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Evaluation of relationship between molecular behaviour and mechanical strength of pullulan films

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ABSTRACT

This study aimed to investigate the relationship between the physical properties and molecular behaviour of pullulan films. To study its thermal behaviour, we obtained a pullulan film by the solvent cast method and analysed it by differential scanning calorimetry (DSC), thermogravimetry analysis (TG), thermomechanical analysis (TMA) and FT-IR spectroscopy. The pullulan film contracted with an increase in temperature, and the film contraction depended on the decrease in water content in the film. The FT-IR spectra of a pullulan film was used as a calibration model set to establish such a model to predict the film contraction and water content by principal component regression (PCR) analysis. The film contraction and water content could be predicted using a calibration model obtained by PCR. The molecular behaviour of pullulan films due to film contraction and decreased water content were demonstrated by analysing the regression vector consisting of PC1 and PC2.

1. Introduction

Capsules are pharmaceutically and universally applicable dosage forms that offer improved drug stability because their contents are tightly enclosed by the capsule shell, and thus, they are protected from oxygen, moisture and light, and also from physiological fluids, until the drug is released. Hard capsules have been used to deliver drugs in the form of powders, granules and pellets to the gastrointestinal tract. The major components of a capsule are gelatin and hydroxypropyl methylcellulose (HPMC); they can be used to form suitable films that dissolve readily in the stomach. In the case of gelatin capsules, incomplete in vitro release problems due to a cross-linking reaction (Digenis et al., 1994; Cadé et al., 1994) have recently attracted attention. This problem was first reported in 1974 for a hard gelatin capsule containing chloramphenicol (Khalil et al., 1974). This case was associated with adverse storage conditions such as elevated temperature, humidity or prolonged storage. Moreover, after the outbreak of bovine spongiform encephalopathy (BSE), HPMC has been attracting increasing attention as an alternative to avoid the risks associated with the use of animal-derived ingredients. HPMC has therefore been commonly used as a film-forming agent. Carrageenan and potassium chloride are added in small quantities to lower the thermal gelation temperature of HPMC and to promote gelation, respectively (Matsuura and Yamamoto, 1993). These hard HPMC capsules exhibit no incompatibilities with most filling materials or powders since the only incompatibility currently known for HPMC is the interaction with certain oxidizing agents. Moreover, Chiwele et al. (2000) reported that in water and dissolution media similar to gastric fluids, at 37 °C, the shell dissolution time of hard HPMC capsules was only slightly greater than that of hard gelatin capsules. On the other hand, Fridrun and Brian (2002) reported that a change from hard gelatin capsules to hard HPMC capsules should not pose problems with respect to drug absorption and bioavailability. Moreover, they reported that using hard HPMC capsules instead of conventional gelatin capsules might be advantageous.

Recently, hard pullulan capsules have been developed; these are water-soluble, neutral polysaccharides with low oxygen permeability. Pullulan is used for numerous applications in food coating (Yuen, 1974; Tsujiska and Mitsuhashi, 1993) and in pharmaceutical industries in addition to its application as hard capsules. It is a linear polymer having martotriosyl units connected by (1–6)-α-D linkages, and it is prepared by the culturing of *Aureobasidium pullulans* with starch and sugar (Wallenfells et al., 1961). The mechanical strength of hard gelatin capsules depends on the relative humidity both inside and outside the capsule. Therefore, we studied the relationship between the water activity and the mechanical strength of hard pullulan capsules filled with potato starch. The mechanical strength was found to decrease with the water activity. Thus far, there have been many reports on the physical, mechanical and dissolution properties of gelatin and hard HPMC capsules, while there have been few reports on the characteristics of hard pullulan cap-

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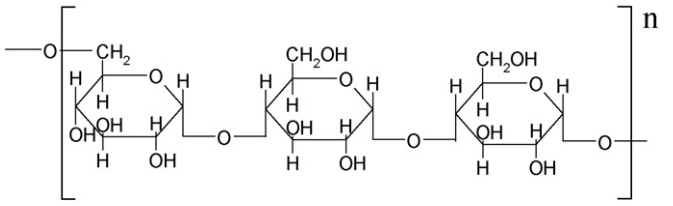

Fig. 1. Chemical structure of pullulan.

sules. In this study, we investigated the relationship between the physical properties and molecular behaviour of pullulan cast films; this is the basic information required to predict the physicochemical properties of pullulan capsules.

2. Materials and methods

2.1. Materials

Pullulan, PF-20-grade [number–average molecular weight (Mn) = 200,000–250,000 Da], was purchased from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The structures of pullulan are shown in Fig. 1. Pullulan capsules and potato starch were purchased from Capsugel Japan, Inc. (Kanagawa, Japan), and Maruishi Pharmaceutical Co. Ltd. (Osaka, Japan).

2.2. Moisture content and water activity

The moisture content of the capsules and the filled potato starch were determined gravimetrically by reweighing the capsules and potato starch after drying at 105 °C for 5 h in a convection dryer (Forced Convection Oven, Advantec Toyo Co. Ltd., Tokyo, Japan). The water activity of the capsules and the filled potato starch was analysed using a water activity meter (HYGROLAB, GSI Creos Co., Tokyo, Japan). The water activity of the capsule wall and potato starch was measured in an airtight chamber.

2.3. Preparation of cast films under aqueous conditions

Pullulan solutions were prepared by weighing the desired amounts of pullulan with distilled water and leaving the solutions to stand overnight. The concentrations of pullulan were maintained constant at 15% (w/w). The cast films were prepared by casting the solutions onto a clean glass plate and leaving them overnight under conditions of 30% relative humidity at 50 °C to allow solvent evaporation. The cast film was then peeled off and used for different tests (DSC, TG, PXRD, TMA and FT-IR analysis).

2.4. DSC and TG analysis

The DSC and TG curves were obtained by thermogravimetric analysis (TG–DTA EXSTRA 6000 using measuring cell DSC 30E; Seiko). Approximately 15–20 mg of the sample was weighed in the DSC pan. The unsealed pan was placed in the sample side of the instrument. An identical reference pan was placed in the reference side. Scans between 25 and 250 °C were carried out at a rate of 2 °C/min using a nitrogen gas purge at 50 ml/min. In the TG curves, the different levels of water content in the pullulan films represent weight loss over the range of 31–70 °C.

2.5. TMA analysis

The TMA curves were obtained by thermo-mechanical analysis (TMA/SS 6300 EXSTRA 6000; Seiko). A film specimen having a length of approximately 20 mm and thickness in the range of 0.20–030 mm was immobilized in the sample holder. Scans between 25 and 230 °C were carried out at a rate of 2 °C/min using a nitrogen gas purge at 50 ml/min.

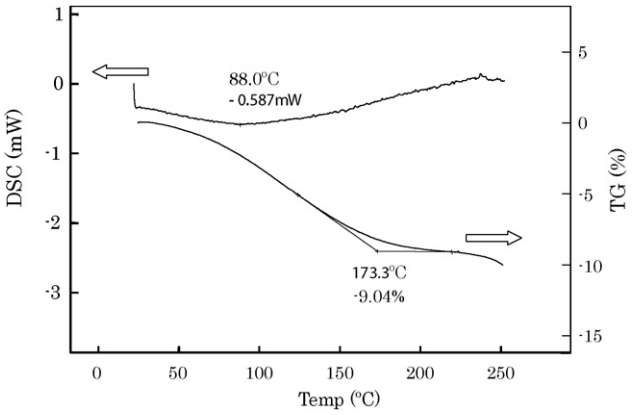

Fig. 2. DSC and TG thermographs of the pullulan film.

2.6. Thermal FT-IR microspectroscopic analysis

The cast film was sliced using SliceMaster® (Jasco, Tokyo, Japan). The sliced film, having a thickness in the range of 50–100 μm, was then sandwiched between 2 KBr pieces using a hydraulic press to form a disc. This sample disc was directly placed on a micro hot stage and then set on the stage of the microscope installed in the FT-IR microscopic spectrophotometer (FT-IR4100, Jasco, Tokyo, Japan) with an MCT-M detector. The system was operated in the transmission mode. The desired sample size was selected and defined by means of an aperture through an optical system (IRT 3000, Jasco, Tokyo, Japan) by using a microscope. The FT-IR spectra were obtained on a FT-IR 4100 spectrometer over the 4000–600 cm^{-1} region. 32 scans were performed at a resolution of 4 cm^{-1}. The heating rate was controlled at 2 °C/min under ambient conditions. The sample disc was equilibrated at the starting temperature (31 °C) and then heated to 70 °C. The thermal-responsive IR spectra were recorded with respect to the temperature. The FT-IR spectra were used as a calibration set to establish a calibration model to predict the film contraction and water content by principal component regression (PCR) analysis. A chemometric analysis was performed by using the PCR program, a part of the Pirouette software (InfoMetrix Co., USA). The conditions were optimized to minimize the standard error of cross-validation (SEV).

3. Results and discussion

3.1. Relationship between film contraction and water content

Thus far, the physical, mechanical and dissolution properties of hard gelatin and hard HPMC capsules have been reported; however, there have been few reports on the characteristics of hard pullulan capsules. Therefore, we prepared hard pullulan capsules containing potato starch in order to study their physical properties. The mechanical strength of these capsules decreased with the water activity in the capsule wall during storage, which led to the contraction of capsule thickness. To clarify the effects of water content on the contraction behaviour of pullulan capsules, we investigated pullulan films using DSC, TG and TMA analysis.

Fig. 2 shows the TG and DSC profiles obtained during the first heating of pullulan films under non-isothermal treatment. The film exhibits weight loss over the range of 35–173.3 °C in open pan TG. When subjected to DSC in an open pan under experimental conditions similar to those for TG, the broad peak temperature in the

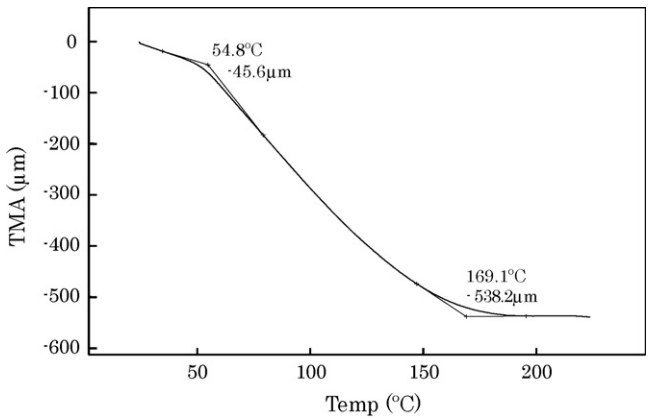

Fig. 3. TMA thermograph of the pullulan film.

DSC curve was found to agree well with that for the weight loss in TG. Since both the TG and DSC profiles obtained during the second heating of the films did not exhibit weight loss and a broad peak, the weight loss in TG and the broad peak in the DSC curve observed during the first heating would indicate the free water in the pullulan films.

The typical TMA profiles of pullulan films under non-isothermal treatment are shown in Fig. 3. The films gradually contracted with increasing temperature, and the flexion point was found to be at 54.8 °C. The flexion point due to the thermal behaviour of pullulan films in TMA results was not found in the DSC and TG results. Above the flexion point, the pullulan film contracted noticeably, whereas the contraction behaviour of the films did not change at temperatures above 169.1 °C.

Fig. 4 shows the relationship between film contraction and water content under non-isothermal conditions. The plot yielded a straight line with a coefficient of determination constant of 0.9882. The mechanical strength of the hard pullulan capsules decreased with the water content in the capsule wall due to water transfer from the capsule wall to the potato starch. This phenomenon was also observed in the case of hard gelatin capsules by Ito et al. (1969). Therefore, the result suggests that the contraction behaviour of the hard pullulan capsule may be attributed to the water content in its wall, which may be related to the change in its mechanical strength.

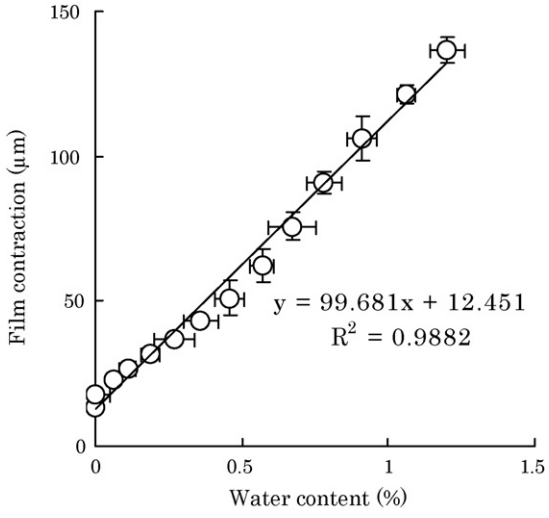

Fig. 4. Relationship between film contraction and water content in pullulan films.

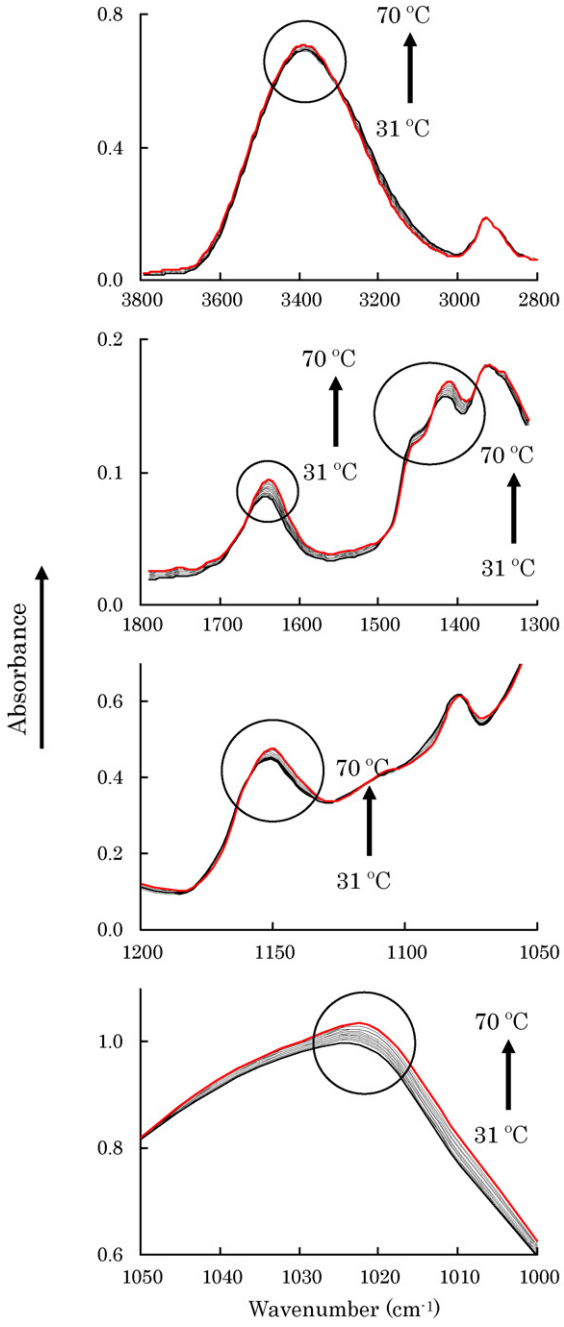

Fig. 5. FT-IR spectra of the pullulan film with respect to the temperature on heating.

3.2. FT-IR spectra of cast films under non-isothermal conditions

It has been reported that the mechanical strength of a pullulan film depends on the preparation temperature of the films prepared using aqueous solutions of pullulan alone (Kawahara et al., 2003). In contrast, there has been no report on the molecular behaviour of pullulan films under heating. To clarify the molecular behaviour of pullulan films under non-isothermal conditions, we used the widely adopted FT-IR approach and studied the chemical and physical changes occurring in the molecular structure of biological materials.

Fig. 5 shows the raw thermal FT-IR spectra of casting films obtained in the range of 31–70 °C under non-isothermal conditions. The functional groups associated with the pullulan polymer were CH, CH_2, C—O—C, C—O and H—O—H. The assignment of each peak

in the film is as follows. The CH and CH_2 stretching absorption bands were found in the 1250–1460 and 2850–2980 cm^{-1} regions. The absorption band at 1640 cm^{-1} corresponded to absorbed water (H—O—H). The broad absorption band in the 3300 cm^{-1} region represented the OH stretching vibration and the hydrogen bonding in the polymer. Moreover, the main bands found in the deconvoluted spectra of pullulan at ca.1155, 1107, 1080, ca.1020 and 1000 cm^{-1} were due to valent vibrations of the C—O and C—C bonds and deformational vibrations of the CCH, COH and HCO bonds (Firsov et al., 1999). The band at 1150 cm^{-1} has been previously assigned to valent vibrations of the C—O—C bond and glycosidic bridge (Kačuráková et al., 2000). The broad peak at 1107 cm^{-1} is probably attributable to the vibration of the C—O bond at the C_4 position of a glucose residue (Kačuráková and Mathlouthi, 1996). The band at 1080 cm^{-1} has been assigned to the vibrations involving the stretching of the C_6—O_6 bond with participation of the deformational vibrations of the C_4—C_5 bond (Shingel, 2002). The bands at approximately 1047 and 1022 cm^{-1} found due to polysaccharides in the spectra of starch were shown to be related to the crystalline and amorphous phase, respectively (van Soest et al., 1994, 1995; Smits et al., 1998). The changes in the intensity of these bands are strongly associated with alterations in the macromolecular order (Wilson and Belton, 1988; Rubens et al., 1999; van Soest et al., 1996). Obviously, the absorbance of FT-IR increased with temperature. In particular, the absorption band corresponding to hydrogen binding (3390 cm^{-1}), adsorbed water (1640 cm^{-1}), CH and CH_2 stretching (1460 and 1409 cm^{-1}) and valent vibrations of the C—O—C bond and glycosidic bridge (1150 cm^{-1}) were strongly affected with increasing temperature. In addition, the intensity of the absorption band corresponding to alterations in the macromolecular order (1030 cm^{-1}) was also strongly affected. Therefore, it is apparent that the increase in temperature affects the molecular behaviour of pullulan films; however, the relationship between these parameters remains unclear.

The frequency shifts observed in Fig. 5 are quite significant. The interpretation of these shifts may clarify the molecular interactions that occur during heating. Chemometrics can be used to decompose raw data profiles and help us understand the significant contributions of specific data groups. Quantitative relationships between objective parameters such as the pharmaceutical properties of films and loadings of the principle components were obtained from the FT-IR spectral results. In the present study, in order to predict the film contraction and water content, calibration models were established based on the FT-IR spectra of the films and their actual film contraction by PCR. Since raw absorbance scans exhibit a baseline in the spectra, the calibration model for the pullulan film was based on the absorbance spectra without any pretreatment.

Table 1
SEV and variance values in the case of the film contraction and the water content prediction.

Number of factors	Film contraction (μm)		Water content (%)	
	SEV	Variance, %	SEV	Variance, %
PC1	9.010	91.784	0.057	91.926
PC2	3.353	5.873	0.035	5.720
PC3	3.555	1.084	0.039	0.876
PC4	3.082	0.567	0.042	0.663
PC5	4.273	0.168	0.050	0.278
PC6	3.677	0.207	0.046	0.209
PC7	3.496	0.082	0.044	0.110
PC8	3.568	0.045	0.046	0.081

Using PCR, we developed a calibration model of the principal components (PCs) in order to predict the film contraction and water content. The calculated results are shown in Table 1. The SEV values of the film contraction and water content decreased with an increase in the number of principal number component factors; however, they were almost constant after factors 2 and 2. Therefore, the minimum SEV values could thus be realized by using two- and two-principal component models for the analysis of the FT-IR spectra. The relationships between the calculated and measured values for film contraction and that for water content are shown in Fig. 6; the plots yielded straight lines with slopes of 0.9761 and 0.9899, *Y*-intercepts of 1.3098 and 0.0014, and multiple correlation coefficient constants, r^2, of 0.9918 and 0.992, respectively. These predicted values indicate that FT-IR has a good predictive potential for film contraction and water content.

3.3. Theoretical analysis of film contraction

The theoretical analysis of the contraction behaviour of pullulan films was performed using chemometrics. Chemometrics can be used to decompose raw data profiles, and it helps us to understand the significant contributions of specific data groups of process variables to the variability in raw data profiles. The quantitative relationships between the objective parameters, such as film con-

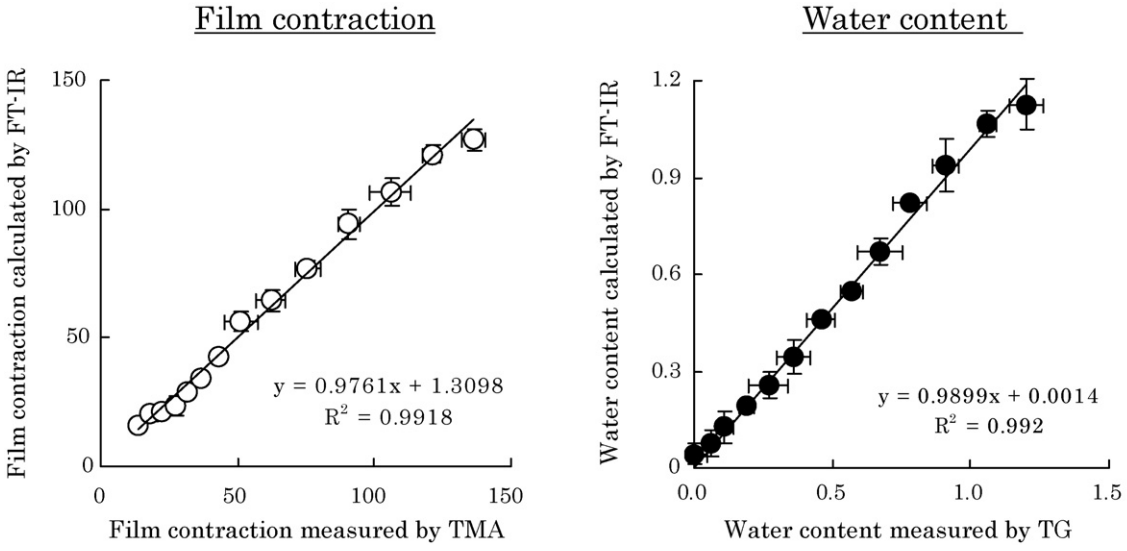

Fig. 6. Relationship between the actual and predicted film contraction and water content of pullulan films.

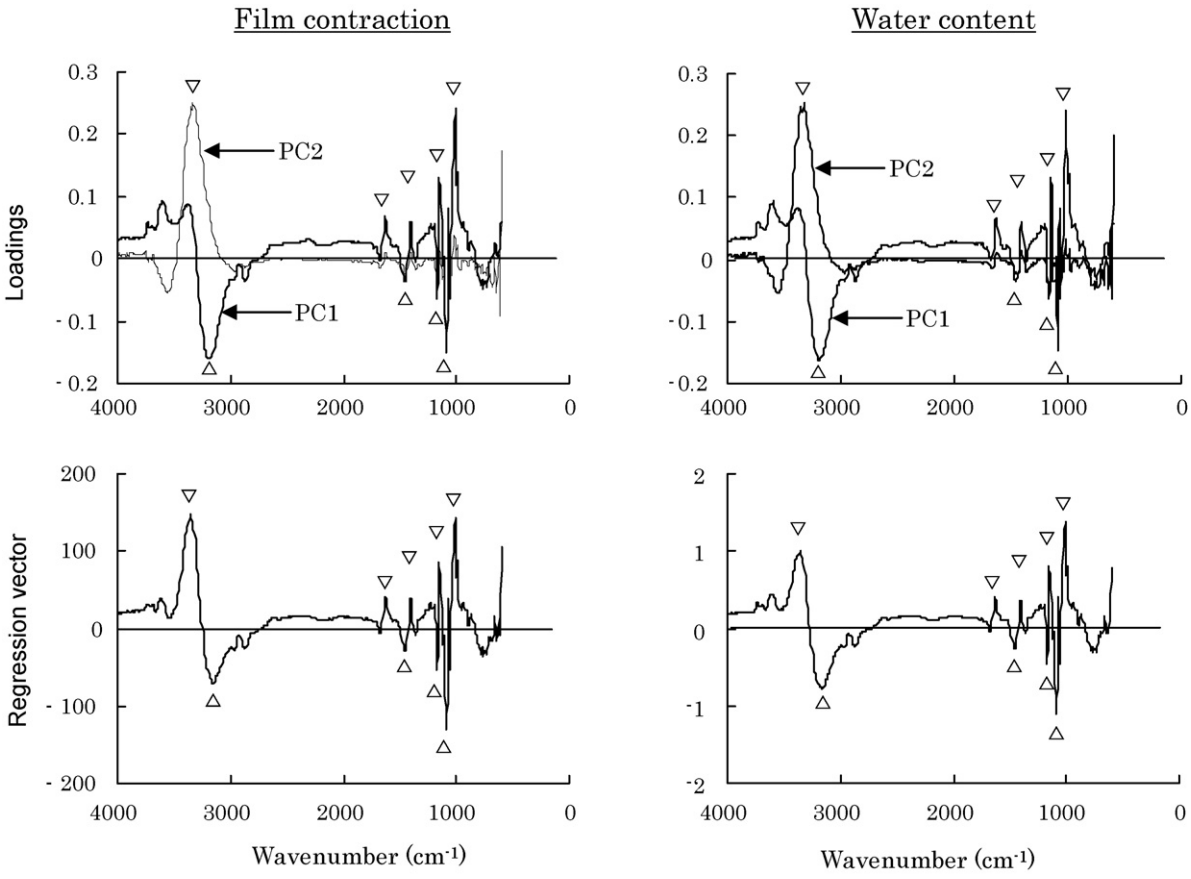

Fig. 7. Loadings and regression vector for predicting the film contraction and water content of pullulan films.

traction and water content, and loadings of the PCs are obtained from the FT-IR spectra results over the 4000–600 cm^{-1} region. The decrease in water content in pullulan capsules resulted in a decrease in their mechanical strength; this, in turn, led to their contraction. In the present study, in order to understand the theoretical analysis of the calibration models used to evaluate the film contraction, the relationships between the SEV values and the number of PCs used and the loading vectors of each PC and film contraction were investigated.

The SEV and variance values of the calibration model for film contraction and water content are shown in Table 1. The SEV value of film contraction decreased within the limits of PC1 and PC2, and the variance values were 91.784 and 5.873 at PC1 and PC2, respectively. The SEV value of water content decreased within the limits of PC1 and PC2, and the variance values were 91.926 and 5.720 at PC1 and PC2, respectively. These results might indicate that PC1 and PC2 are closely related to the pharmaceutical properties related to film contraction and water content.

Fig. 7 shows the regression vector and loading vector related to film contraction and water content. The regression vector is the spectrum pattern comprising the loading vectors of PC1 and PC2. In the case of film contraction, the loading vector of PC1 exhibited negative peaks at 3190, 1470, 1170 and 1089 cm^{-1} (Fig. 7) and positive peaks at 1630, 1410, 1150 and 1010 cm^{-1} (Fig. 7) that might be attributable to a combination of the hydrogen binding (3190 cm^{-1}), CH and CH_2 stretching (1410 and 1470 cm^{-1}, respectively), valent vibrations of the C—O—C bond and glycosidic bridge (1150 and 1170 cm^{-1}, respectively), stretching vibrations of the C_6—O_6 bond with participation of the deformational vibrations of the C_4—C_5 bond (1089 cm^{-1}) and alternations in the macromolecular order (1010 cm^{-1}). PC2 had a positive peak at 3360 cm^{-1} that might be attributable to a hydrogen bonding in the polymer. Moreover, PC1, the loading vector of water content, exhibited negative peaks at 3190, 1470, 1170 and 1090 cm^{-1} and positive peaks at 1630, 1410, 1150 and 1010 cm^{-1} (Fig. 7), and PC2 exhibited a positive peak at 3360 cm^{-1} (Fig. 7). Therefore, the main peaks for the regression vector of water content were observed to be similar to those for film contraction. Therefore, the result suggested that the calibration model for film contraction could be predicted based on that for water content.

On the other hand, the regression vector comprising both PC1 and PC2 exhibited absorption bands at 3190–3200 cm^{-1} and at 3360–3370 cm^{-1} corresponding to hydrogen bonding. In fact, the mechanical strength of the hard pullulan capsules filled with potato starch decreased with the water content in the capsules, as observed in the present study. Usually, the water in the film is known to play the role of a plasticizer. Therefore, these results obtained by FT-IR, TMA and TG suggest that the change in molecular behaviour due to film contraction would be strongly related to the water content in the films.

4. Conclusions

To evaluate the relationship between film contraction and water content in pullulan films, calibration models were constructed based on the transmission FT-IR spectroscopic method using PCR. It was suggested that the film contraction and water content could be predicted based on the FT-IR spectra. Since the regression vector of film contraction and water content was a spectrum pattern comprising 2 PCs, the calibration models for film contraction could be predicted using that of water content. On analysing the regression vectors of film contraction and water content, we observed that the

molecular behaviour of pullulan films might be strongly related to their water content.

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