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International Journal of Pharmaceutics 237 (2002) 15–22

international journal of pharmaceutics

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Application of acid-treated yeast cell wall (AYC) as a pharmaceutical additive. III. AYC aqueous coating onto granules and film formation mechanism of AYC

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Received 3 November 2001; received in revised form 18 December 2001; accepted 27 December 2001

Abstract

From the viewpoint of effective utilization of natural resources and development of new pharmaceutical materials, acid-treated yeast cell wall (AYC) was prepared via a novel approach involving acidification of brewers' yeast cell wall. AYC aqueous dispersion containing 5% (w/v) AYC and 0.5% (w/v) glycerol was prepared. Subsequently, AYC was coated onto core granules containing acetaminophen (AAP). Spray mist size under various spray conditions and viscosity of the AYC aqueous dispersion at various AYC concentrations were measured. AYC spray mists were optically observed. The surface of AYC cast film and AYC-coated granules were observed with a confocal scanning laser microscope. We attempted to show the utility of AYC as a novel material for granule coating, following the tablet coating in our previous report. In addition, the film formation mechanism of AYC was investigated. A smooth surface of the AYC-coated granules was obtained at a coating ratio of only 5%, which generally requires approximately 15–30% coating against the core granule weight, with no aggregation. These results are attributable to the fact that the granules were coated with a large number of small mists of AYC and the coating progressed efficiently, and the thin film layer of AYC was formed on the granules by mutual tangling of the hydrogel layers of AYC polysaccharides. AAP release from AYC-coated granules was obviously rapid, suggesting the high utility of AYC as a coating material for the rapidly releasing granules. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Acid-treated yeast cell wall; film coating; Spray mist; Granule; Film formation mechanism; Pharmaceutical additive

1. Introduction

Recently, natural materials have been studied as pharmaceutical additives from the perspective of utilization of available resources and safety

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PII: S0378-5173(02)00008-X

(Watanabe et al., 1991; Hou et al., 1991; Ashford et al., 1994; Kaneko et al., 1997 and Kaneko et al., 1999). Previously, we reported a novel preparation for acid-treated yeast cell wall (AYC) via acidification of brewers' yeast cell wall in order to discover novel utilities regarding its unique functions. We found that AYC exhibits high utility as a novel aqueous coating material for tablets. According to these findings, AYC was dispersed as independent hydrogel particles in water unlike other polymers generally employed as a solution; moreover, medicine release from tablets coated with AYC was scarcely affected by pH of the dissolution fluid or by storage at room temperature for 120 days. The AYC film demonstrated an extremely small oxygen permeability coefficient corresponding to the value for aluminum foil laminated with polyethylene and polyethyleneterephtalate as well as a sufficiently low water permeability coefficient, which protects the medicine from moisture. (Kasai et al., 2000) Acetaminophen (AAP) release from AYC-coated tablets showed a sigmoidal release profile with an initial lag time; furthermore, it was feasible to control the lag time and the release rate of AAP by varying curing time and temperature. (Yuasa et al., 2000)

In film coating on granules, agglomeration is likely due to their small gravity; moreover, it is considerably difficult to obtain the coated granule possessing a completely covered smooth surface as the surface of core granules is not smooth; additionally, it is rougher compared to that of tablet (Motoyama, 1991; Fukumori et al., 1991, 1992, 1993; Sakamoto, 1995; Yuasa et al., 1997; Kage et al., 1998; Nakano et al., 1999).

This investigation attempted to apply AYC as a novel material for granule coating via application of its aforementioned unique functions and to clarify the film formation mechanism of AYC.

2. Materials and methods

2.1. Materials

Brewers' yeast (*Saccharomyces cerevisie*, Kirin Brewery, Tokyo), following actual use in beer manufacturing, was employed as a raw material. Acetaminophen (AAP, Tokyo Kasei Kogyo, Tokyo) served as a model drug. Hydroxypropylcellulose (HPC-L, density = 1.21 g/cm^3, Shin-Etsu Chemical, Tokyo), magnesium stearate (Wako Pure Chemical Industries, Osaka) and glycerol (Wako Pure Chemical Industries, Osaka) were utilized as binder, lubricant and plasticizer, respectively. Lactose (DMV Japan, Tokyo) was used as an excipient. Hydroxypropylmethylcellulose (HPMC, TC-5R, density = 1.29 g/cm^3, Shin-Etsu Chemical, Tokyo) was utilized as a coating agent.

2.2. Preparation of AYC

AYC was prepared in the same manner as previously reported. (Kasai et al., 2000) The intracellular components of intact yeast were solubilized by reaction with intracellular or external enzymes, such as protease and glucanase, and the soluble components were removed. The acidification reaction was then conducted with 5% (w/v) aqueous dispersion of the residual fraction and 0.5 N HCl at 80 °C for 20 min. Following centrifugation, precipitates were thoroughly washed with water. The pH of the system was adjusted to 9.0 to remove bitterness substances originating from hops as the brewers' yeast utilized in this study was residue that had been employed in the actual manufacture of beer. The pH was readjusted to 3.8–4.2 and AYC was subsequently obtained following centrifugation and washing with water.

2.3. Preparation of core granule

The powdered mixture of AAP, lactose and HPC (6.0/93.4/0.6) was granulated with a fluidized bed (MP-01, Powlex, Osaka). Operating conditions for coating were as follows: the mixed powder, 500 g; inlet and outlet air temperatures, 80 and 42–45 °C, respectively; fluidization air flow rate, 45–70 m^3/h; spray pressure, 1.5 kg f/cm^2; spray rate, 15 g/min; spray air flow rate, 25 l/min; nozzle insert diameter, 0.8 mm. The granules obtained were sieved at 300–1000 μm.

2.4. Preparation of AYC-coated granule

An AYC aqueous dispersion containing 5% AYC and 0.5% glycerol was employed for coating. Coating of the core granules by the AYC aqueous dispersion was performed with a fluidized bed (MP-01, Powlex, Osaka) by the top spray method.

The operating conditions for coating were as follows: core granules, 500 g; inlet and outlet air temperatures, 60 and 32–34 °C, respectively; fluidization air flow rate, 50 m^3/h; spray pressure, 3 kg f/cm^2; spray rate, 13–14 g/min; spray air flow rate, 40 l/min; nozzle insert diameter, 0.8 mm.

2.5. Observation of core and AYC-coated granule

Core and AYC-coated granules were observed under a scanning electron microscope (SEM) (S-2250N, Hitachi, Tokyo).

2.6. Measurement of viscosity of AYC and HPMC aqueous coating dispersion and solution

Viscosity of AYC aqueous dispersion and HPMC aqueous solution was measured with a corn–plate viscometer (Brookfield Engineering Laboratories, a digital viscometer model DV-II +) at various concentrations of AYC and HPMC.

2.7. Measurement of spray mist size

Spray mist size at various concentrations of AYC and HPMC was measured with a phase doppler particle analyzer (TSI/Aerometrics, USA) in open air under room temperature and humidity.

The operating conditions of the spray gun were as follows: spray pressure, 0.5–2.5 kg f/cm^2; spray rate, 1–7 g/min; spray air flow rate, 42.5 l/min; nozzle insert diameter, 0.8 mm. The number-basis geometric diameter was determined from approximately 10 000 mist sizes.

2.8. Observation of spray mist

AYC aqueous dispersion and HPMC aqueous solution were sprayed on a glass plate in open air under operating conditions identical to those of the spray gun in the case of core granule coating. Upon spraying, spray mists were observed with an optical microscope (Digital microscope VH-7000C, Keyence Tokyo).

2.9. Observation of surface of AYC cast film and AYC-coated granule

The AYC cast film was prepared with the AYC aqueous dispersion. After degassing, the dispersion containing 0.45 g AYC was placed in a teflon petri dish with the diameter of 75 mm and dried at 40 °C and 0% RH for 3 days (Yuasa et al., 2000). A confocal scanning laser microscope (VL2000D, Lasertec, Yokohama) was used to observe the surface of AYC cast film and AYC-coated granule.

2.10. Release study

The release profiles of AAP from the AYC-coated tablet were studied with a disolution tester (NTR-6100A, Toyama Sangyo, Osaka), according to the paddle method (JP13), involving 900 ml of distilled water at 37 ± 0.5 °C and a rotating paddle at 100 rpm. The quantity of AAP was determined spectrophotometrically via measurement of absorbance at 242 nm.

3. Results and discussion

3.1. AYC aqueous coating on core granules

Fig. 1 shows SEM photographs of core and AYC-coated granules at coating ratios of 5 and 20%. Smooth and wholly-covered surfaces of granules were observed at a coating ratio of only 5%, which generally required at least 15–30% coating against the core granule weight (Bechard and Leroux, 1992; Lopez-Rodriguez et al., 1993; Maganti and Çelix, 1994; Williams et al., 1997; Kokubo et al., 1997; Tasaka et al., 1999; Yuasa et al., 2001). Agglomeration of granules during the coating process was scarcely observed.

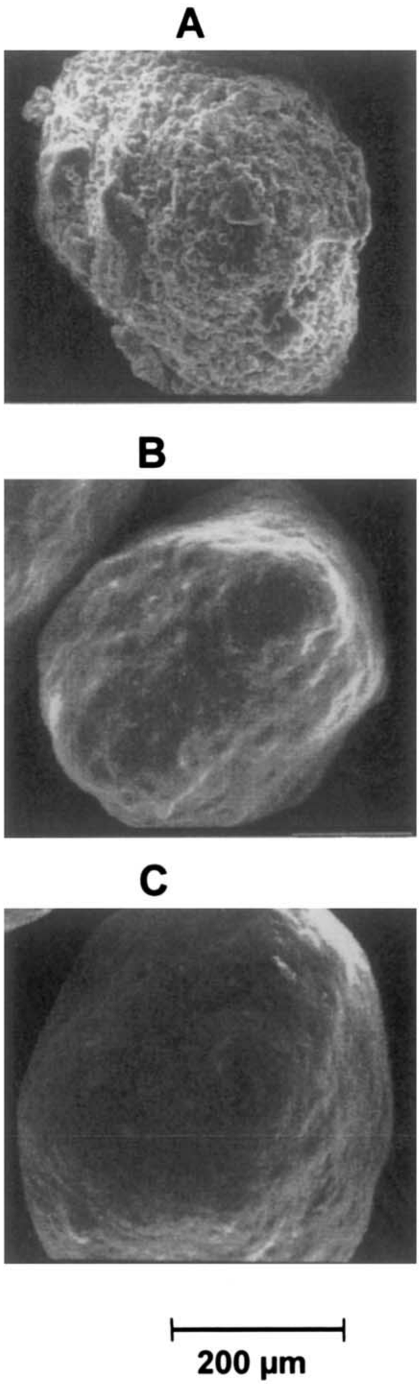

Fig. 1. SEM photographs of core and AYC-coated granules. (A) Core granule AYC-coated granules; (B) 5% coating; (C) 20% coating.

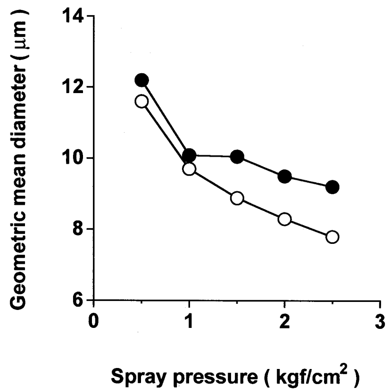

Fig. 2. Geometric mean diameter of spray mist of AYC aqueous dispersion and HPMC aqueous solution at various spray pressures. Concentration of AYC and HPMC is 5% (w/v), spray rate is 5 g/min. AYC (○); HPMC (●).

3.2. Measurement of spray mist size and viscosity of AYC aqueous dispersion and HPMC aqueous solution and observation of spray mist

To investigate the characteristics of AYC aqueous coating dispersion, measurements of spray mist size under various spray conditions and viscosity of AYC aqueous dispersion at various AYC concentrations were performed. In addition, morphological observations of AYC spray mists were conducted. These properties were compared to those of HPMC, which is widely utilized for film coating.

Fig. 2 presents the effect of spray pressure on the geometric mean diameter of spray mist of AYC aqueous dispersion and HPMC aqueous solution. The mist size decreased with increasing spray pressure in both AYC and HPMC; moreover, AYC mist size was smaller than that of HPMC at all spray pressures. Fig. 3 illustrates the effect of spray rate on the geometric mean diameter of spray mist. Mist size increased with increasing spray rate in both AYC and HPMC; additionally, AYC mist size was smaller than that of HPMC at all spray rates. Fig. 4 shows the effect of AYC or HPMC concentration on the geometric mean diameter of spray mist. The mist size of AYC was smaller than that of HPMC at all concentrations. An increase in mist size of HPMC with increasing concentration was scarcely

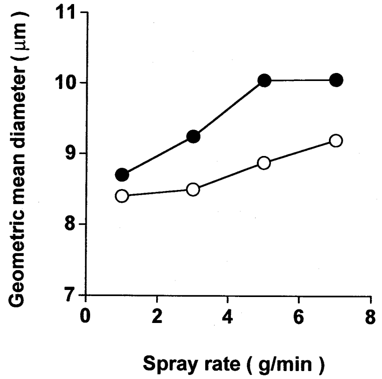

Fig. 3. Geometric mean diameter of spray mist of AYC aqueous dispersion and HPMC aqueous solution at various spray rates. concentration of AYC and HPMC is 5% (w/v), spray pressure is 1.5 kg f/cm^2. AYC (○); HPMC (●).

observed. Conversely, mist size of AYC decreased with increasing concentration. Fig. 5 displays the effect of AYC or HPMC concentration on the viscosity of AYC aqueous dispersion or HPMC aqueous solution. The viscosity of HPMC solutions slightly increased with increasing concentration, whereas viscosity of AYC aqueous dispersion markedly increased with increasing concentration. Fig. 6 exhibits the optical photographs of spray mists of AYC and HPMC. HPMC mists were observed as transparent droplets. In contrast, AYC mists were composed of one–several AYC hydrogel particles and their

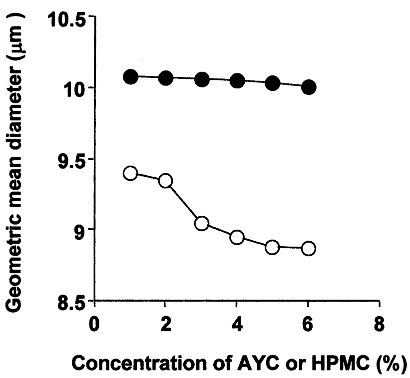

Fig. 4. Geometric mean diameter of spray mist of AYC aqueous dispersion and HPMC aqueous solution at various concentrations of AYC or HPMC. spray rate is 5 g/min, spray pressure is 1.5 kg f/cm^2. AYC (○); HPMC (●).

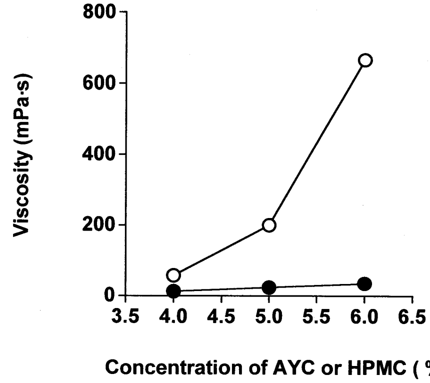

Fig. 5. Viscosity of AYC aqueous dispersion and HPMC aqueous solution at various concentrations of AYC or HPMC. AYC (○); HPMC (●).

hydrogel layers, consisting of the graft chains of the glucan and mannan-protein complex, were in mutual contact (Northcote and Horne, 1952; Lampen, 1968; Kidby and Davies, 1970; Fleet and Manners, 1976).

As we have previously reported, AYC maintains the shape of the original yeast and displays the baggy structure. In water, AYC disperses including the water inside the structure. (Kasai et al., 2000; Yuasa et al., 2000). Unlike a linear polymer such as HPMC in which long chains are mutually tangled in solution, AYC disperses as a hydrogel particle contacting one another through weak binding forces between AYC particles in the AYC coating dispersion. Therefore, AYC hydrogel particles were readily separated at spraying and the mists including one–several AYC hydrogel particles were generated and the mist size of AYC may be smaller than that of HPMC at every spray condition.

As shown in Fig. 5, the increase in the viscosity of HPMC solution in concentrations of 4 to 6% was very slight. In the case of AYC dispersion, AYC dispersed including the water inside the structure as mentioned above. The increase in the concentration of AYC caused the fluidity of the AYC hydrogel particles to decrease due to the decline in the amount of dispersion medium. Thus, the viscosity might markedly increase with increasing AYC concentration. The viscosity, a rheological characteristic value indicative of flow resistance, increased with increasing AYC concentration, whereas the spray mist size decreased. This finding may be attributable to the fact that AYC becomes easy to disperse at spraying as a result of the decrease in the amount of dispersion medium existing between AYC hydrogel particles.

3.3. Observation of surface of AYC cast film and AYC-coated granule

Fig. 7 shows confocal scanning laser microscopic photographs of surfaces of AYC cast film and AYC-coated granules. In the case of the AYC cast film, AYC particles overlapped one another and the hydrogel layers were mutually tangled; subsequently, the film was formed. In the case of AYC-coated granules, the AYC hydrogel was expanded as compared with the AYC cast film. These results suggest the following. The AYC mists were composed of one–several AYC hydrogel particles. In the coating process, the AYC hydrogel particles including sufficient moisture adhered to the surface of granules and expanded by shearing stress generated by fluidization of the granules. The expanded hydrogels were tangled; additionally, the AYC was coated on granules. As a result, the thin AYC coating layer was formed on the surface of the granule, causing the smooth surface of the granule at a small coating ratio.

As shown in Fig. 4, the geometric mean diameters of spray mist of AYC aqueous dispersion and HPMC aqueous solution at 5% of concentration used for granule coating were 8.8 and 10 μm, respectively. When the mist volume is calculated from the geometric mean diameter and the number of mists at spraying of an equal volume are compared, the number of spray mists of AYC is approximately 1.47 times greater than that of HPMC. This result indicates that the granule was coated with a large number of small mists and the coating progressed efficiently with no aggregation

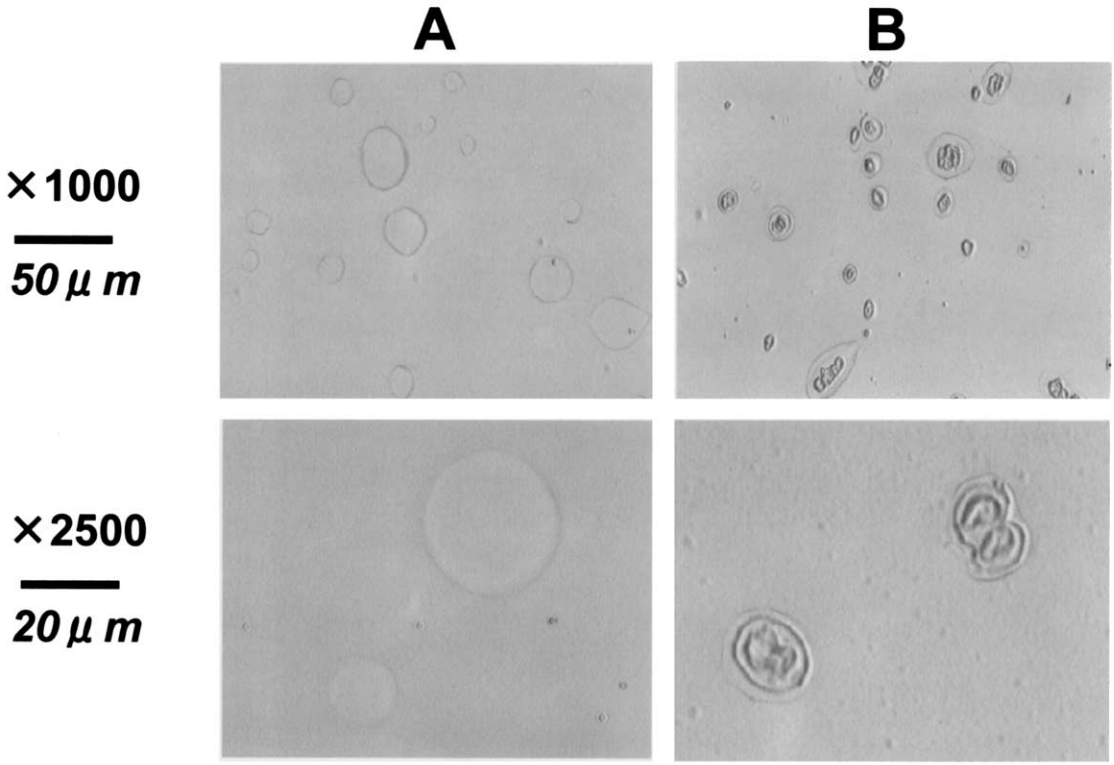

Fig. 6. Photographs of spray mist of AYC aqueous dispersion and HPMC aqueous solution. (A) HPMC, (B) AYC.

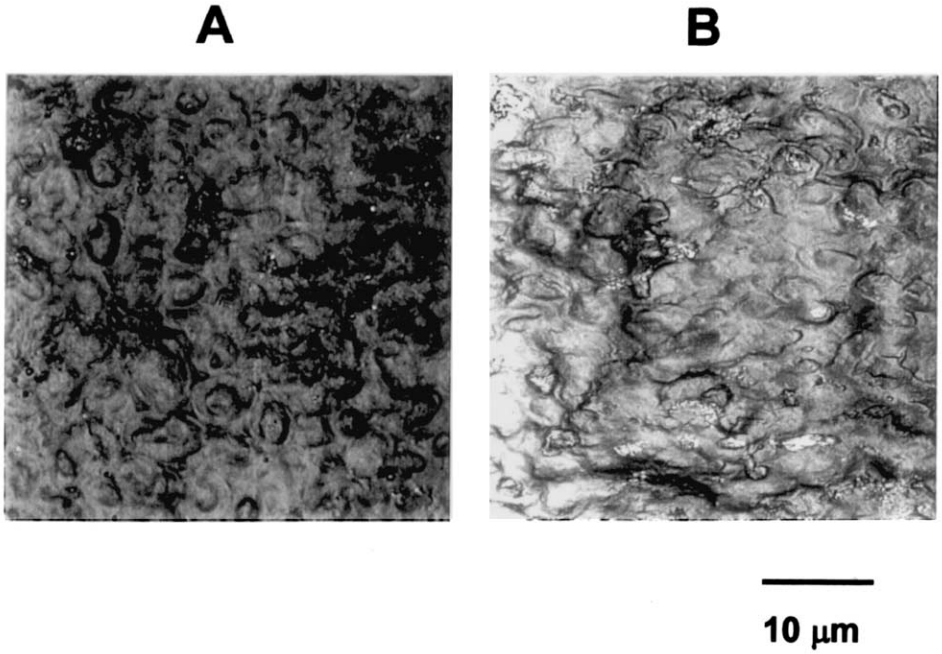

Fig. 7. Confocal scanning laser microscopic photographs of the surface of AYC cast film and AYC-coated granule. (A) AYC cast film, (B) AYC-coated granule.

(Schoefer and Wørts, 1977; Sakamoto, 1995; Yuasa et al., 1999).

3.4. Release profiles of AAP from AYC-coated granules

Fig. 8 shows AAP release profiles from the core and AYC-coated granules. AAP release from all AYC-coated granules was obviously rapid, suggesting that AYC possesses high utility as a coating material for the rapidly releasing granules.

4. Conclusion

It was feasible to obtain the smooth surface of the AYC-coated granules at a coating ratio of only 5% with no aggregation. These findings arise due to the fact that the granules were coated with a large number of small spray mists and the coating progressed efficiently, and the thin film layer of AYC was formed on the granules by the mutual tangling of the hydrogel layers of AYC. AAP release from the AYC-coated granules was obviously rapid. These results suggest that AYC possesses high utility as a coating material for the rapidly releasing granules, e.g. the bitter taste masking.

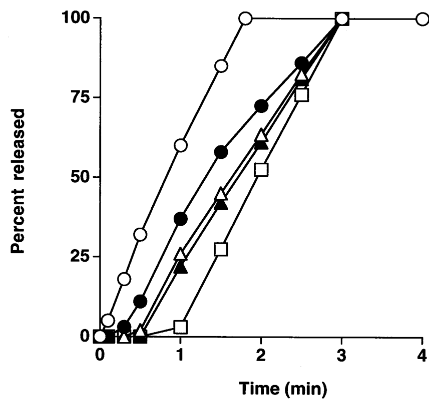

Fig. 8. Release profiles of AAP from core and AYC-coated granules. Core granule (○); AYC-coated granule (coating ratio: 5%, ●; 10%, △; 15%, ▲; 20%, □).

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