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Application of acid-treated yeast cell wall (AYC) as a pharmaceutical additive I. AYC as a novel coating material

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Abstract

Acid-treated yeast cell wall (AYC) was newly prepared by acidifying the cell wall of brewer's yeast and the potential to use AYC as a novel coating material was studied. AYC had an oval shape with the diameter of several μm. The rheogram of AYC aqueous dispersion showed the plastic fluid property that is generally observed in the suspension. Core tablets containing 3% of acetaminophen (AAP) were coated with the AYC aqueous dispersion containing 5% (w/v) of AYC and 0.35% (w/v) of glycerol at various coating percents. The AAP release profile from the AYC-coated tablets was studied by the JP13 paddle method using solutions at various pH. Tensile strength and permeability of oxygen and water vapor of AYC cast film were measured. The AAP release from the AYC-coated tablets showed sigmoidal release profile with an initial lag time and the duration of the lag time depended on the coating percent of AYC. The pH of the dissolution fluid or the storage at room temperature for 120 days had little affect on AAP release from the AYC-coated tablets. These results suggest that it is possible to control the start time of medicine release independent of the pH by coating of AYC, that is the time-controlled release. The AYC cast film showed a large tensile strength and an extremely small oxygen permeability coefficient and a sufficient level of water permeability coefficient in order to protect from moisture. These results present that AYC has the high utility as a novel aqueous coating material for DDS preparations.

Keywords: Yeast; Coating material; Lag time; Sigmoidal release; pH; Oxygen permeability

1. Introduction

Synthetic polymers such as cellulose, methacrylic acid copolymers or polyvinyl polymers are generally used for film coating of phar-

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maceuticals. Recently, however, natural materials have been attended from the viewpoint of utilization of available resources and safety. Reportedly, sodium alginate (Kaneko et al., 1999), xanthan gum (Watanabe et al., 1991), locust bean gum (Watanabe et al., 1991), chitosan (Hou et al., 1991) and pectin (Ashford et al., 1994) have been attempted for use as natural coating materials. In this study, as well as these polymers, we noticed beer yeast cell wall as a natural material for coating.

Yeast is a micro-organism, which has been used for brewing and baking since ancient times, but recently applications based on its physiological, metabolic or nutrient-rich characteristics have appeared in food and pharmaceutical industries. These applications, however, mainly utilized the characteristics and components of the yeast cytoplasm.

In the series of this study, in order to find a new and effective use for yeast cell wall, we have prepared the acid-treated yeast cell wall (AYC) and developed the application of AYC to pharmaceutical additives. In this report, we attempted to apply AYC as a novel coating material and investigated the possibility of actual coating of AYC and the functions of the AYC-coated layer.

2. Materials and methods

2.1. Materials

Brewer's yeast (*Saccharomyces cerevisie*, Kirin Brewery, Tokyo) collected after the manufacturing of beer was used as a raw material. Acetaminophen (AAP, Tokyo Kasei Kogyo, Tokyo) was used as a model drug. Hydroxypropylcellulose (HPC-L, viscosity 6.0–10.0 cps, Shin–Etsu Chemical, Tokyo), magnesium stearate (Wako Pure Chemical Industries, Osaka), glycerol (Wako Pure Chemical Industries, Osaka) and Lactose (DMV Japan, Tokyo) were used as a binder, lubricant, plasticizer and excipient, respectively.

2.2. Preparation of AYC

Manufacturing of AYC was performed through an acid treatment process because lysinoalanine, which is noxious for the human body, was generated by heat alkali treatment. Intracellular components of the intact yeast were solubilized by reaction with intracellular or external enzyme, such as proteases and glucanases, and the soluble components were removed. The acidifying reaction was then carried out with 5% (w/v) of the residual fraction and 0.5 N HCl at 80°C for 20 min. After centrifugation, the precipitates were thoroughly washed with water. The pH of the system was adjusted to 9.0 in order to remove the bitterness substances from hops as the brewer's yeast used in this study was actually the residue used from the manufacture of beer. The pH was adjusted to 3.8–4.2 and the AYC was then obtained after centrifugation and washing with water.

2.3. Observation of surface of yeast and AYC

The surface of the brewer's yeast and AYC was observed by scanning electron microscopy (SEM) using an ultra-high revolution, low-velocity scanning electron microscope (UHR-SV SEM/ S-900LV 1KV, Hitachi, Tokyo).

2.4. Analysis of AYC components

The protein content of the AYC was analyzed by Kjeldahl method (Nakajima et al., 1988) and the lipid content was measured using the method described by Folch et al. (Folch et al., 1957). The crude fiber content was analyzed by the standing method (Sample Analysis Standard Workshop, 1998) and the mineral content was analyzed by the direct ashing method. The amount of nitrogen free extract was calculated by subtracting the content of the protein, lipid, crude fiber and ash from the total content.

2.5. Dispersion state and rheological properties of AYC aqueous dispersion

An AYC aqueous dispersion containing 6% of

AYC was centrifuged at 5000 rpm for 5 min and shaken by hand for several seconds to disperse AYC again. The dispersion state of AYC before and after shaking was observed. The rheological property of the AYC aqueous dispersion was evaluated from the relationship between shearing-rate and shearing-stress using a corn-plate viscometer (Brookfield Engineering Laboratories, a digital viscometer model DV-II +) at various concentrations of AYC.

2.6. Preparation of core tablets

The formulation of the core tablet was shown in Table 1. A mixed powder of AAP and lactose was granulated with a fluidized bed (MP-01, Powlex, Osaka) using HPC-L aqueous solution as a binder by the top spray method. The granules obtained were mixed with magnesium stearate and compressed with a rotating tabletting machine (HT-22P HATA, Tokyo) equipped with a 7 mm diameter and 4.5 mm radius of curvature die.

2.7. Preparation of AYC-coated tablets and measurement of thickness of AYC-coated layer

The AYC aqueous dispersion containing 5% of AYC and 0.35% of glycerol was used for coating. The coating of the core tablets was performed with Driacoater (Powlex, Osaka) at the coating % of 10, 25, 43 and 67 based on the weight of the core tablet. The operating conditions for coating were as follows: core tablets, 250 g; inlet and outlet air temperatures, 70 and 45–47°C respectively; air volume, 0.83 m^3/min; spray pressure, 1.5 kgf/cm^2; spray rate, 5–7 g/min; spray air volume, 28 l/min; pan revolution, 30 rpm; curing temperature, 80°C; curing time, 90 min. The thickness of the AYC-coated tablet was measured with a dial thickness gauge (Mitsutoyo, Tokyo). The thickness was determined as the distance between the top of the curvature of the AYC-coated tablet. The thickness of AYC-coated layer was calculated by subtracting the thickness of the core tablet from that of the AYC-coated tablet.

Table 1
Formulation of core tablet (mg)

Acetaminophen (AAP)	3.6
Lactose	112.8
Hydroxypropyl cellulose	3.0
Magnesium sterate	0.6
Total weight per tablet	120.0

2.8. Release study

The release profiles of AAP from the AYC-coated tablet were studied with a dissolution tester (NTR-6100A, Toyama Sangyo, Osaka), according to the paddle method (JP13) using 500 ml of dissolution fluid at 37 ± 0.5°C and a rotating paddle at 100 rpm. Distilled water (pH 5.8), buffer solutions composed of NaCl and HCl of pH 1.2, CH_3COOH and CH_3COONa of pH 4.0, CH_3COOH and CH_3COONa of pH5. 0, KH_2PO_4 and Na_2HPO_4 of pH 6.0, KH_2PO_4 and NaOH of pH 7.0 and $CH_2(NH_2)CH_2OH$ and HCl of pH 8.0 were used for the dissolution fluid. The quantity of AAP was determined spectrophotometrically by measuring the absorbance at 242 nm.

The apparent change of the AYC-coated tablet during the release process was observed with optical microscope (Nikomat, Nikon, Tokyo).

2.9. Preparation of AYC cast films

The AYC cast film was prepared with the AYC aqueous dispersion containing 3.5% of AYC and 0.35% of glycerol. After degassing, the dispersion containing 1 g of AYC was placed in a plastic plate with the dimensions of 9.0×13.0 cm and drying at 40°C for 24 h. The AYC cast film obtained was dark brown and translucent. The thickness of the AYC cast film was about 60 μm.

2.10. Measurement of tensile strength and oxygen and water vapor permeability coefficients of AYC cast film

The tensile strength of the AYC cast film cut in the dumbbell shape was measured by the JIS Z1702 method with a universal testing machine (CATY1001-ZS, Yonekura Seisakusho, Osaka) at

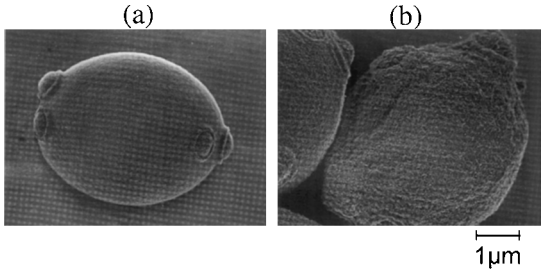

Fig. 1. SEM photographs of crude brewer's yeast (a) and AYC (b).

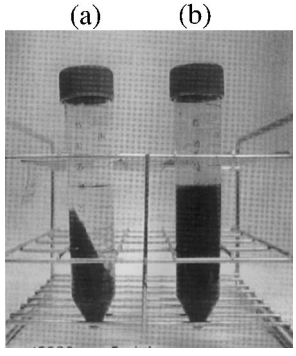

Fig. 2. Appearance of AYC aqueous dispersion. (a), centrifuged at 5000 rpm for 5 min; (b), after shaking (a) by the hand.

23°C, 50% RH and 50 mm/min of a cross-head speed. The oxygen permeability coefficient of the AYC cast film was measured by JIS K7126B method with a TRAN10/50 (MOCON, Minneapolis) at 23°C and 0% RH. The area of AYC cast film tested was 50 cm^2 and oxygen concentration was 100%. The water vapor permeability coefficient of the AYC cast film was measured by JIS Z0208 method with a PL4SP incubator (Tabaiesupekku, Osaka) and balance (AE200, Mettler-Toledo, Greifensee) at 40°C and 50% RH. The area of AYC cast film tested was 28.26 cm^2.

3. Results and discussion

3.1. Shape and components of AYC

SEM photographs of crude brewer's yeast and AYC are shown in Fig. 1(a) and (b), respectively. Brewer's yeast is classified as Ascomycetes and has an oval shape with the diameter of about 6–10 μm. By comparing these two photographs it can be seen that the size of crude brewer's yeast and AYC are almost the same. The crude yeast has a smooth surface, but AYC has a rough surface and a distorted shape.

The chemical components of the yeast cell wall are different depending on the species. In the case of *S. cerevisiae* (known as a brewer's yeast and a baker's yeast) is composed of mainly polysaccharides such as glucan and mannan and a little protein (Northcote and Horne, 1952; Fleet and Manners, 1976). A double-layer model is advocated for the structure of the yeast cell wall, composed of a mannan-protein complex as the upper layer and of glucan as the lower layer (Lampen, 1968; Kidby and Davies, 1970). Therefore, the change in the surface and shape may be due to loss of mannan and protein of the yeast outer layer during the acidifying process.

The component of AYC was as follows: nitrogen free extract content, 58%; crude fiber, 30%; protein, 10%; lipid, 0.3% and ash, 0.7%. Dietary fiber is generally defined as the nitrogen-free extract and crude fiber. So, dietary fiber content was estimated as 88%.

3.2. Dispersion state and rheological properties of AYC aqueous dispersion

Fig. 2 (a) shows the AYC aqueous dispersion containing 6% of AYC after centrifugation at 5000 rpm for 5 min. It was observed that the cake of AYC had settled at bottom of the centrifuge tube. When this was shaken gently by the hand for several seconds, AYC was homogeneously re-dispersed (b) and the dispersion state was maintained for at least 6 h. This may be due to only a slight difference in the apparent density between water and sufficiently water-absorbed AYC.

Fig. 3 shows the rheogram of the AYC aqueous dispersion at various concentrations of AYC. All dispersions have the yield value and a linear relationship was observed between the shearing-stress and the shearing-rate without hysteresis, indicating a plastic fluid property. Polymer solutions

generally used for coating show the quasi-viscous fluid property without the yield value (Martin et al., 1983; Ichibagase et al., 1997). This is because of the mutual folding and tangling of the linear polymer molecules at low shearing-stress is reduced with the increased shearing-stress, and then the polymer molecules align in the direction of flow. In contrast, AYC was insoluble to water and AYC aqueous dispersion was a suspension that AYC swollen particles dispersed in. Therefore, the plastic flow property was observed.

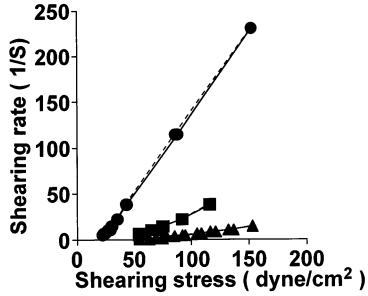

Fig. 3. Rheogram of AYC aqueous dispersion at various AYC concentrations. AYC concentration: ●, 4%; ■, 5%; ▲, 6%. Full line: increase in shearing-stress. Broken line: decrease in shearing-stress.

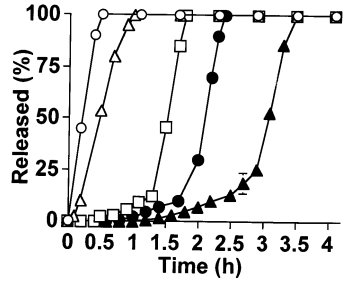

Fig. 4. Effect of AYC coating percent on AAP release from AYC–coated tablets. Coating %: ○, 0 (core tablet); △, 10; □, 25; ●, 43; ▲, 67. Each point represents the mean ± S.D. (n = 3).

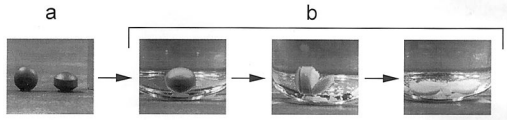

Fig. 5. Apparent change of AYC-coated tablet during release process. a, before the dissolution test; b, during the dissolution test.

It was found that AYC was dispersed as an independent particle in water unlike other polymers generally used as a solution for the film coating, and the dispersion state of AYC aqueous dispersion was maintained for a long time. These results suggest that the AYC aqueous dispersion is useful for the actual coating without the necessity for the agitation during the coating process.

3.3. AAP release profile from core tablet and AYC-coated tablets at various coating percents of AYC

Fig. 4 shows AAP release profiles from the core tablet and the AYC-coated tables at various AYC coating % in the distilled water. The thickness of the AYC-coated layer at coating percents of 10, 25, 43 and 67 were about 210, 470, 710 and 1140 μm, respectively. Rapid release of AAP from the core tablet was observed. In contract, the AAP release from the AYC-coated tablets showed sigmoidal release profile with an initial lag time and the duration of the lag time depended on the coating percent of AYC. This result indicates that AYC film is certainly formed on the surface of the core tablet and functions as a coating layer, which makes the release a sigmoidal profile with a lag time. This sigmoidal profile with an initial lag time may be useful for masking bitter taste and offensive smell (Shirai et al., 1993; Kaneko et al., 1997; Sugao et al., 1998). Reportedly, the sigmoidal release profiles are obtained by blending the polymers of different kinds or adding other materials such as a swelling agent or an organic acid (Narisawa et al., 1994; Ueda et al., 1994a,b; Yamakita et al., 1996; Narisawa et al., 1996, 1997). In this study, the sigmoidal release profile was obtained by coating only the AYC aqueous dispersion.

Slight difference in the release rate of AAP after the lag time was observed. This result is caused by the fact that AAP release from the AYC-coated tablet began with a collapse of the AYC-coated layer as shown in Fig. 5, although the drug is released by the dissolution of the coating film in the case of other coating materials. The increase in the coating percent of AYC caused the increase in the thickness of the AYC-coated layer. There-

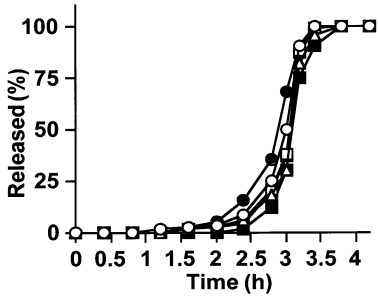

Fig. 6. Effect of pH of dissolution fluid on AAP release from AYC-coated tablets. pH: ○, 1.2; △, 4.0; □, 5.0; ●, 6.0; ▲, 7.0; ■, 8.0.

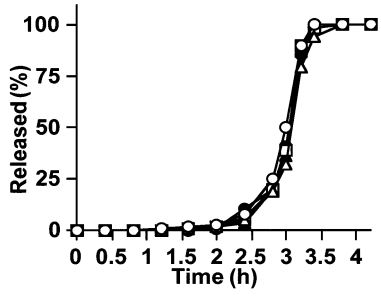

Fig. 7. Effect of storage period on AAP release profiles from AYC-coated tablets. Storage period (d): ○, 0; △, 30; □, 60; ●, 90; ▲, 120.

Table 2
Tensile stength and oxygen and water vapor permeability coefficients for AYC cast films

Tensile strength (Mpa)	39.6 ± 2.5[a]
Oxygen ($cm^3 \cdot mm/m^2 \cdot 24\ h \cdot atm$)	$5.0 \times 10^{-3} \pm 1.6 \times 10^{-3}$[a]
Water vapor ($g \cdot mm/m^2 \cdot 24\ h$)	17.1 ± 0.6[a]

[a] The values represent the mean $\pm$ SD ($n = 5$)

fore, the extension in the lag time with the increasing coating percents of AYC may be due to the increase in the strength of the AYC-coated layer and the decrease in the permeation rate of the dissolution fluid.

3.4. Effects of pH of dissolution fluid and storage period on AAP release

Figs. 6 and 7 show the effects of the pH of dissolution fluid and the storage period on AAP release from the AYC–coated tablets, respectively. The AYC coating percent was 67. The AYC-coated tablets had been stored at room temperature under 50% RH. The pH and the storage for 120 days had little affect on AAP release from the AYC-coated tablets. These results indicate that the coating of AYC can make the time-controlled release system independent of pH of the dissolution fluid.

3.5. Tensile strength and permeability coefficients of oxygen and water vapor into AYC cast film

The tensile strength and the oxygen and water vapor permeability coefficients for the AYC cast films are listed in Table 2. The tensile strength was 39.6 ± 2.5 MPa (e.g. The values of hydroxypropylmethylcellulose (HPMC) and HPC are 43.0 and 43.2 MPa, respectively, which are estimated from the product brochures of Shin–Etsu Chemical and Nippon Soda). The oxygen permeability coefficient was an extremely small value, as it was equal to the value of the aluminum foil laminated with polyethylene and polyethyleneterephthalate (1.8×10^{-3} $cm^3 \cdot mm/m^2 \cdot 24\ h \cdot atm$, Yoshii and Yamaguchi, 1998). The water vapor permeability coefficient was a sufficient level in order to protect it from moisture (e.g. The values of HPMC and HPC are 81.0 and 168.6 $g \cdot mm/m^2 \cdot 24$ h, respectively, which are estimated from the product brochures of Shin–Etsu Chemical and Nippon Soda).

4. Conclusion

The sigmoidal release profile with an initial lag time was obtained by coating the AYC aqueous dispersion and the release profile was hardly affected by pH or the storage period for 120 days. The AYC cast film had an extremely low oxygen permeability and a sufficient water vapor permeability level in order to protect it from moisture. Our results suggest that the AYC has a high potential utility as a novel aqueous coating material for DDS preparations.

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