Molecular encapsulation of ascorbyl palmitate in preformed V-type starch and amylose

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Molecular encapsulation of bioactive molecules in preformed V-type amylose

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ABSTRACT: Starch-guest inclusion complexes have been known for over 200 years as the subject of hundreds of scientific studies. However, few technical applications have been successfully commercialized, despite structural similarity to cyclodextrin-guest inclusion complexes that are employed in numerous high-value applications in the pharmaceutical and other industries. One of the greatest hindrances of successful exploitation of starch-guest complexes is a lack of cost-efficient and reproducible methods for their preparation. Existing methods to produce starch-guest complexes generally require high temperature processing, complicated procedure, and waste of guest materials. In the present study, we introduce a simple method to prepare inclusion complex by "inserting" guest molecules into preformed "empty" V-type amylose helices. Ascorbyl palmitate was used as a model guest material to investigate the

effect of solvent environment, complexation temperature, annealing and guest concentration on the efficiency of inclusion complex formation. Results showed that high complexation temperature is not necessary for encapsulating guest molecules in amylose helices. This method would also avoid the wasting of guest materials because uncomplexed guest can be reused instead of being washed away in other methods.

KEYWORDS: starch, amylose, inclusion complex, bioactive, molecular encapsulation.

Introduction

Starch, especially its amylose component, is well known to form inclusion complexes with a variety of small molecules, e.g. iodine,[1] alcohols,[2] fatty acids, aromas,[3,4] salicylic acid[5] and its analogues,[6] ibuprofen, and warfarin.[7] In the presence of guest molecules, amylose forms a 6-fold left-handed single helix stabilized by hydrogen bonds.[4] The amylose helix has a hydrophilic outer surface and a hydrophobic helical channel that accommodates the guest molecules. The amylose helices may then pack together and form a crystalline structure known as V-type. There are a number of V subtypes. The intra-helical inclusion complex is known as subtype V_{6I} (or V-hydrate/V_h). It can then convert to anhydrous V (Va) upon losing water between the helices. Alternatively, guest molecules can also be entrapped between amylose helices (interhelical association) in subtype V_{6II} (or $V_{butanol}$) and V_{6III} (or $V_{isopropanol}$), which have larger inter-helical space than V_{6I}.[8,9] These two types can be converted to V_{6I} upon solvent departure. With α-naphthol as the guest, amylose is thought to form 8 fold helices (V_8 or $V_{\alpha\text{-naphthol}}$).[10]

Starch-guest inclusion complexes may be useful as a delivery system for guest molecules. For instance, amylose complexed with conjugated linoleic acid,[11] genistein,[12] esters of vitamin and fatty acid,[13] and long chain unsaturated fatty acids[14,15] have been produced for controlled

release purposes. By forming an inclusion complex with amylose or starch, it is expected that the active ingredients, such as essential fatty acids, lipophilic vitamins, and soy isoflavones, can be protected against the acidic environment of the stomach, and their bioavailability may be increased, since the bioactive guest compounds can be released in the small intestine when amylose breaks down by the action of enzymes or saccharolytic bacteria.[16,17]

Current techniques to form starch inclusion complex involve first converting amylose to its random coil conformation and inducing coil to helix transition with the presence of guest molecules. There have been primarily three methods to form amylose random coils or loose helices, and thus three methods to prepare amylose-guest inclusion complexes, namely dimethyl sulfoxide (DMSO) method, alkali method, and high temperature method. In the DMSO method, starch or amylose is dissolved in a DMSO aqueous solution (e.g. 95% (v/v) DMSO) and mixed with the desired guest. The dispersion is diluted with water at an elevated temperature and allowed to cool slowly. The inclusion complexes formed will then precipitate. In the alkali method, starch or amylose is dissolved in an alkali aqueous solution (e.g. 0.01 M potassium hydroxide) and mixed with the desired guest compound. The dispersion is then neutralized and slowly cooled prior to precipitation. In the high temperature method, a starch or amylose aqueous solution is heated to a high temperature (e.g. 155 ^{o}C) and mixed with guest compounds at a lower temperature (e.g. 90 ^{o}C). The dispersion is allowed to cool and inclusion complex precipitate can be collected.

Starch-guest inclusion compounds have been known for over 200 years as the subject of hundreds of scientific studies, yet no technical applications have been successfully commercialized (with the possible exception of dough conditioners). A homologous structure to the V-type amylose-guest inclusion complex is the cyclodextrin-guest inclusion complex, which

have been employed in numerous high-value applications, for instance, in the pharmaceutical industries to improve drug stability, solubility, bioavailability, and control drug release.[18,19]

One of the greatest hindrances of successful exploitation of starch-guest complexes is a lack of cost-efficient and reproducible methods for their preparation. As aforementioned, current techniques to produce starch-guest inclusion complex require a relatively high processing temperature (at least 70 to 80 ^{o}C) and a long time to allow complexation, crystallization and precipitation (hours to days). In contrast, the preparation of cyclodextrin-guest inclusion complexes generally require much less time and lower temperature.[20] Common methods include co-crystallization, co-evaporation, co-grinding, kneading and etc. In most cases, guest molecules enter the cyclodextrins cavity spontaneously when particular solvent environment is satisfied. Despite easy preparation and successful commercialization, the use of cyclodextrins have certain drawbacks. First, compared with starch and amylose, cyclodextrins are costly, because they are produced from starch degradation by cycloglycosyl transferase amylases produced by various bacilli.[21] Secondly, when complexation efficiency is low, precipitated product is a mixture of the inclusion complex, empty cyclodextrin, and free guest. Empty cyclodextrin is wasted and production cost is increased. Thirdly, certain methods (e.g. co-crystallization) using aqueous solution are restricted to β-cyclodextrin, because low solubility is required for easy precipitation. Though the product is inclusion only, the yield is very low. Above all, these considerations are all associated with the high cost of cyclodextrins, which definitely increase the price of nutraceutical products and pharmaceutical drugs. If an inclusion forming method as easy as that of cyclodextrins is available for low cost starch, production cost of encapsulation products can be significantly reduced.

Here we introduce a new method to create amylose-guest inclusion complex, which involves first precipitating “empty” V-type amylose helices and then “inserting” guest molecules into the helices at temperatures well below the melting point of the V-type amylose. This method is similar to the preparation of cyclodextrin-guest inclusion complex and has several advantages over the traditional techniques for amylose inclusion complex. First, this method does not require a high temperature at the step of forming the inclusion complex, such that oxidation or degradation of heat-labile compounds can be minimized. Secondly, uncomplexed compounds in the solvent can be reused for the next batch. This makes it appropriate for expensive compounds, which would otherwise be washed away in previous methods. Finally, complexation takes place immediately when the V-type amylose and guest are mixed together. Hence, this simplified technique to produce amylose inclusion complex has easy scalability and great potential to replace the costly cyclodextrins in a number of applications.

Specifically in this study, we propose to prepare “empty” V-type amylose/starch and take advantage of their empty helical cavity as a novel method of forming inclusion complex with model bioactive guest compound, i.e. ascorbyl palmitate (AP). AP is an ester form of ascorbic acid (vitamin C) with palmitic acid and has been used as a source of vitamin C and an antioxidant food additive. We are to investigate the effect of complexation temperature, solvent quality, AP concentration, and annealing of V-amylose on the ability and efficiency of inclusion complex formation. In still another report, we will demonstrate method variations for a variety of guest compounds.

Materials and Methods

Materials. High amylose corn starch (Hylon VII) was kindly provided by Ingredion Incorporated (Bridgewater, NJ) and used as received. Potato amylose (essentially free from

amylopectin), ascorbyl palmitate (AP) were purchased from Sigma-Aldrich, Inc (St. Louis, MO). Acetone (HPLC) was obtained from EMD Millipore Chemicals (Billerica, MA). Ethanol (200 proof) and dimethyl sulfoxide (DMSO) were obtained from VWR International (Radnor, PA).

"Empty" V-amylose and V-starch preparation. The "empty" V-amylose and V-starch were prepared following two routes. In the first method, amylose or starch (5%, w/v) was dissolved in 95% (v/v) DMSO aqueous solution in a boiling water bath with constant stirring for at least one hour. Then the hot amylose or starch dispersion was mixed into 2.5 volumes of ethanol with vigorous stirring. The mixed suspension was then centrifuged (2000 g, 10 min). The precipitate was washed with ethanol twice and finally dried. In the second method, amylose or starch (1%, w/v) was dispersed in water at 150 ^{o}C for 30 min in a stirred pressure reactor (Parr-4592, Parr Instrument Company, Moline, IL). The amylose or starch dispersion was cooled to 90 ^{o}C and mixed into 2.5 volumes of ethanol. The V-amylose and V-starch were recovered by the same centrifugation, washing and drying procedure as described in the first method.

Annealing of the "empty" V-amylose and V-starch. The dry powders of V-amylose and V-starch were heated in different ethanol:water mixtures at different temperatures for 10 min. The combination of these conditions resulted in different crystallinities of the annealed V-amylose and V-starch. Annealed V-amylose and V-starch were recovered by the same centrifugation, washing and drying procedure as described earlier.

Inclusion complex formation. Only one method (co-mixing in solvent) is used in this study, while other methods will be demonstrated in another report. 0.05 grams of guest compounds and 0.1 grams of the preformed "empty" V-amylose (or V-starch) were mixed together into 10 mL of different solvents. The solvents used were ethanol:water mixtures (0/100 to 100/0, v/v) and acetone:water mixtures (0/100 to 100/0, v/v). The suspension was kept at 20,

45, or 70 ºC for 10 min. In the section to study the effect of AP concentration, 0.05 grams of V-amylose in 2 mL of 80% (v/v) ethanol that contains 0.01, 0.02, 0.1, and 0.2 grams of AP were kept at 45 ºC for 10 min. 80% ethanol and 45 ºC were used to balance the solubility of AP at high concentrations and the ability to form inclusion complex. Samples will be collected by the same centrifugation, washing and drying procedure as described earlier.

Wide angle X-ray diffraction (XRD). Wide angle X-ray diffraction patterns were obtained with a Rigaku MiniFlex II desktop X-ray diffractometer (Rigaku Americas Corporation, TX). Samples were exposed to Cu Kα radiation (0.154 nm) and continuously scanned between 2θ = 4 and 30 º at a scanning rate of 2º/min with a step size of 0.02 º. A current of 15 mA and voltage of 30 kV were used. Data were analyzed with Jade v.8 software (Material Data Inc., Livermore, CA). The area of the amorphous halo generated by Jade© software using the cubic spline fit option was subtracted from the total X-ray diffraction area to obtain the crystalline fraction. The degree of crystallinity was then calculated as the crystalline fraction over the total area multiplied by 100.

Differential scanning calorimetry (DSC). Approximately 5 to 6 mg of sample was weighed into a 60 µL stainless steel pan (Perkin-Elmer Instruments, Norwalk, CT) and water was added to obtain a 10% (w/v) dispersion. Pans were hermetically sealed. Samples were equilibrated to 10 ºC, and then heated to 170 ºC at 2 ºC/min in a Thermal Advantage Q100 DSC (TA Instruments, New Castle, DE). The DSC was calibrated with indium, with an empty sample pan used as the reference. Data was analyzed using the TA Universal Analysis software (Universal Analysis 2000 v.4.2E, TA Instruments-Waters LLC, New Castle, DE).

Morphological Characterization. Scanning electron microscopic observation was performed using a Phenom G2 Pro SEM (Eindhoven, The Netherlands) at an accelerating

voltage of 5 keV. Three-dimensional images of the V-amylose powder surfaces were reconstructed with the 3D Roughness Reconstruction application in the Phenom Pro Suite package.

Results and Discussion

Characterization of V-amylose and V-starch. Through both the DMSO and high temperature preparation routes, ethanol precipitated starch and amylose demonstrated V-type X-ray diffraction (XRD) patterns (Figure 1a, 2a, & 2b), characterized by three major peaks at 8, 13.8, and 21º 2θ. Annealed V-amylose and V-starch showed sharpened XRD patterns (Figure 1b, 2c & 2d), which will be discussed in detail in section "effect of annealing" below. Since no guest compounds were mixed before precipitation, most amylose helices formed should have empty helical channels. High temperature method resulted in V-amylose with same crystallinity (approximately 40%) as that from the DMSO method. Using the DMSO method, crystallinity of V-starch was estimated to be lower than that of V-amylose, i.e. 33% versus 40%. This could be attributed to the presence of amylopectin in starch that constitutes about 30% (w/w) in Hylon VII starch and is structurally difficult to form V-type single helices.[13] Thermograms of the precipitated V-starch show a broad and low endotherm (Figure 3a), indicating little complexation took place between starch and native lipids. Approximately 1% (w/w) monoacyl lipids, e.g. palmitic, stearic, and linoleic acid, exist in native high amylose corn starch. These lipids are potentially able to form inclusion complexes with starch. The presence of various lipids resulted in different structures of inclusion complexes, e.g. length of helices, and thus different thermal stabilities of the inclusion complexes. The precipitated V-amylose did not show any noticeable endotherm during heating (Figure 3b), and thus no dissociation of amylose-guest inclusion complex could be detected.

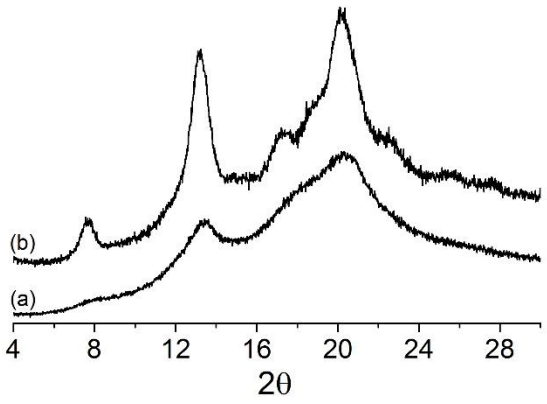

Figure 1. X-ray diffraction patterns of (a) V-starch and (b) V-starch annealed in 50% (v/v) ethanol at 70 ^{o}C.

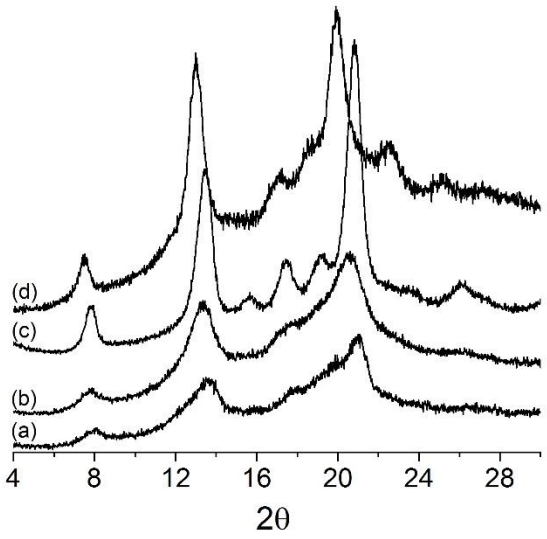

Figure 2. X-ray diffraction patterns of (a) V-amylose precipitated by high temperature method, (b) V-amylose precipitated by DMSO method, and V-amylose annealed in (c) 40% and (d) 50% (v/v) ethanol at 70 ^{o}C.

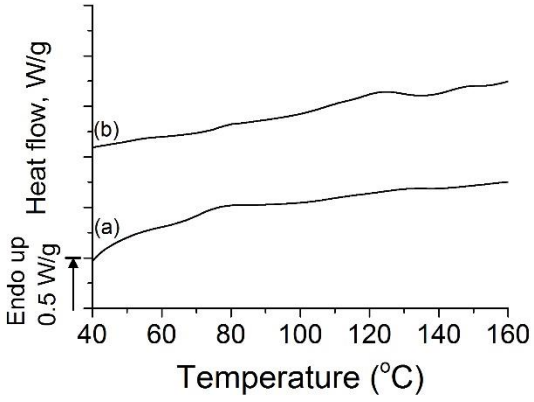

Figure 3. DSC thermograms of (a) V-starch and (b) V-amylose.

V-type starch formation by alcohol precipitation can be dated back to 1933, when Katz and Derksen suggested the V-type as "an intermediate step between the amorphous conditions of the pasted starch and the B configuration".[22] This may be true because the V-type starch could eventually transform into B-type when sufficient water is provided. However, later studies, especially X-ray diffraction and molecular modeling, regarded the V-type as a particular configuration, which differs much from the B-type.[23] Instead of precipitating starch from aqueous solutions, Zobel et al.[24] described precipitation of amylose-DMSO complex using alcohol to prepare the V-type (V_a). The V_a could then be transformed to V-hydrate (V_h) by exposure to a humid environment (e.g. 85% relative humidity). The only difference between V_a and V_h is the extent of water hydration, which brings slight modifications in their crystal structure. Although this helical structure of V-type with empty helical cavity has been known for many decades, no other technical applications have ever been described, with the possible exception of the "sealed heating" treatment (> 100 °C) utilized by a group of researchers. The enzymatically synthesized amylose employed showed V_{6I} pattern before "sealed heating" treatment. The inclusion of 2-naphthol,[25] salicylic acid,[5] and its analogues[6] converted the V_{6I} helical structure to either V_7 or V_8 structure, depending on the size of the guest molecule.

However, according to other evidences, the V_7 structures are actually V_{6II} or V_{6III}, where guest molecules are entrapped between helices instead of inclusion within the helical cavity.[8] In the present study, we used V-amylose and V-starch from native starch to molecularly encapsulate model bioactive compound, ascorbyl palmitate, at lower temperatures.

Effect of complexation temperature and solvent quality. In theory, the complexation ability and efficiency is affected by guest intrinsic solubility (S_0) and complex affinity constant (K), which can be expressed as: *complexation efficiency* = KS_0.[20] An increase in the complexation efficiency can be achieved by increasing either the guest intrinsic solubility or the complex affinity constant, or both parameters simultaneously.[26] The guest solubility is mainly affected by solvent quality, whereas the complex affinity constant is governed by the driving force for complex formation. The main driving force for amylose-guest complexation may involve hydrophobic interaction, hydrogen bonding, and concentration gradient (diffusion). Ascorbyl palmitate was chosen as a model compounds, because its complexation with amylose has been demonstrated and it has higher solubility in ethanol:water mixtures than fatty acids, e.g. palmitic acid.

Practically, there are numerous process parameters that can influence the complexation ability and efficiency in this method, for instance, solvent composition, complexation temperature, time duration, guest concentration, V-amylose concentration, V-amylose physical properties (surface area, porosity, and crystallinity), to name but a few. At the first step, the effect of ethanol concentration and temperature was studied on V-amylose complexation with AP. XRD patterns showed the presence of V-patterns after treating in all ethanol concentrations except for in pure water (Figure 4). All V-type structure was converted into B-type in water even in the presence of guest compound, regardless of complexation temperature. 20% (v/v) of

ethanol resulted in a mixture of V- and B-type, while only trace amount of B-type is detectable above 40% (v/v) of ethanol. Sharpening effect of the V-pattern was noticeable when intermediate ethanol concentrations (40% to 80%, v/v) were used. Especially, 40% (v/v) of ethanol was able to increase the crystallinity of V-amylose to about 60% at 70 ^{o}C. While V-amylose would probably lose its complexation ability after it was converted to B-type, whether AP was complexed into V-amylose or V-amylose remained "empty" in all other conditions could not be resolved from XRD patterns.

According to previous studies, the dissociation of amylose-AP inclusion complex would produce an endotherm with a peak temperature around 95 ^{o}C on a DSC thermogram.[13] After complexation at 20 ^{o}C, V-amylose samples from 40% to 60% (v/v) ethanol solutions demonstrated an endotherm representing the dissociation of amylose-AP inclusion complex (Figure 5). At higher temperatures, i.e. 45 and 70 ^{o}C, 80% ethanol was also feasible to induce complexation between amylose and AP. The variation in complexation ability could be explained by two competitive mechanisms, i.e. a competition between AP solubility in solvent and AP-amylose inner channel hydrophobic interaction, and a competition between mobility and water susceptibility of amylose helices, all of which were affected by ethanol concentration and temperature. When high ethanol concentrations (80% to 100%, v/v) were used, neither the high solubility of AP nor the lack of amylose mobility favored the insertion of AP into amylose helices. At lower ethanol concentrations (<20%, v/v), less AP molecules were available at the liquid-solid (solvent-V-amylose) interface due to poor solubility. A further reduction in ethanol concentration (0%, v/v) would result in too much flexibility of amylose helices that they transformed into B-types. Balancing conditions had to be met. At intermediate ethanol concentrations (40% to 60%, v/v), the plasticizing effect of water gave enough chain mobility for

easy insertion of AP molecules, while not too flexible to disrupt the helical configuration. Similarly, a reduction in AP solubility produced constantly high concentration gradient that favored diffusion of AP from solvent phase into V-amylose for hydrophobic interaction, the main driving force in complexation. In the case of 80% ethanol, an increase in temperature changed it to a favorable condition by increasing the mobility of amylose helices. Besides the endotherm for amylose-AP dissociation, all of the thermograms showed an endotherm with a peak temperature about 150 ºC, which can be attributed to the dissociation of retrograded amylose. Uncomplexed amylose molecules were subject to retrogradation in the DSC pans before heating scan. The small portion of B-type component in the V-amylose samples would also contribute to this endotherm.

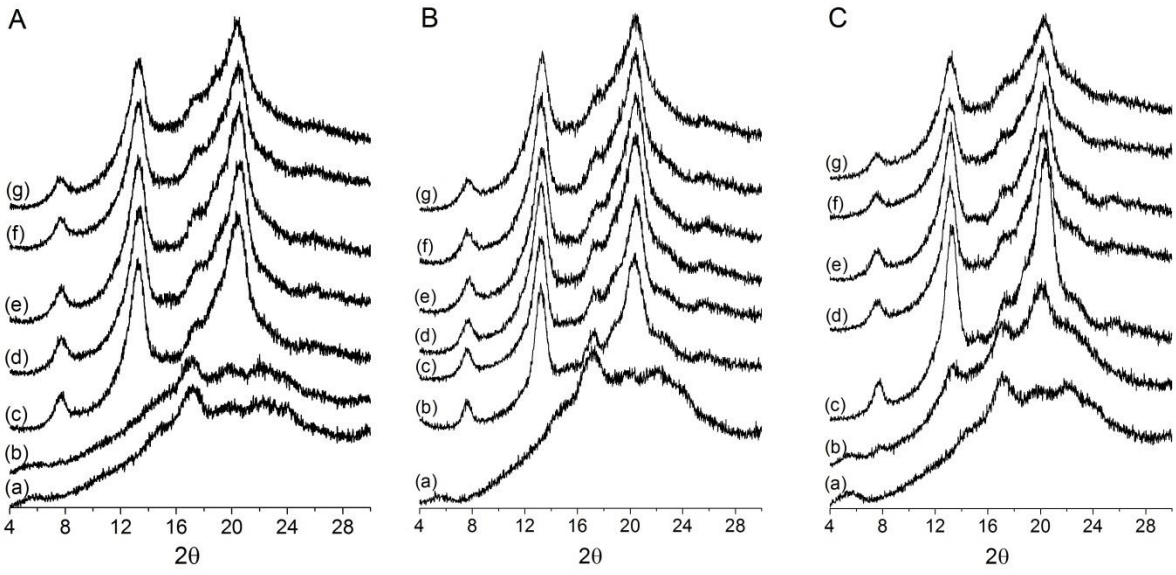

Figure 4. X-ray diffraction patterns of V-amylose complexation with AP at (A) 20, (B) 45, and (C) 70 ºC in different ethanol concentrations: (a) 0%, (b) 20%, (c) 40%, (d) 50%, (e) 60%, (f) 80%, and (g) 100% (v/v) ethanol aqueous solutions.

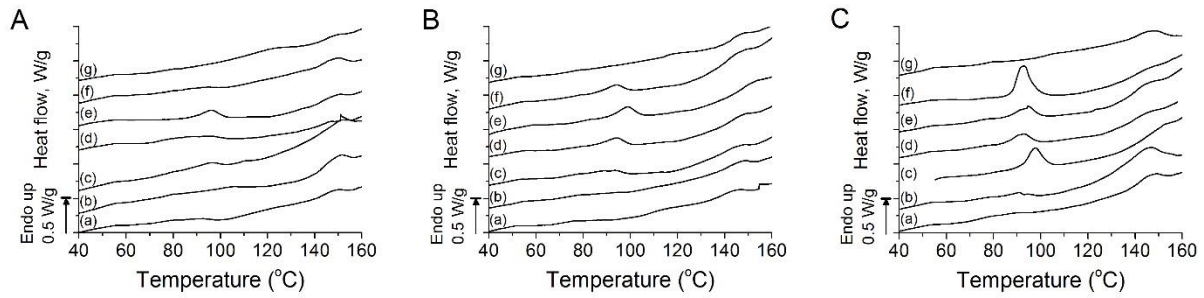

Figure 5. DSC thermograms of V-amylose complexation with AP at (A) 20, (B) 45, and (C) 70 °C in different ethanol concentrations: (a) 0%, (b) 20%, (c) 40%, (d) 50%, (e) 60%, (f) 80%, and (g) 100% (v/v) ethanol aqueous solutions.

In addition to the relatively pure amylose component of starch, it was worthy of exploring complexation using V-starch. The separation of pure amylose from starch is a complicated and costly process, and thus it may be more economical to use high amylose starches for most practical applications. The trends of XRD patterns and thermograms as affected by ethanol concentration and temperature were similar as those of V-amylose (Figure 6). This was expected because amylose was the main component in this specialty starch. Yet V-starch did show slightly different behaviors. A very broad and flat endotherm in the range from approximately 50 to 100 °C was always observable (Figure 7). As discussed above, this could be due to the presence of native lipids with different chain length. There were no endotherms that accounted for dissociation of retrogradated starch. The structural irregularity, especially branched amylopectin molecules, might impede perfection of retrogradated starch phase and result in diffused thermal events that spread along the rising baselines.

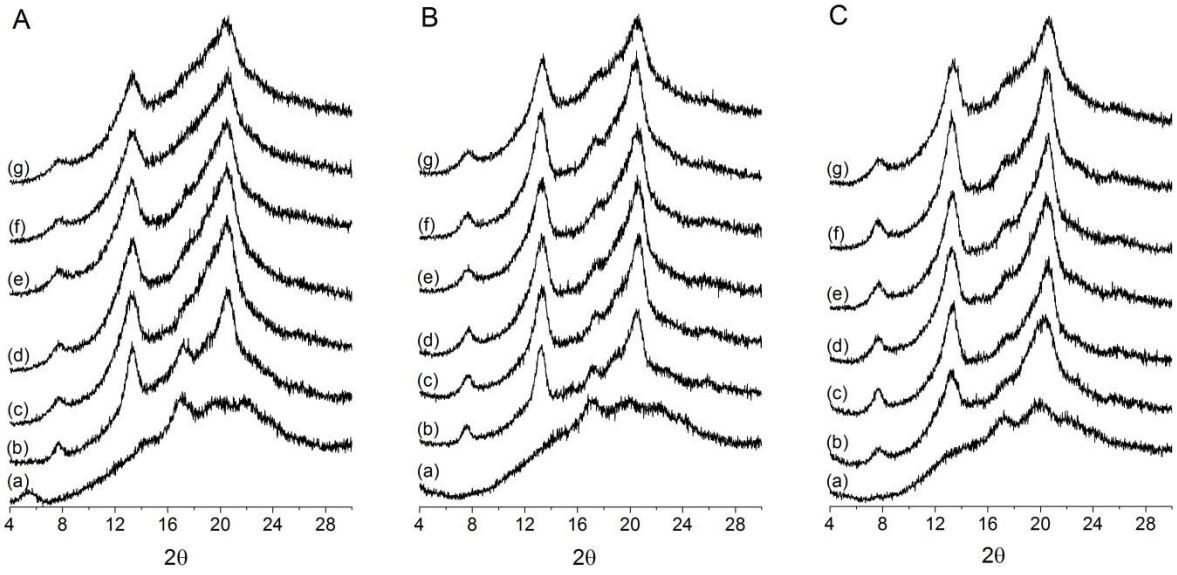

Figure 6. X-ray diffraction patterns of V-starch complexation with AP at (A) 20, (B) 45, and (C) 70 ºC in different ethanol concentrations: (a) 0%, (b) 20%, (c) 40%, (d) 50%, (e) 60%, (f) 80%, and (g) 100% (v/v) ethanol aqueous solutions.

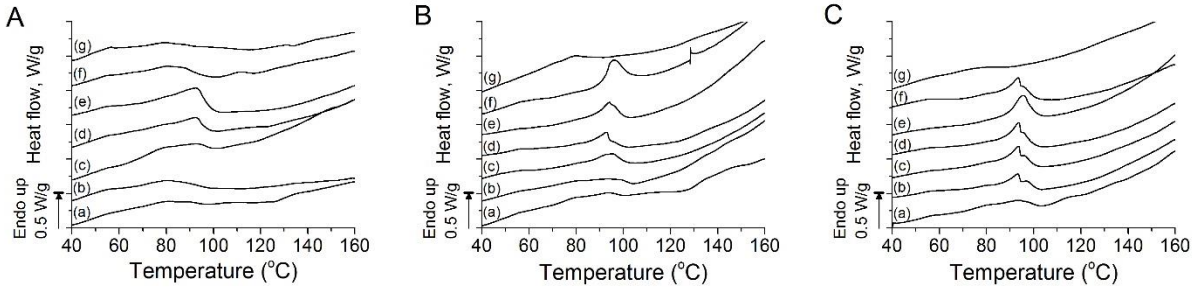

Figure 7. DSC thermograms of V-starch complexation with AP at (A) 20, (B) 45, and (C) 70 ºC in different ethanol concentrations: (a) 0%, (b) 20%, (c) 40%, (d) 50%, (e) 60%, (f) 80%, and (g) 100% (v/v) ethanol aqueous solutions.

The solubility of guest compounds is one of the important parameters for successful complexation in this method. Solvents other than ethanol:water mixtures can also be employed, especially when guest compounds have low solubility in both ethanol and water. Here we chose to study whether acetone:water mixtures could be used for complexation purpose. Thermograms

showed endotherms for V-amylose samples recovered from 40% to 60% (v/v) acetone solutions where AP was dissolved. 50% (v/v) was by far the most effective acetone concentration, since 40% and 60% (v/v) only allowed small amount of complexation (low endotherms).

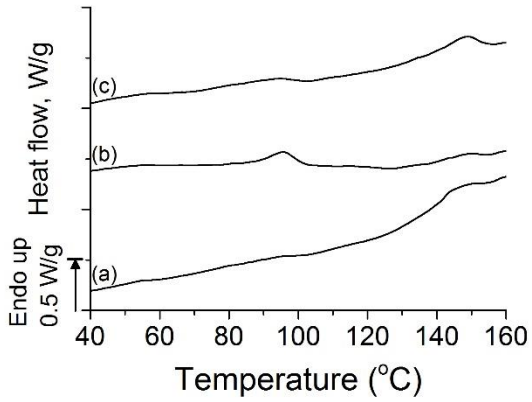

Figure 8. DSC thermograms of V-amylose complexation with AP at 45 °C in different acetone concentrations: (a) 40%, (b) 50%, and (c) 60% (v/v) acetone aqueous solutions.

Effect of annealing. The V-pattern of the precipitated V-type starch could be enhanced by a further heat treatment (or referred to as annealing) in an appropriate media. Bear in 1942 stated that "Starches which give V patterns after alcohol precipitation in the cold can be made to yield excellent ones if properly treated".[27] This treatment took place in 75% to 80% hot alcohol at 70 °C with vigorous stirring. Precipitated and dried starch showed "three almost unbelievably sharp diffraction rings". Zobel et al. employed a similar treatment, i.e. boiling in 85% methanol, and suggested that "molecular chains conceivably would have ample opportunity to arrange into the most stable structure" by this treatment.[24] Other than these examples, no other reports have been found to systematically study the effect of heat treatment on the arrangement of amylose molecules in V-type structure. In our previous study,[28] we obtained thermograms of electrospun starch fibers (V-type from XRD evidence) in different ethanol:water mixtures during heating. When the V-type starch fibers were scanned in 40% to 60% ethanol solutions, we observed an

exotherm followed by an endotherm, the positions of which depended on the ethanol concentration. The exotherm disappeared during a reheating scan. We suggested that the exotherm was attributed to the crystallization of starch helices that is evidenced as a sharpened V-pattern. The endotherm could be attributed to the dissociation of the V-type starch single helices or retrograded starch molecules (double helices).

In the current study, we found that annealing of V-amylose and V-starch powders in 40% to 50% (v/v) ethanol at 70 ^{o}C significantly increase the crystallinity (Figure 1b, 2c & 2d). The crystallinities of annealed V-starch and V-amylose were estimated to be 44% and 47%, respectively. Nevertheless, crystallinity of V-amylose could be enhanced to as high as 65% by annealing in 40% (v/v) ethanol at 70 ^{o}C. This suggested that 40% (v/v) ethanol could provide a better solvent environment, where amylose helices are flexible enough to arrange into more stable structure, but not too flexible to transition to B-type amylose as in the case of 0% and 20% (v/v) ethanol. A small portion of B-type component was present as characterized by the peaks at 17, 22, and 24^{o} 2θ. The B-pattern was also sharpened compared with that without annealing. The annealed V-amylose samples should have more well-formed helices (higher crystallinity) and larger crystal size (smaller full width at half maximum) than V-amylose without annealing. While more empty helices available would provide more sites to capture guest molecules, larger crystal size might bury more helices in the crystal, which were inaccessible to guest molecules. Therefore, it would be necessary to elucidate whether high crystalline V-amylose would facilitate inclusion complexation or not.

Compared with V-starch and V-amylose without annealing, inclusion complex formation by annealed V-amylose demonstrated a similar trend as a function of ethanol concentration when complexation at 45 ^{o}C (Figure 9). The enthalpies ranged from 1.02 J/g (sample from 20%

ethanol) to 7.79 J/g (sample from 50% ethanol). This value is greater than the largest enthalpy of V-amylose inclusion complex formed at 45 ^{o}C, i.e. 2.62 J/g. It appeared that high crystalline V-amylose was more efficient in capturing AP molecules, probably due to more regular crystal structure. However, conclusion shall not be easily drawn without considering other possible variables that brought much variation in the values of enthalpies in this study. Microscopic observation showed that the V-amylose sample powders are very porous with very rough surface (Figure 10). We suggest that surface area may be an important parameter. We found that the surface area of powders can vary a lot depending on how fine the powders were ground. The variation in surface area might give difference in the amount of V-amylose helices available to accommodate guest molecules, since guest molecules would only enter the helices on the surface of the crystals. It will be necessary in the future to evaluate the effect of particle size and distribution on the complexation efficiency.

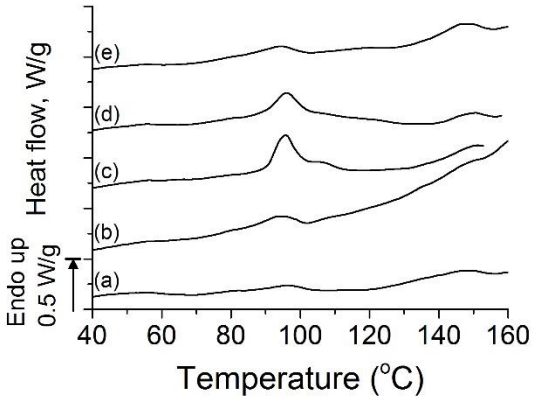

Figure 9. DSC thermograms of annealed V-amylose complexation with AP at 45 ^{o}C in different ethanol concentrations: (a) 20%, (b) 40%, (c) 50%, (c) 60%, and (c) 80% (v/v) ethanol aqueous solutions.

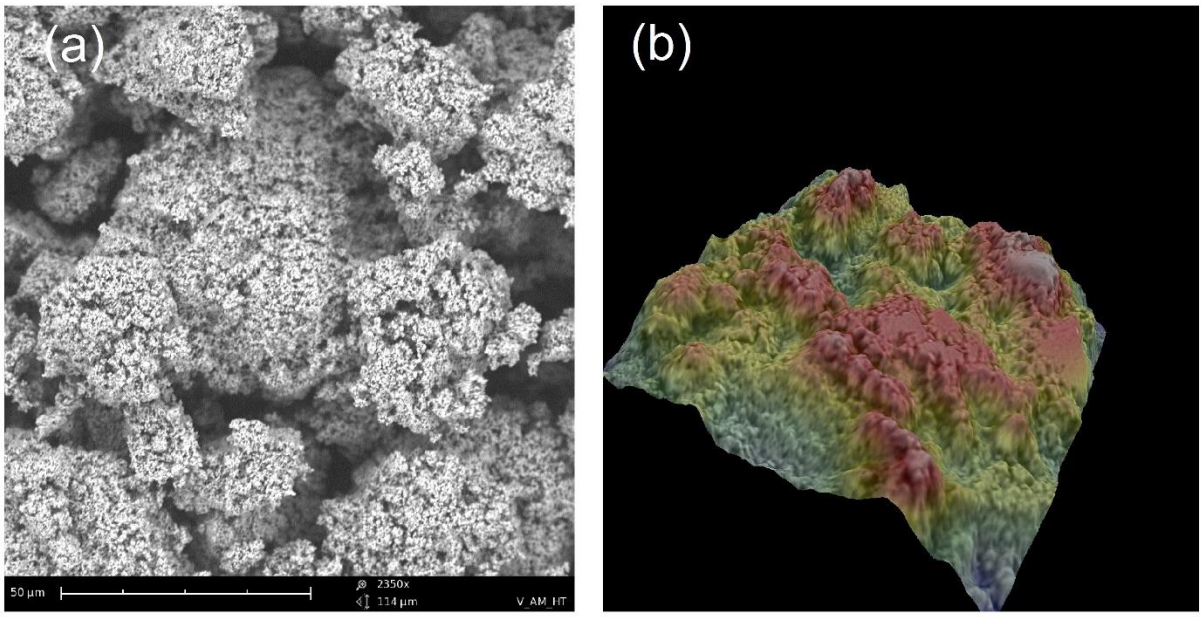

Figure 10. Scanning electron micrograph (a) and reconstructed three-dimensional surface (b) of the V-amylose sample powders.

Effect of AP concentration. In general, the endothermic enthalpies of V-amylose-AP complexes obtained in this study is smaller than the enthalpy by DMSO method. AP concentration may be responsible for the low complexation efficiency, even though the weight ratio of AP to V-amylose seemed saturated given the maximum % AP complexed by starch is around 6.3% (w/w). In practical, AP more than this number is required. For example, it was found that yield of starch-AP inclusion complex increased as higher amount of guest molecules were added, reaching a maximum at 15% (w/w) weight of starch. In the AP concentration range studied here, complexation efficiency, represented as endothermic enthalpy, increased significantly with AP concentration without reaching a maximum plateau (Figure 11). It should be noted that AP concentration above 0.5% (w/v) was not fully soluble in the solvent, i.e. 80% (v/v) ethanol. The dissociation temperature experienced a slight decrease with AP concentration used.

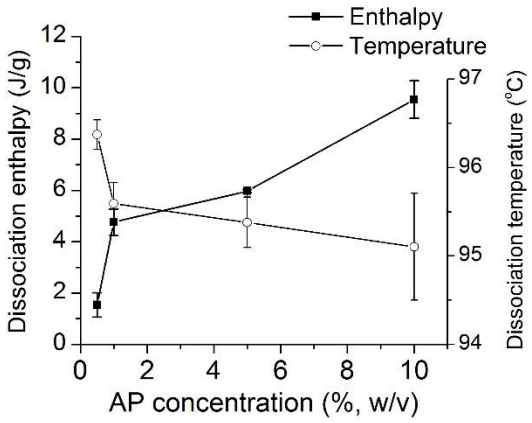

Figure 11. Dissociation enthalpy and temperature of V-amylose complexation with different AP concentrations at 45 ^{o}C in 80% (v/v) ethanol aqueous solution.

Conclusions

In conclusion, preformed V-type amylose and starch with empty helical channels could be synthesized and utilized to readily form inclusion complex with selected model guest compound, ascorbyl palmitate. Complexation could take place in intermediate ethanol and acetone concentrations (generally 40% to 60%, v/v) at room temperature. Annealing, i.e. heat treatment in ethanol solutions at elevated temperatures, was able to significantly increase the crystallinity of V-amylose and V-starch. A crystallinity up to 65% was obtained by annealing in 40% ethanol at 70 ^{o}C for 10 min. High crystallinity did facilitate complexation, probably due to more regularly arranged helical cavities in larger crystals. Complexation efficiency was also affected by guest concentration. Nevertheless, there may be other factors, e.g. particle size, porosity, and surface area, which need to be investigated in the future.

This new method of forming starch-guest inclusion complex is easy, economical and potentially scalable for mass production. It is worthwhile to try to encapsulate more types of nutrients, drugs, and bioactive molecules, intended for food and pharmaceutical applications, especially where cyclodextrins have been heavily used.