Original Article

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Development of mucoadhesive buccal films from rice for pharmaceutical delivery systems

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Summary **The aim of this work was to investigate the suitable rice varieties for developing pharmaceutical buccal films. Two rice varieties with extreme difference in amylose content were used. Rice powders were chemically modified to yield the carboxymethyl rice prior to film preparation. Scanning electron microscope (SEM) and X-ray diffractometer (XRD) were used to investigate the solid structure of rice powders. The results indicated that amylose content in the rice grains played the effects on the morphology and crystalline structure of the modified rice powders as well as the film properties. The modified rice powders of low amylose content showed halo pattern XRD whereas some crystalline peaks could be observed from the high amylose content modified rice powders. Adding of glycerin caused the films better properties of more transparency and getting rid of air bubbles. High amylose rice films showed more transparency and higher mucoadhesive property and was considered to be suitable for incorporating the drug. Adding of surfactant caused the increase in tensile strength and decrease in elongation of the rice films. The most suitable surfactant for diclofenac buccal rice film is Tween 20. This study demonstrates that rice grains are the promising natural source for pharmaceutical film forming agent. Suitable pharmaceutical buccal films could be developed from the rice with high amylose content.**

Keywords: Rice film, mucoadhesive, buccal mucosa, modified starch, carboxymethyl starch

1. Introduction

The systemic delivery of pharmaceutical active ingredients through buccal mucosa is receiving increased attention as for avoiding acid hydrolysis in the gastrointestinal (GI) tract and the hepatic first-pass effect. Buccal films are the most recently developed dosage form for buccal administration. Moreover, buccal mucoadhesive films show several advantages and popular for local therapy (*1,2*). Films are preferred over adhesive tablets in terms of flexibility and comfort. Films can help protect the wound surface, thus helping to reduce pain and treat the disease more effectively. In addition, they can circumvent the relatively short residence time of oral gels on the mucosa, which are easily washed away and removed by saliva. Many pharmaceutical buccal delivery bases are made of synthetic polymers, *e.g*. copolymers of acrylic acid, polyethylene glycol and monomethylether monomethacrylate (*3,4*), copolymer of polyisobutylene and polyisoprene (*5,6*), polyvinyl pyrrolidone (7,8), and eudragit (*9-11*). Blooming use of these polymers has caused serious environmental problems and petroleum as an important resource for originating such polymers is limited. Biodegradable polymers produced from natural raw materials are of great interest nowadays because of environmental benefits and sustainability (*12,13*). Among these, starch is considered as a promising candidate for developing sustainable materials owing to its complete biodegradability (*14*), low cost and renewability (*15*).

Rice (*Oryza sativa* L.) is the most prized cereal crop plant that is cultivated extensively worldwide since it is the principal staple food for half the world's population

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(*16*). Rice grain is considered as an important environmentally friendly raw material for starch. Two major components, amylose and amylopectin, are exist in the rice starch (*17,18*). Many previous studies have demonstrated great variations in the amylose content in rice grains of different varieties, that allow their classification as waxy (1-2% amylose), very low amylose content (2-12%), low amylose content (12-20%), intermediate amylose content (20-25%) and high amylose content (25-33%) (*19-21*). It was reported that amylose content played an important role on physicochemical properties of the derived rice products (*22*). Up to date, less of the earlier study reports the effect of rice variety as on the physicochemical property of the rice films in the view point of pharmaceutical delivery system. In the present study, the rice films prepared from two different rice varieties having far different amylose content was firstly compared in order to select the variety for developing the pharmaceutical films.

2. Materials and Methods

2.1. *Materials*

Milled white rice grains of two different rice varieties from Thailand; a non-glutinous Saohai and a glutinous Niaw Sanpatong harvested in 2013 were used. Monochloroacetic acid and glycerol were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium hydroxide and glacial acetic acid were from RCI Labscan Co., Ltd. (Bangkok, Thailand). All other chemicals and solvents were of AR grade or the highest grade available.

2.2. *Preparation of rice powder and analysis of amylose content*

Rice powder was prepared by the wet milling method. The rice grains were firstly cleaned and soaked in water at room temperature overnight. The soaked rice was washed twice with deionized water and blended with addition of water in a blender for 10 min. The filtrate passed through the 80-mesh sieve was centrifuged at 15,000 rpm for 15 min. The solid residue was washed with water and filtered through an 80-mesh and received with a 200-mesh screen. The residue collected on the 200-mesh screen was dried at 55°C for 48 h and ground. The ground rice powder after passing through the 80-mesh sieve was kept in a desiccator for further analysis. Amylose content of the rice powders was assayed using the iodine binding method described by Juliano (*23*).

2.3. *Modification of rice*

The raw rice powder of each variety was subjected to chemical modification prior to prepare the films. The reaction was carried out in a 500 mL three necked round-bottom flask, equipped with motor-driven stirrer. An aqueous solution of 50 g sodium hydroxide in 100 mL solution was prepared and firstly mixed with ethanol at a weight ratio of 1:4. The raw rice powder was added and the mixture was stirred. The temperature was raised to 40°C and the mixture was further stirred at 300 rpm for 30 min. Then, monochloroacetic acid was added. The temperature of the mixture was raised to 50°C and further stirred for 3 h. The solid mass was separated and neutralized using glacial acetic acid and washed several times with 85% ethanol until the silver nitrate test for chloride of the filtrate was negative (*24*). The solid obtained was dried in an oven at 45°C for 48 h, and then pulverized. The white powder of the modified rice after passing the 80-mesh sieve was kept in a desiccator for further experiments.

2.4. *Solid structure characterization*

The internal solid structure of the rice powders was investigated by X-ray diffractometry (XRD) using a Siemens D-500 X-ray diffractometer with Cu Kα radiation at a voltage of 30 kV and 15 mA. The samples were scanned between 2θ = 5-60° with a scanning speed of 5°/min. Prior to testing, the samples were dried and stored in a desiccator.

The external structure of the samples was investigated by SEM using a JEOL JSM-5410LV (Japan) equipped with a large field detector. The acceleration voltage was 10-20 kV under low vacuum mode (0.7-0.8 torr).

2.5. *Film preparation*

Rice films were prepared from the modified rice powders obtained from each rice variety by casting technique. Exact weight of 5 g of the modified rice powder was dispersed in distilled water. The final volume was adjusted to 100 mL. The dispersions were heated to 90°C in a closed chamber for 2 h and gently stirred in order to obtain clear homogenous liquid gel and avoiding of air bubble formation. In order to study the effects of other additional substances including drugs on the film properties, they were added to this gel before going to further step. Exact portion of the obtained rice gel was poured into a glass petri dish. The gels were spread uniformly over the entire surface and dried at 60°C for 6 days. After drying, cast films were removed. The physical appearance of the films was observed visually.

2.6. *Film thickness*

Thickness of the films was measured using a precision digital micrometer (Fowler, model FOW52-229-001,

Pennsylvania, USA) with an accuracy of 0.0001 mm. The mean thickness of each film was determined from an average of ten random locations on the film.

2.7. *Mechanical properties of the films*

The examined mechanical properties of the films included tensile strength and elongation at break were determined using an Instrong Universal Testing Machine Model 1000 (H1K-S,UK) with the procedure according to ASTMD 882-80a (25) with the 24 h preconditioning and testing performed at 27 ± 2 °C, 65 ± 2% RH according to TIS949-2533 (*26*). The film samples were cut with sharp scissors into 10 × 70 mm rectangles for each film and used as a test specimen. The initial grip separation and cross-head speed was set at 100 mm and 20 mm/min, respectively. There were ten sample measurements for each kind of rice film.

2.8. *Mucoadhesive study of the films*

The films were cut into 20 mm × 20 mm dimensions and subsequently investigated for the mucoadhesive property using a method described by Kundu *et al*. (*27*) with some modification. After 30 s immersing in water, the wet films were placed on a freshly excised porcine intestinal mucosa, fixed on a glass slide with very thin cellotape so that the exposed mucous membrane was 20 × 20 mm. Similarly, fresh porcine intestinal mucosa was also fixed on another glass slide, superimposed on the free film surface so that the film laid between the two mucosa bars. The surface area of the film plates and the intestinal mucosa were exactly the same to avoid direct attachment of the mucosa to each other. Finally, the plates were subjected to a little pressure for 2 min. The lower slide was fixed while the other slide was attached to a thread which passed over a system of pulley and was connected to a small plastic container filled with water to confer load. The force of detachment was measured from the load at which detachment of the film from the mucosa occurred.

2.8. *Statistic analysis*

Descriptive statistics for continuous variables were calculated and reported as a mean±standard deviation. Data were analyzed using a one-way analysis of variance (ANOVA) and Duncan's multiple range test ($p < 0.05$) using Statistic a software version 11.

3. Results and discussion

3.1. *Solid structure of rice and amylose content*

The raw rice flours have similarly outer appearance in comparison with their modified powders when observed visually as seen in Figure 1. The amylose content of the glutinous NSP was found to be 4% whereas that of the non-glutinous SH was 21%. This extreme difference of amylose content is considered to be due to the variety of the rice. Observation by SEM demonstrates different morphology between NSP and SH varieties as illustrated in Figure 2. Layered organization with some small pieces of supposedly "broken particles" possibly as a result of the preparation process (*28*) was clearly observed in NSP particles. The chemical method and conditions used in the present study to modify the rice starch to carboxymethyl type caused a significant change to the rice particles and this effect was clearly seen under SEM investigation. The modified SH particles were swollen and the surface edges were obviously unsharpened which some of them were either clustered or merged together. The modified rice particles of NSP displayed more prominent change in shape and surface with extremely higher swelling and merging than SH which some part appeared as complete fusion that the individual particles could not be observed. The surface of the modified NSP also displayed rough and wrinkle. Internal structure investigated by XRD demonstrates some crystalline identical peaks of the raw starch particles indicating that the raw rice powders of both varieties have crystalline structure as seen in Figure 3. After modification, the internal structure of the rice was obviously changed as shown in Figure 4. The XRD

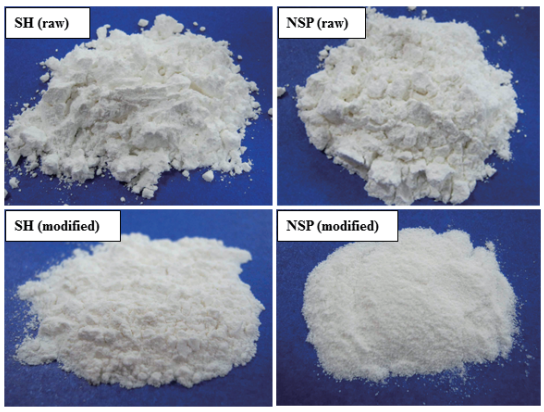

Figure 1. Outer appearance of the rice powders.

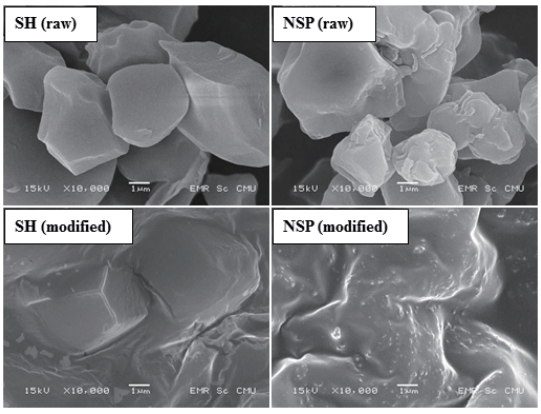

Figure 2. SEM morphology of the rice powders.

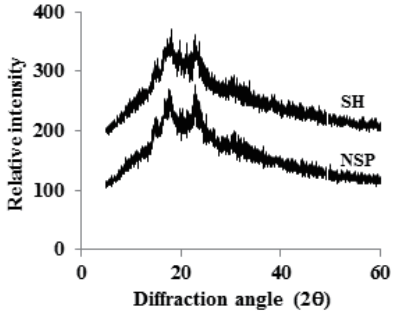

Figure 3. XRD diffractograms of the raw rice powders.

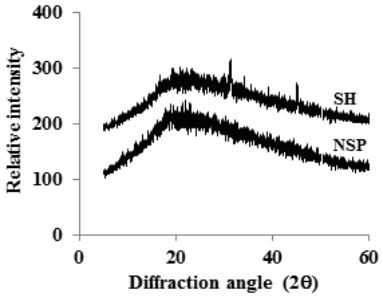

Figure 4. XRD diffractograms of the modified rice powders.

halo patterns were found indicating the destruction of crystalline structure into amorphous form. However, the level of crystalline destruction was different. The internal structure of NSP was completely changed to amorphous structure whereas some crystalline peaks were observed in that of SH. It was concluded that the low amylose content (NSP) rice had higher swelling and obviously higher microstructure change than the high amylose content (SH) variety. Our results indicate the influence of amylose content in the rice starch.

3.2. *Effect of additive substance on film appearance and thickness*

The film opacity is used to assess the transparency of the films. Preferable pharmaceutical buccal film bases should be transparent, odorless, tasteless, and colorless. The films obtained from 5% modified rice showed slightly white opaque with some small air bubbles and brittle. The received films exhibited rice odor and tasteless. Three common film plasticizers; glycerin, propylene glycol, or polyethylene glycol 400 was used in order to improve film quality. As shown in Figure 5. The plasticizers displayed a role to solve these problems but in different level. No air bubble was seen in the films with all additives but the films added with propylene glycol or polyethylene glycol 400 was still opaque and fragile. NSP films gave similar results but more opaque than those of SH. The best films of both rice varieties were obtained after added with glycerin. Further investigation was done with the effect of glycerin concentration on film thickness. It was found that adding glycerin in the range of 0.75-2.5% caused

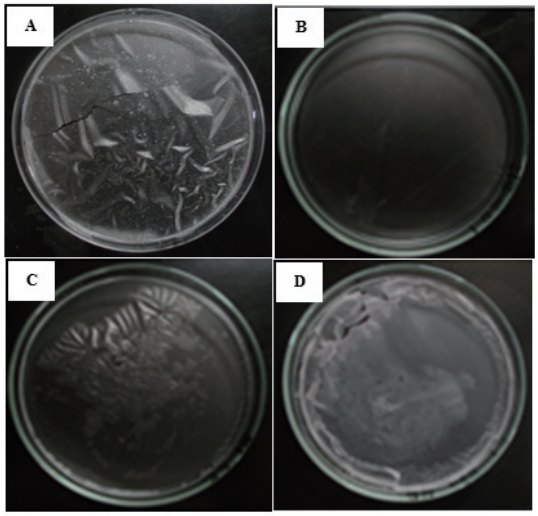

Figure 5. Outer appearance of SH Films without additive substance (A), with 1.5% glycerin (B), 1.5% propylene glycol (C), and 1.5% polyethylene glycol 400 (D).

Table 1. Effect of glycerin on film thickness

Glycerin (%)	Film thickness (mm)	
	SH	NSP
0.00	0.057 ± 0.005	0.058 ± 0.004
0.75	0.054 ± 0.009	0.051 ± 0.016
1.00	0.054 ± 0.008	0.049 ± 0.009
1.25	0.051 ± 0.007	0.047 ± 0.005
1.50	0.051 ± 0.007	0.048 ± 0.009
2.50	0.050 ± 0.004	0.049 ± 0.007

the decrease in film thickness in both rice varieties as seen in Table 1. However, it was observed that the films with more than 1.5% glycerin were slightly liquefied and difficult to get off from the casting mold. Therefore, glycerin 1.5% was selected to add in the film formulation for further study.

3.3. *Mucoadhesive property of the films*

Mucoadhesive films have been designed to remain in contact with the buccal mucosa for therapeutic purposes for prolonged periods of time. Hence, the measurement of the mucoadhesive strength or time of mucoadhesion is important to respond the desirable property of the buccal films. In these experiments, instead of measuring time of mucoadhesion, the study was focused on determining the mucoadhesive strength or the force of adhesion of the films. The mucoadhesive force of the developed rice films of both rice varieties was found to be obviously different. SH film demonstrated the significantly higher mucoadhesive stength of 191.5 ± 6.2 Kg/m^2 whereas NSP film exhibited the strength of 137.1 ± 5.1 Kg/m^2. It was reported that films with high amylose content forms a network of stiff strands and pores present in the network could possibly entrap more water (*29*). This leads to increase the adhesive bond between the starch polymer in the films and

Table 2. Tensile strength and elongation of the films

Film composition	Tensile strength (kPa)	Elongation (%)
SH 5%, Glycerin 1.5%	0.76 ± 0.12	153 ± 23
SH 5%, Glycerin 1.5%, Drug 0.25%	0.67 ± 0.17	241 ± 21
SH 5%, Glycerin 1.5%, Drug 0.25%, Tween-20 0.1%	2.39 ± 0.19	139 ± 11
SH 5%, Glycerin 1.5%, Drug 0.25%, Tween-80 0.1%	1.23 ± 0.14	101 ± 18
SH 5%, Glycerin 1.5%, Drug 0.25%, Triton-X-100 0.1%	1.63 ± 0.18	132 ± 14

the biological substrates in the mucosa layer such as hydrogen bonds and van der Waals forces (*30,31*).

3.4. *Tensile strength and elongation of the films*

A good pharmaceutical buccal film must withstand the normal stress encountered during its application. Tensile strength is the maximum tensile stress sustained by the sample during the tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated tensile strength at yield or at break, respectively (*32*). In this experiment, the film of SH was selected because of its better properties than that of NSP, such as more transparency and higher mucoadhesive strength. Diclofenac sodium (DS) was used as a model drug because the drug can be used in the buccal cavity for anti-inflammatory and analgesic activity. DS was incorporated into the films formulation to yield the drug percentage of 0.25% in the films. According to enhance the solubility of the drug to be dispersed molecularly and thoroughly the films, three kinds of surfactant; Tween-20, Tween-80, or Triton-X-100 at a concentration of 0.1% was added. The results are shown in Table 2. It was found that the unloaded rice film had high strength with a breaking point at 0.76 ± 0.12 kPa. After drug loading, the tensile strength of the films was slightly decreased with a breaking point at 0.67 ± 0.17 kPa. Interestingly, adding surfactant caused the significant increase in tensile strength of the films. The increasing power was depended on the surfactant type. It was found that among the three surfactants used, Tween-20 showed the highest activity on enhancing of the film strength with a breaking point at 2.39 ± 0.19 kPa. The obvious increasing tensile strength by surfactant was considered to be due to a high formation of intermolecular bonding inside the film. The tensile strength of the films prepared from different type of surfactant was different. This phenomenon indicated the critical type of the surfactant suitable for film-forming components.

Elongation at the break is an indication of the films' flexibility and stretch ability (extensibility), which is determined at the point when the film breaks under tensile testing and is expressed as the percentage of change of the original length of the specimen between the grips of a film to stretch (extend). It was found that elongation of the films was affected by the incorporated drug and surfactant. The elongation of the drug loading rice film without surfactant was higher than that of the free film. However, adding surfactant caused the decrease of film elongation property. Among three surfactants used, Tween-20 gave the least decrease of elongation.

4. Conclusion

The present study explored the development of pharmaceutical mucoadhesive buccal films using rice as film forming agent. Amylose content in the rice grains showed the effects on the morphology and crystalline structure of the modified rice powders and the film properties. Adding of glycerin caused the films better properties of more transparency and getting rid of air bubbles. High amylose rice films showed more transparency and higher mucoadhesive property and was considered to be suitable for incorporating the drug. Adding of surfactant caused the increase in tensile strength and decrease in elongation of the rice films. The most suitable surfactant of diclofenac buccal rice film is Tween 20.

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References

1. Jones DS, Medlicott NJ. Casting solvent controlled release of chlorhexidine from ethylcellulose films prepared by solvent evaporation. Int J Pharm. 1995; 114:257-261.
2. Senel S, Ikinci G, Kas S, Yousefi-Rad A, Sargon MF, Hincal AA. Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery. Int J Pharm. 2000; 193:197-203.
3. Shojaei AH, Berner B, Li X. Transbuccal delivery of acyclovir: I. *In vitro* determination of routes of buccal transport. Pharm Res. 1998; 15:1182-1188.
4. Shojaei AH, Zhuo SL, Li X. Transbuccal delivery of acyclovir (II): Feasibility, system design, and *in vitro* permeation studies. J Pharm Pharm Sci. 1998; 1:66-73.
5. Guo JH. Bioadhesive polymer buccal patches for buprenorphine controlled delivery: Formulation, *in-vitro* adhesion and release properties. Drug Dev Ind Pharm. 1994; 20:2809-2821.
6. Guo JH, Cooklock KM. The effects of backing materials and multilayered systems on the characteristics of bioadhesive buccal patches. J Pharm Pharmacol. 1996; 48:255-257.
7. Anders R, Merkle HP. Evaluation of laminated mucoadhesive patches for buccal drug delivery. Int J Pharm. 1989; 49:231-240.

8. Save T, Shah MU, Ghamande AR, Venkitachalam P. Comparative study of buccoadhesive formulations and sublingual capsules of nifedipine. J Pharm Pharmacol. 1994; 46:192-195.
9. Cui Z, Mumper RJ. Buccal transmucosal delivery of calcitonin in rabbits using thin-film composites. Pharm Res. 2002; 19:1901-1906.
10. Cui Z, Mumper RJ. Bilayer films for mucosal (genetic) immunization via the buccal route in rabbits Pharm Res. 2002; 19:947-953.
11. Jay S, Fountain W, Cui Z, Mumper RJ. Transmucosal delivery of testosterone in rabbits using novel bi-layer mucoadhesive wax-film composite disks. J Pharm Sci. 2002; 91:2016-2025.
12. Kinci GI, Senel S, Akincibay H, Kas S, Ercis S, Wilson CG, Hincal AA. Effect of chitosan on a periodontal pathogen *Porphyromonas gingivalis*. Int J Pharm. 2002; 235:121-127.
13. Rossi S, Sandri G, Ferrari F, Bonferoni MC, Caramella C. Buccal delivery of acyclovir from films based on chitosan and polyacrylic acid. Pharm Dev Technol. 2003; 8:199-208.
14. Araújo MA, Cunha A, Mota M. Enzymatic degradation of starch-based thermoplastic compounds used in prostheses: Identification of the degradation products in solution. Biomaterials 2004; 25:2687-2693.
15. Zhang JF, Sun XZ. Mechanical properties of PLA/ starch composites compatibilized by maleic anhydride. Biomacromol. 2004; 5:1446-1451.
16. Hagenimana A, Ding X, Fang T. Evaluation of rice flour modified by extrusion cooking. J Cereal Sci. 2006; 43:38-46.
17. Zhou Z, Robards K, Helliwell S, Blanchard C. Composition and functional properties of rice. Int J Food Sci Tech. 2002; 37:849-868.
18. Yu S, Ma Y, Sun DW. Impact of amylose content on starch retrogradation and texture of cooked milled rice during storage. J Cereal Sci. 2009; 50:139-144.
19. Coffman WR, Juliano BO. Rice. In: Nutritional quality of cereal grains: Genetic and agronomic improvement (Olson RA, Frey KJ, eds.). Madison, American Society of Agronomy, 1987; pp. 101-131.
20. Ong MH, Blanshard JMV. Texture determinants in cooked, parboiled rice. I: Rice starch amylose and the fine structure of amylopectin. J Cereal Chem. 1995; 21:251-260.
21. Frei M, Siddhuraju P, Becker K. Studies on *in vitro* starch digestibility and the glycemic index of six different indigenous rice cultivars from the Philippines. Food Chem. 2003; 83:395-402.
22. Lourdin D, Della Valle G, Colonna P. Influence of amylose content on starch films and foams. Carbohyd Polym. 1995; 27:261-270.
23. Juliano BO. A simplified assay for milled-rice amylose. Cereal Sci Today 1971; 16:334-360.
24. Pfeiffer K, Heinze Th, Lazik W. Starch derivatives of high degree of functionalization. 5. Stepwise carboxymethylation of amylose. Chem Pap. 2002; 56:261-266.
25. ASTM, American society of testing and materials. Standard test methods for tensile properties of thin plastic sheeting. D 882 Annual book of ASTM standards, 1995.
26. TIS 949-1990, Thai Industrial standards institute. Standard for oriented polypropylene films, 1990.
27. Kundu J, Patra C, Kundu SC. Design, fabrication and characterization of silk fibroin-HPMC-PEG blended films as vehicle for transmucosal delivery. Mater Sci Eng C. 2008; 28:1376-1380.
28. Wang LF, Pan SY, Hu H, Miao WH, Xu XY. Synthesis and properties of carboxymethyl kudzu root starch. Carbohyd Polym. 2010; 80:174-179.
29. Muscat, D, Adhikari B, Adhikari R, Chaudhary DS. Comparative study of film forming behaviour of low and high amylose starches using glycerol and xylitol as plasticizers. J Food Eng. 2012; 109:189-201.
30. Kinloch AJ. The science of adhesion. Part 1: Surface and interfacial aspects. J Mater Sci. 1980; 15:2141-2166.
31. Chickering D, Mathiowitz E. Definitions, mechanisms, and theories of bioadhesion. In: Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches, and Development (Mathiowitz E, Chickering D, Lehr C, eds.). Marcel Dekker Inc, New York, USA, 1999.
32. ASTM. Standard test method for tensile properties of plastics. D638. In ASTM. Annual book of American standard testing methods (Vol. 15.09, pp. 159e171). Philadelphia, 1991.

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