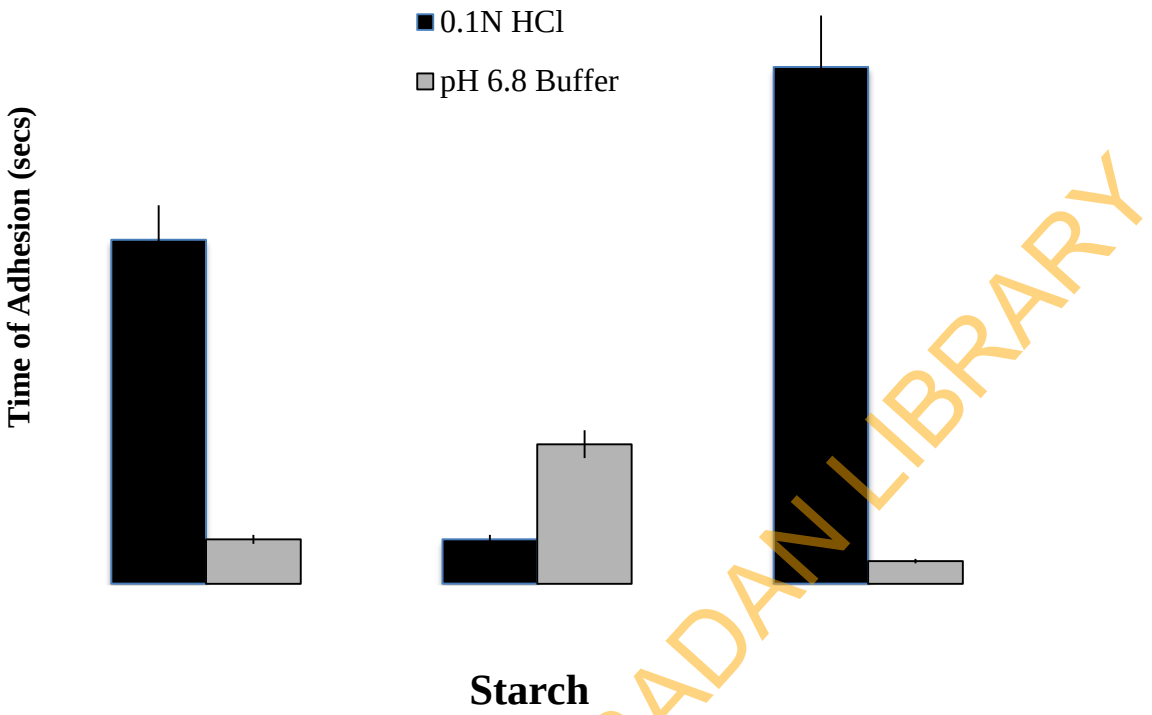
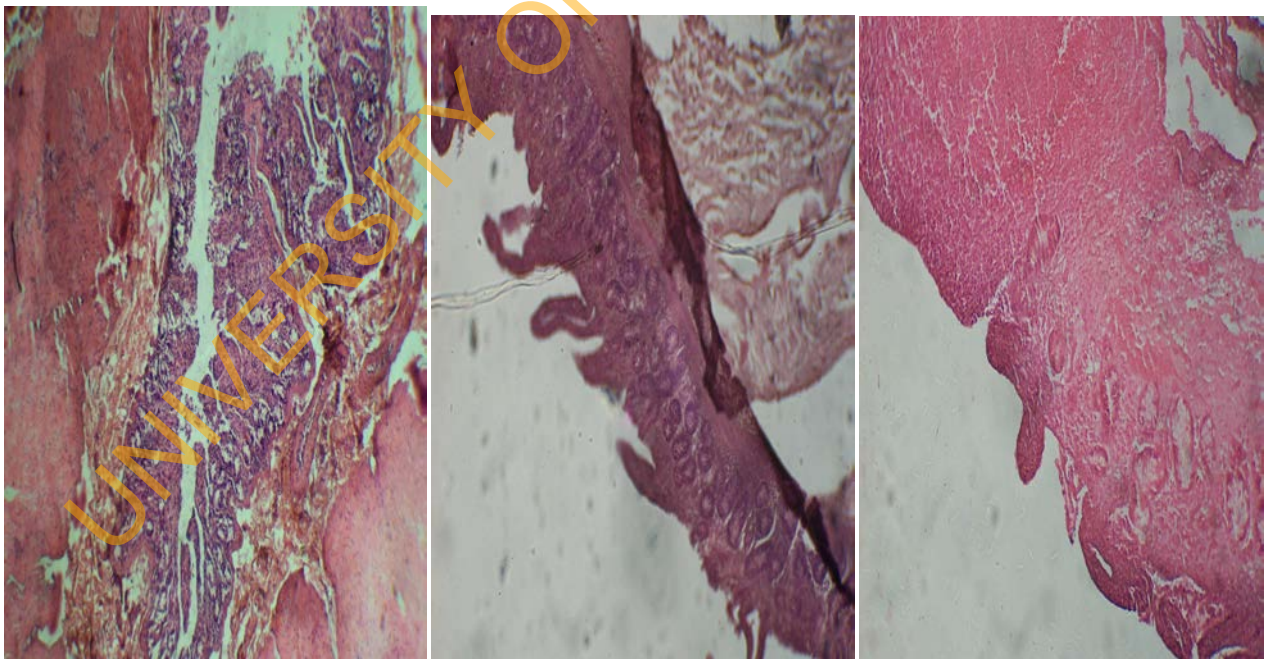


Figure 1:
Bioadhesion time of the various starches on excised pig's ileum in 0.1N HCl and phosphate buffer pH 6.8 (mean ± SD, *n* = 3).



(a) (b) (c)

Figure 2:
Light Photomicrograph of the pig's ileum Mucosa Normal Mucosa (a), Starch treated mucosa in phosphate buffer pH 6.8 (b) and Starch treated mucosa in 0.1M HCl (c)

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In targeting drugs for release in the intestines however, the pregelatinized cocoyam starch will be preferred to serve as a carrier for drugs since it is shown in the results obtained that the tablets of the pregelatinized cocoyam starch took the highest time to detach from the ileum in the phosphate buffer (which is an in-vitro representation of the intestines). The possible mechanism of adhesion is hydrogen bonding. Further work investigating the blending of cocoyam starch with other polymers could serve to extend the time of adhesion while exploiting the specificity of the starch.

REFERENCES

Adedokun MO, Itiola OA. (2010). Material properties and compaction characteristics of natural and pregelatinized forms of four starchea. *Carbohydrate Polymers* .79: 818–824.

Adriaens E, Ameye D, Dhondt MMM, Foreman P, Remon JP. (2003). Evaluation of the mucosal irritation potency of co-spray dried Amioca/Poly (Acrylic Acid) and Amioca/Carbopol 974P mixtures. *Journal of Controlled Release* 88:393-399.

Alves RML, Grossmann MVE, Silva RSSF.(1999). Gelling properties of extruded yam (Dioscorea alata) starch. *Food Chem*. 67: 123-127.

Ameye D, Musa D, Foreman P, Remona JP. (2005). Spray-dried Amioca® starch/Carbopol® 974P mixtures as buccal bioadhesive carriers. *Int. J. Pharm*. 301: 170–180.

Barimah J, Amankwah EA, Oldham JH, Amagloh FK. (2009). Effect of acetylation and carboxylation on some physicochemical properties of cassava starches. *Journal of Ghana Science Association.* 11:1,109-115.

Bowen FE, Vadino WA.(1984). A simple method for differentiating sources of pregelatinized starch NF. *Drug Dev. Ind. Pharm.* 10 (3): 505–514.

Chowdary KPR, Rao YS. (2004). Mucoadhesive microspheres for controlled drug delivery. *Biological and Pharmaceutical Bulletin*, *27*:(11), 1717

Chun M-K, Cho C-S, Choi H-K.(2002). Mucoadhesive drug carrier based on interpolymer complex of poly(vinyl pyrrolidone) and poly(acrylic acid) prepared by template polymerization. *J. Control. Release* 81:327–334.

Di Colo G, Burgalassi S, Chetoni P, Fiaschi MP, Zambito Y, Saettone MF. (2001). Gel-forming erodible inserts for ocular controlled delivery of ofloxacin. *Int. J. Pharm*. 215:101–111.

Doane WM. (1992). USDA research on starch-based biodegradable plastics. *Starch*. 44:293–295.

Dubolazov AV, Nurkeeva ZS, Mun GA, Khutoryanskiy VV. (2006). Design of mucoadhesive films based on poly(acrylic acid) and hydroxypropyl cellulose, *Biomacromolecules*, 7: 1637-1643.

Edsman K, Hagerstrom H. (2005). Pharmaceutical applications of bioadhesion for the non-oral routes. *J. Pharm. Pharmacol*. 57:3–22.

Grabovac V, Guggi D, Bernkop-Schnurch A. (2005).Comparison of the mucoadhesive properties of various polymers. *Adv Drug Deliv Rev*. 3;57(11):1713-23.

Hägerström H. Uppsala, 2003. 76 f. [Dissertation for the degree of Doctor of Philosophy in Pharmaceutics. Uppsala University]

Lee JW, Park JH, Robinson JR.(2000). Bioadhesive-based dosage forms: the next generation. *J Pharm Sci.* 89:850-866.

Lehr C-M. (2000). Lectin-mediated drug delivery: the second generation of bioadhesives. J. *Control. Release* 65:19–29.

Rios M. (2006). Developments in powder flow testing. *Pharm. Technol*. 30 (2): 38–49.

Quintanar-Guerrero D, Villalobos-Garcia R, Alvarez-Colin E, Comejo-Bravo JM. (2001). In vitro evaluation of the bioadhesive properties of hydrophobic polybasic gels containing ,*N*dimethylaminoethyl methacrylate-co-methyl methacrylate. *Biomaterials* 22: 957–961.

Shogren RL, Fanta GF, Doane WM (1994). Development of starch based plastics—a re examination of selected polymer systems in historical perspective. *Starch* 45:276–280

Singh J, Kaur L, McCarthy OJ. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications - A review. *Food Hydrocolloids*. 21,(1):1-22.

Singla AK, Chawla M, Singh A. (2000). Potential applications of carbomer in oral mucoadhesive controlled drug delivery system: a review. *Drug Dev. Ind. Pharm.* 26: 913–924.

Suzuki Y, Makino Y. (1999). Mucosal drug delivery using cellulose derivatives as a functional polymer. *J. Control. Release*. 62:101–107.

Tiwari D, Goldman D, Town C, Sause R, Madan PL. (1999). In vitro–in vivo evaluation of a controlled release buccal bioadhesive device for oral drug delivery. *Pharm. Res.* 16:1775–1780.

Werle M, Hsu Y T, Chang FC, Lee CH.(2007). Analytical methods for the characterisation of multifunctional polymers for oral drug delivery. *Current Pharmaceutical Analysis*, 3:1-
.

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