Precipitation Behaviour of Debranched Waxy Rice Starch in the Presence of Fatty Acid

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ABSTRACT

Debranched waxy rice starch (DBS) and fatty acid (FA; i.e., lauric acid and stearic acid) were dissolved and co-precipitated in acetate buffer solutions pH 3-7. The X-ray powder diffraction (XRD) patterns of precipitants obtained displayed B-type at low pH (3-5) but V_h-type at pH 7, indicating that DBS precipitated as free form at pH 3-5 but as inclusion complexes with FA at pH 7. The precipitation behaviour of DBS in the presence of FA at pH 3-7 was evaluated, using rapid visco analyzer (RVA). RVA was able to detect the increased viscosity, resulting from both the precipitations of free DBS and DBS/FA inclusion complexes. DBS/FA complexes precipitated at a higher temperature than did free DBS. RVA profiles also revealed that DBS favorably precipitated as free form at pH 3 and as DBS/FA inclusion complexes at pH 7. It was thought that more FAs dissociated at pH 7 and resulted in the increased solubility which facilitated more FA to form inclusion complex with DBS. RVA profiles clearly demonstrated that lauric acid had less potential to form inclusion complex with DBS, particularly at a pH below the pKa. In addition, it was likely that the formation and the precipitation of DBS/FA complexes occurred at a higher temperature when the pH of the mixture was increased.

Key words: Debranched waxy rice starch, Lauric acid, Stearic acid, Complex, RVA

INTRODUCTION

Starch is mainly consisted of two types of polysaccharides, amylose and amylopectin. Amylose is an essentially linear polymer with molecular weights ranging from 10^5-10^6 and with the number of glucose residues per molecule ranging up to 5000 (Galliard and Bowler, 1987). Many chemicals that consist of a non-polar moiety, such as fatty acids, iodine and surfactants are known to form inclusion complex with amylose and precipitate from the solution (Kuge and Takeo, 1968). A part or whole of their molecules are usually incorporated inside amylose helices in which the interior surface is built up by C-H groups and glycosidic oxygen atoms, forming a lipophilic core. Nuclear magnetic resonance (NMR) and molecular

modeling results demonstrated that an aliphatic chain of fatty acid (FA) is included in amylose single helices while the carboxylic group is located near the entrance of the helical cavity (Godet et al., 1993; 1995; Snape et al., 1998). Two types of polymorphs of DBS/lipid complexes, i.e., form I and form II have been discovered. Biliaderis and Galloway (1989) proposed that form I, having lower melting points and amorphous-like in XRD pattern, is formed in the presence of strong complexing agents or at low crystallization temperature (T_C). Form II, having higher melting points and displaying a well-defined V-pattern, is formed at high T_C in which complex formation is allowed to progress as a conventional crystallization process, thereby leading to partially-crystalline structure. Form II is generated from the single helices of amylose/lipid complexes, arranging into hexagonal lattice (Mikus et al., 1946; Brisson et al., 1991) and forming lamella-like crystals with a folding length of about 100 A˚ (Biliaderis and Galloway, 1989). Form I can be transformed into form II if it is heated at the temperature slightly above its dissociation temperature (T_m). Karkalas et al., (1995) studied the thermal properties of amylose/stearic acid complexes, prepared under 2 T_C and found that form I complexes, formed at ≤ 60˚C had T_m in the range of 96-104˚C. Form II polymorph, formed at ≥ 90˚C had T_m ranging from 114-121˚C.

Although much information on the structures and precipitation behaviours of amylose/FA complexes has been disclosed as mentioned above, little is known concerning the complex formation between debranched amylopectin and FA. The chain length of debranched amylopectin is relatively short (the number of glucose residue per molecule is usually less than 100), which may contribute to the difference in the complex formation behaviour from amylose. Debranched amylopectin of high purity can be easily prepared by hydrolyzing waxy rice starch with debranching enzymes such as pullulanase (Yotsawimonwat et al., in press). In the present study, the complex formation between DBS and FA as well as their precipitation behaviours were evaluated, using rapid visco analyzer (RVA). The information of this study would be useful for the preparation of DBS/drug complexes in the future. It is expected that DBS/drug complexes might be useful for drug solubility and stability enhancement in a similar manner as found in cyclodextrin/drug complexes.

MATERIALS AND METHODS

Materials

DBS was obtained by debranching waxy rice starch with pullulanase (Promozyme®; 45 Pullulanase Unit NOVO/ g of starch) at pH 5, 55˚C for 19 h. The beta-amylolysis limit of the DBS was 97.5%, indicating that DBS was almost linear (Yotsawimonwat et al., in press). Lauric acid and stearic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). The purities of both FAs were ≥ 99%. All other reagents used in this study were of analytical grade.

Methods

Evaluation of FA:DBS ratio for DBS/FA complex formation

The appropriate FA:DBS ratio that yielded the maximum DBS/FA complex formation was evaluated. DBS (2.5 g, dry basis) and various amounts of lauric acid (0.025, 0.05, 0.25 and 0.5g, equivalent to FA:DBS ratios (w/w) of 1:100, 1:50, 1:10 and 1:5, respectively) were

added into an Erlenmeyer flask 250 ml. Deionized water was added to adjust the total weight of the mixture to 50g. The mixture was sealed and heated in boiling water until the clear solution was obtained. The mixture was then incubated in a water bath shaker at 90˚C and 180 rpm for 30 min, slowly cooled down to 30˚C in 8 h and then was left standing at 30˚C for 16h. The whole mixture was dried at 40˚C for 2 days. The dried sample was ground and subjected to X-ray powder diffraction (XRD) analysis.

Effect of pH on the precipitation of DBS/FA mixture

A mixture of DBS (2.5 g, dry basis) and the suitable amount of FA (lauric acid and stearic acid), obtained from the above study, was added into an Erlenmeyer flask 250 ml. Acetate buffer solution (0.1 M) pH 3-7 was added to adjust the total weight of the mixture to 50 g. The mixture was sealed and heated in a boiling water bath until the solution became clear. The mixture solution was incubated in a water bath shaker at 90˚C and 180 rpm for 30 min, slowly cooled down to 30˚C in 8 h and then left to stand at 30˚C for 16 h for DBS to precipitate. The mixture was centrifuged at 10,000g for 15 min at 30˚C. The precipitant was collected and dried in an oven at 40˚C for 2 days. All samples were ground and kept in a desiccator at room temperature until XRD analysis.

X-ray powder diffraction analysis

XRD patterns were recorded, using an X-ray diffractometer (Diffraktometer Siemens D500, Germany) with Cu Kα as a radiation source at 20 kV. All samples were scanned between diffraction angles (2θ) 5˚ and 30˚.

Precipitation behaviour of DBS/FA mixtures by Rapid Visco Analysis

The effect of pH on the changes in the apparent viscosity characteristics of the DBS/FA mixtures were measured, using RVA (Model # 3-D, NewPort Scientific, Sydney, Australia). DBS (6.3 g, dry basis) and 0.125 g of FA were added into a canister. Acetate buffer solution of pH 3-7 was added to adjust the final weight of the sample to 31.5 g; the final DBS concentration was 20%, w/w. A plastic paddle was inserted into the canister and rotated to disperse the material. The canister, along with the paddle, was placed in the RVA which was used along with the accompanying software (Thermocline). After the initiation of the test, the sample temperature was immediately increased to 95˚C and was held isothermally for 10 min with the paddle speed of 960 rpm. After 10 min, the paddle speed was decreased and maintained at 160 rpm throughout the study and a cooling cycle was applied simultaneously, a linear gradient from 95˚C to 30˚C in 13 minutes (cooling rate: 5˚C/min) followed by a final isothermal step at 30˚C for 27 min. All apparent viscosity values were expressed as centipoise (cP). The number of replications was two.

RESULTS AND DISCUSSION

Suitable amount of FA to form inclusion complex with DBS

The XRD patterns of the dried mixtures of DBS and lauric acid at different concentrations are shown in Figure 1. The major peaks at diffraction angles (2-theta) of 16.9˚, 21.9˚, 23.8˚ and two minor peaks at around 14.0˚ and 15.0˚ in the XRD patterns of all DBS/lauric acid mixtures appeared at the same diffraction angles as those of DBS alone.

These peaks, therefore, belonged to B-type free 'DBS'. The observation of strong B-type peaks indicated that most DBS molecules did not form inclusion complex with lauric acid and precipitated as free DBS from the solution. The small peaks at 2-theta ~19.8(observed in the XRD pattern of the dried DBS/lauric acid mixtures were attributed to V_h-type DBS/ lauric acid complexes. The V_h-peak of the dried mixture of 1:50 was larger than that of 1:100, but not different from those of 1:10 and 1:5 indicating that the amount of DBS/lauric acid complexes formed in the mixtures, having ratios of 1:50, 1:10 and 1:5 were not different. It was evident from the experiment that the amounts of lauric acid added into the mixtures of

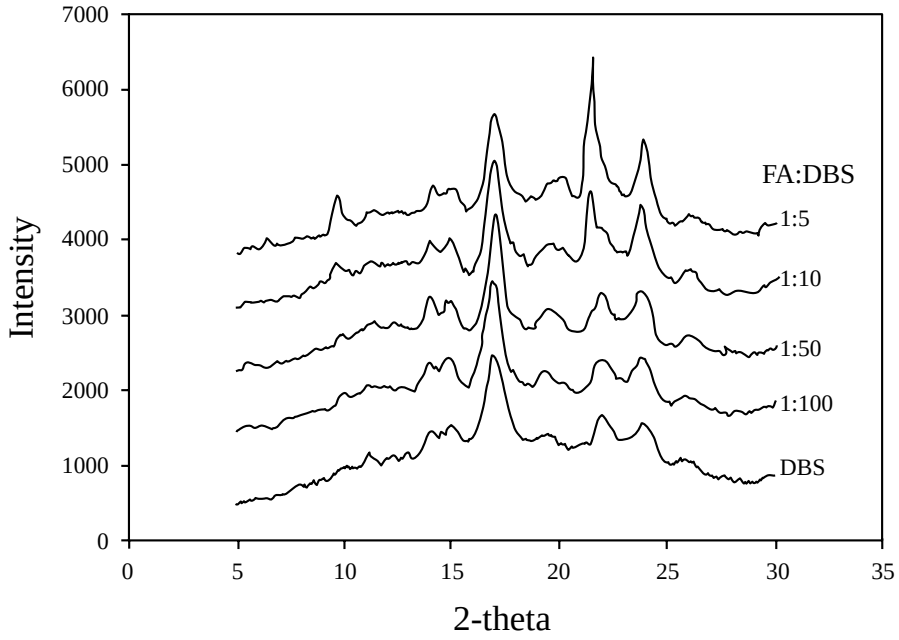

Figure 1. X-ray powder diffraction patterns of dried DBS/lauric acid mixtures obtained from various lauric acid:DBS ratios (w/w).

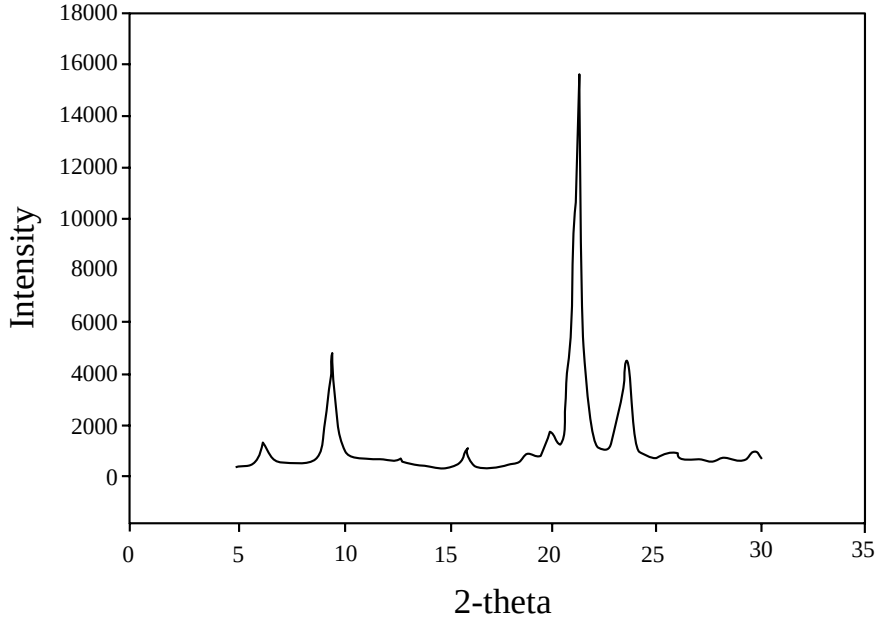

Figure 2. X-ray powder diffraction pattern of lauric acid.

1:10 and 1:5 ratios were much excessive because significant amount of lauric acid was observed on the top surface of the mixtures after the incubation, while only a tiny amount of lauric acid particles remained in the mixture of 1:50 ratio. These contributed to the appearance of narrow peaks at 2-theta 9.6˚, 21.5˚ and 23.9˚ in the XRD patterns of the dried DBS/lauric acid mixtures of 1:10 and 1:5 ratios. The peaks at those diffraction angles were in accordance with the major peaks of lauric acid (Figure 2). Therefore, they are attributed to the diffraction peak of free remaining lauric acid. For the above reasons, the appropriate ratio of lauric:DBS was chosen at 1:50 and it was used throughout the following experiments. The suitable ratio of stearic acid:DBS was also selected at 1:50, since only tiny amount of stearic acid particles was also found on the top surface of the mixture at this ratio.

Effect of pH on the precipitation of DBS/FA mixtures

The XRD patterns of precipitants obtained from mixture solutions of DBS and lauric acid at pH 3-7 are shown in Figure 3. At pH 3-5, DBS/lauric acid precipitants displayed B-type XRD pattern, similar to that of the precipitant of DBS alone. These results demonstrated that most DBS molecules in DBS/lauric acid solutions formed double helices on crystallization and precipitated as free 'DBS'. A small peak at 2-theta 19.8˚, corresponding to the peak of V_h-type, suggested the existence of a small amount of DBS/lauric acid inclusion complexes in the DBS/lauric acid precipitants obtained at pH 3-5. The V_h-type XRD patterns of DBS/lauric acid precipitants obtained at pH 6 and 7 indicated that primarily DBS/lauric acid inclusion complexes precipitated from the solutions. A tiny peak of B-type crystallites at 2-theta 17.2˚ was also detected in the XRD patterns.

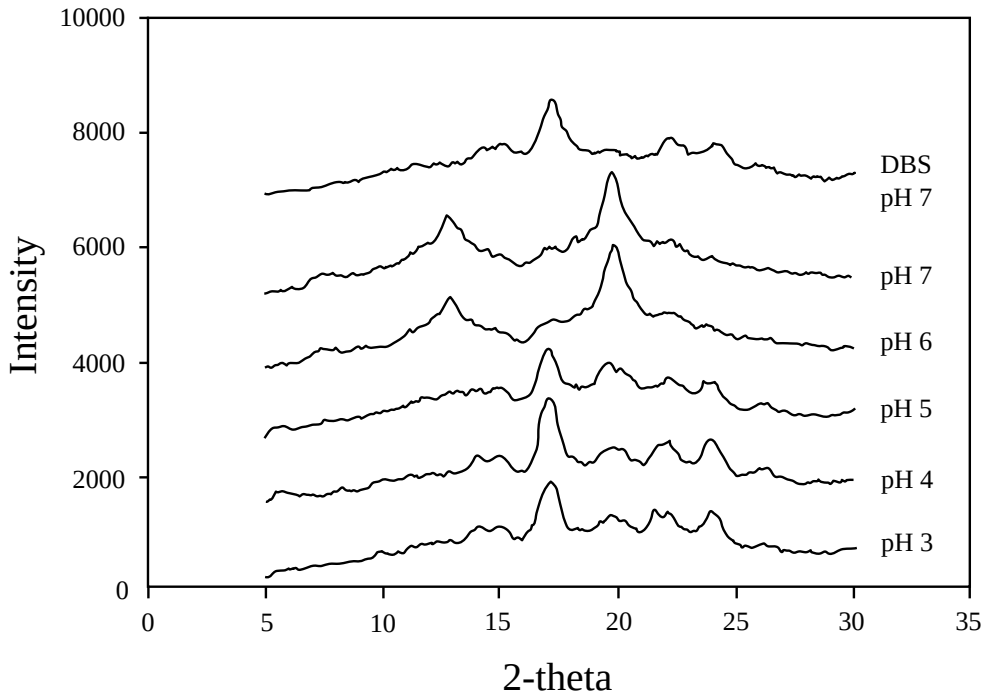

Figure 3. X-ray powder diffraction patterns of dried DBS/lauric acid precipitants prepared in acetate buffer solutions pH 3-7 (0.1 M).

The XRD patterns of the DBS/stearic acid precipitants obtained from mixture solutions at pH 3-7 are shown in Figure 4. The XRD patterns of DBS/stearic acid precipitants are B-type at pH 3-6 and V_h-type at pH 7. These are attributed to the fact that most DBS crystallized and precipitated as free form from the solutions at low pH and as inclusion complexes at pH 7. At pH 3-5, the complex peaks at 2-theta 19.8˚ of DBS/stearic acid precipitants are smaller than those of DBS/lauric acid precipitants at a selected pH, which suggest that the ability of stearic acid to form inclusion complex with DBS is lower than that of lauric acid in these conditions. A peak at 2-theta ~17.2˚ of B-type crystallites was observed clearly in the XRD patterns of DBS/stearic acid precipitants at pH 7. Due to the fact that the pKa of FAs is approximately 4.8, it can be suggested that DBS favorably form inclusion complex with both FAs at pH above pKa. At these pHs, more FAs dissociate and result in the increased solubility, which facilitate more FA to form inclusion complex with DBS. The results of these studies are in agreement with those reported by Fanta et al., (1999); the sodium salts of most FAs (dissociated form) have more potential to form inclusion complex with high-amylose starch than its free form.

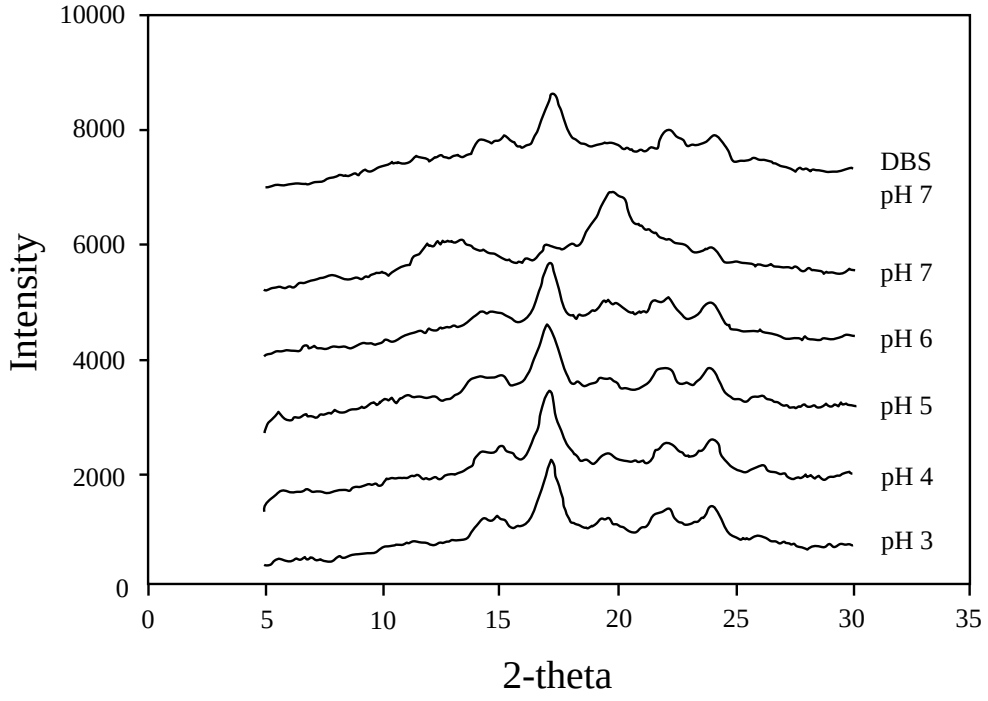

Figure 4. X-ray powder diffraction patterns of dried DBS/stearic acid precipitants prepared in acetate buffer solutions pH 3-7 (0.1 M).

Effect of pH on RVA profiles of DBS/FA mixtures

The viscosity profiles of DBS alone at various pHs are shown in Figure 5. The condition for running RVA to measure the viscosity changes from DBS precipitation was investigated quite extensively. The concentration of DBS used in the RVA experiment needed to be increased up to 20% in order that the viscosity generated from the precipitation of DBS could be detected by RVA. It might be due to the low degree of polymerization of DBS. The temperature and the paddle speed were set at 95˚C and 960 rpm at the first 10 minutes in order to completely dissolve DBS and FA. Then, the paddle speed was

decreased to 160 rpm to measure the change in viscosity due to the precipitation of DBS when the mixtures were cooled down. The abrupt change in a paddle speed led to the immediate decrease in the viscosity from about 100 cP to slightly above 0 cP. It can be seen in Figure 5 that the viscosities of DBS solutions at every pH were almost constant until the temperature was decreased to around 43˚C at which the viscosity started to increase. This was attributed to the fact that DBS began to form double helices on crystallization and started to precipitate from the solutions. The temperature at this point was considered as the onset temperature of precipitation of DBS as detected by the RVA. It can be observed that pH had only small effect on the viscosity profiles of DBS.

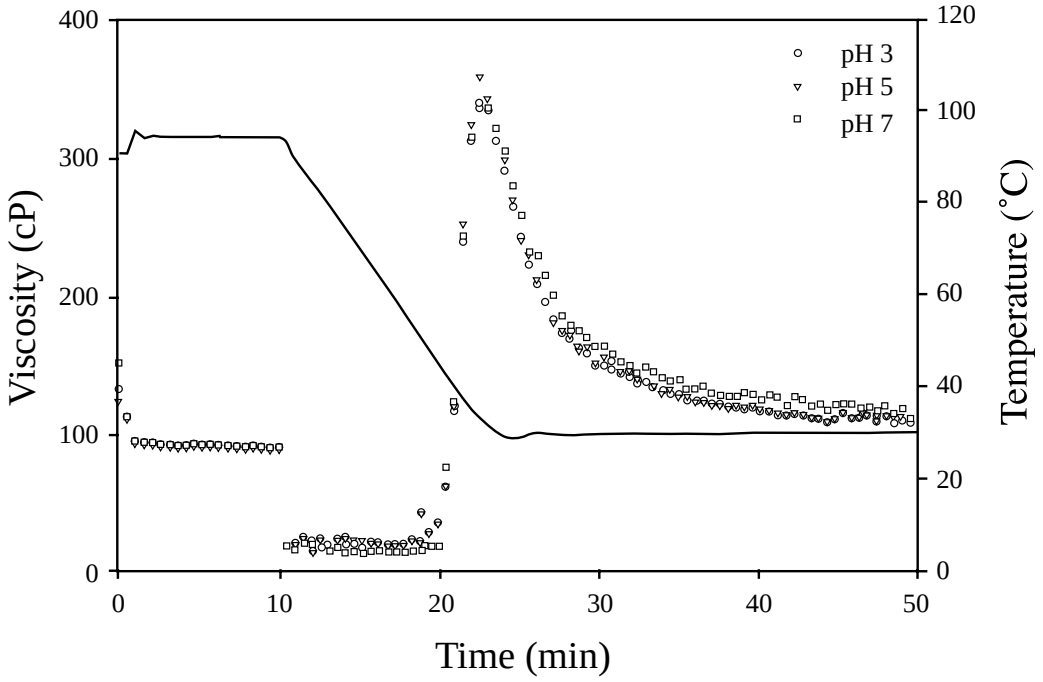

Figure 5. RVA profiles of DBS (20%, w/w) in acetate buffer solutions pH 3, 5 and 7 (0.1 M). The RVA profiles of pH 4 and 6 are not shown. The solid line represents the temperature program.

Figure 6 shows the viscosity profiles of the DBS/lauric acid mixtures at various pHs. The RVA profile of DBS/lauric acid mixture at pH 3 displays a gradual increase of viscosity at the temperature above the precipitation temperature of DBS alone. The viscosity increases substantially at the precipitation temperature of free DBS, but the maximum viscosity of DBS/lauric acid mixture at pH 3 was dramatically lower than that of the DBS alone. For DBS/lauric acid mixture at pH 7, the viscosity increases rapidly at the temperature higher than the precipitation temperature of DBS alone and remains almost constant throughout the run. A slight increase in viscosity was still observed at the temperature close to the precipitation temperature of DBS alone. Since the XRD pattern of DBS/lauric acid precipitant obtained at pH 7 is V-type, the increased viscosity at the temperature higher than the precipitation temperature of DBS can be attributed to the precipitation of DBS/lauric acid complexes. From this information, it can be described that a small amount of DBS/lauric acid complexes was formed at high temperature in the mixture of pH 3. Subsequently, when

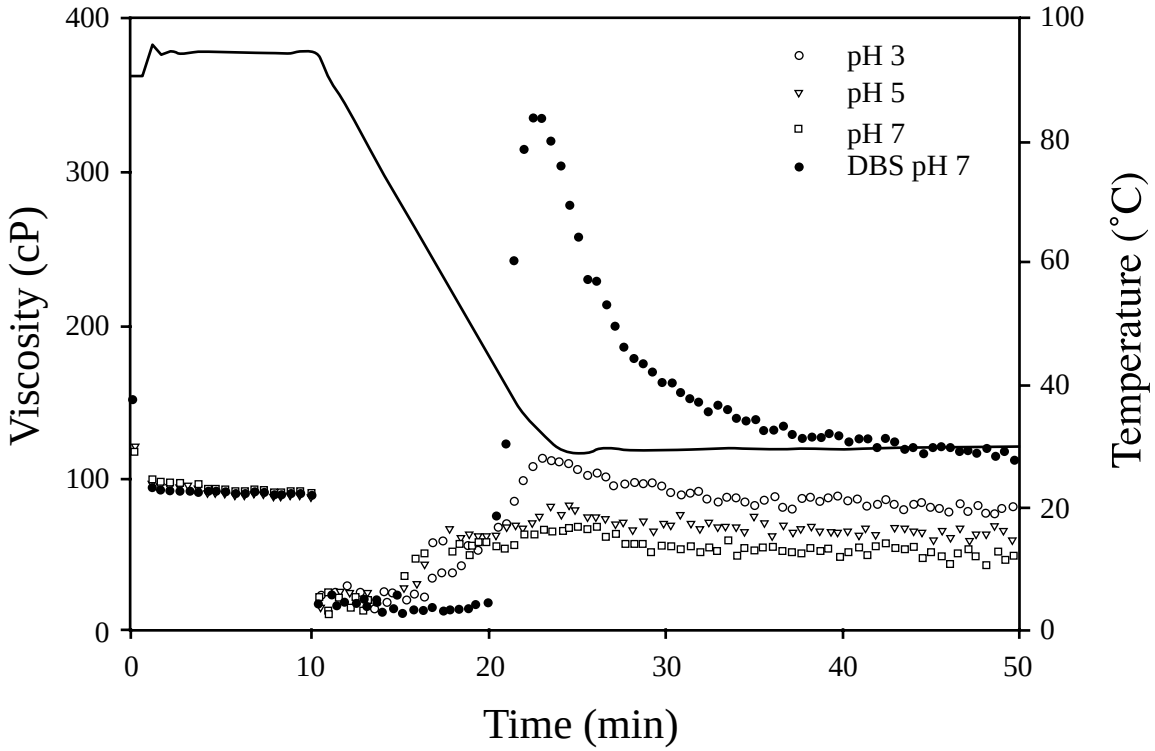

Figure 6. RVA profiles of DBS/lauric acid mixtures in acetate buffer solutions pH 3, 5 and 7 (0.1 M). The RVA profiles of pH 4 and 6 are not shown.

the mixture was cooled down, free remaining DBS formed double helices, precipitated and gave a high viscosity at low temperature. On the contrary, the RVA profile of DBS/lauric acid at pH 7 displays almost exclusively the viscosity from the precipitation of DBS/lauric acid complexes. It is obvious that more DBS formed inclusion complex with lauric acid at pH 7 than at pH 3, and a small amount of free DBS precipitated when the mixture solution was cooled down. The RVA profile of pH 5 is in between those of pH 3 and pH 7.

Figure 7 shows the viscosity profiles of the DBS/stearic acid mixtures at various pHs. The RVA profiles of DBS/stearic acid mixtures are similar to those of DBS/lauric acid mixtures. The viscosity profile of DBS/stearic acid mixture at pH 3 is only slightly different from that of the DBS alone, particularly at temperature above the precipitation temperature of DBS, which indicates that most DBS did not form inclusion complex with stearic acid. This corresponds to the XRD result in which the DBS/stearic acid precipitant displays almost exclusively B-type XRD pattern at pH 3. The RVA profile of pH 7 displayed mainly the increased viscosity at high temperature, which is in agreement with the Vh-type XRD pattern of DBS/stearic acid precipitant obtained at pH 7. It is obvious from the RVA profiles that, at pH 3 and 5, the viscosities arisen from the precipitation of DBS/lauric acid complexes (Figure 6) are greater than those of DBS/stearic acid complexes (Figure 7) while the viscosities generated from free DBS precipitation in DBS/stearic acid mixtures are greater than in DBS/lauric acid mixtures. As a result, it is confirmed that lauric acid has more potential to form inclusion complex with DBS at pH 3 and 5 than stearic acid. These results provide useful information on using RVA as another tool for investigating the complex

formation between DBS and FA, particularly when there is only a small amount of DBS/FA complexes formed.

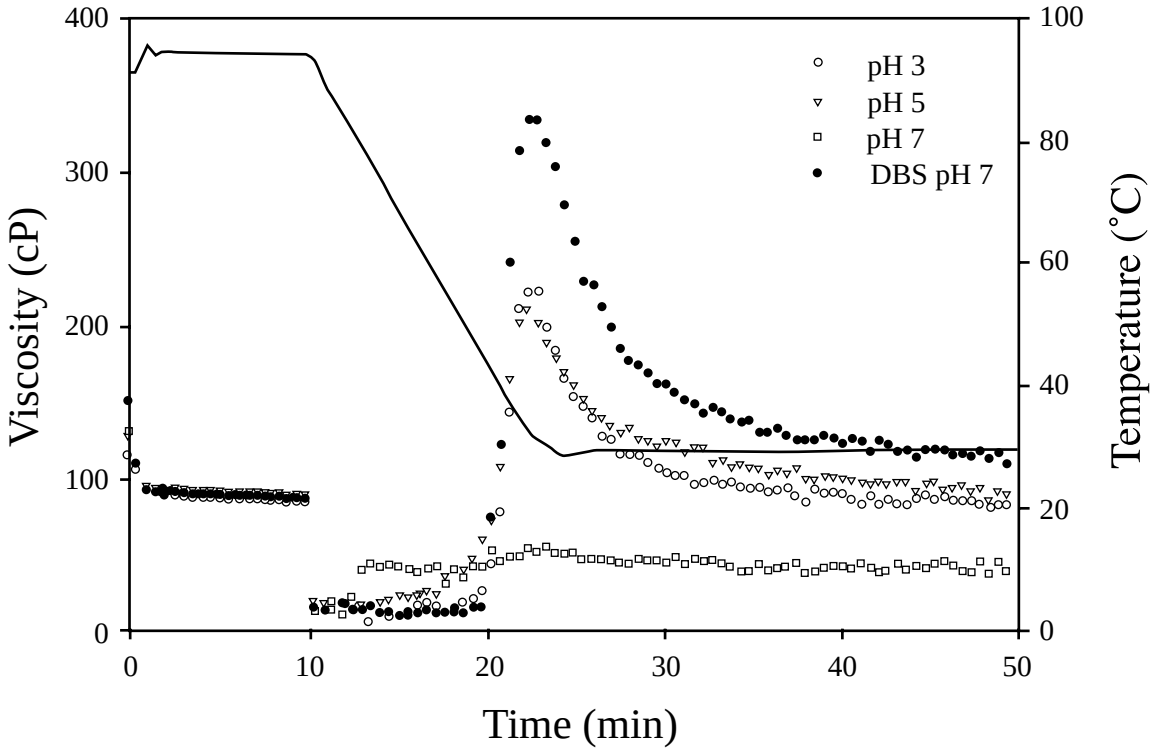

Figure 7. RVA profiles of DBS/stearic acid mixtures in acetate buffer solutions pH 3, 5 and 7 (0.1 M). The RVA profiles of pH 4 and 6 are not shown.

The onset temperatures of the precipitation of all samples were determined from the RVA profiles and were plotted in Figure 8. The onset temperatures of precipitation of DBS were independent on pH. However, the onset temperatures of precipitation DBS/lauric acid mixtures slightly increased as the pH was increased. The onset temperatures of precipitation of DBS/stearic acid mixtures at pH 3-5 were not different from those of DBS alone, but increased substantially at pH 6 and 7. It is likely that the formation and the precipitation of DBS/FA complexes occurred at a higher temperature when the pH of the mixture was increased. In addition, it can be observed that the onset temperatures of precipitation of DBS/stearic acid complexes were greater than those of DBS/lauric acid complexes at pH 6 and 7.

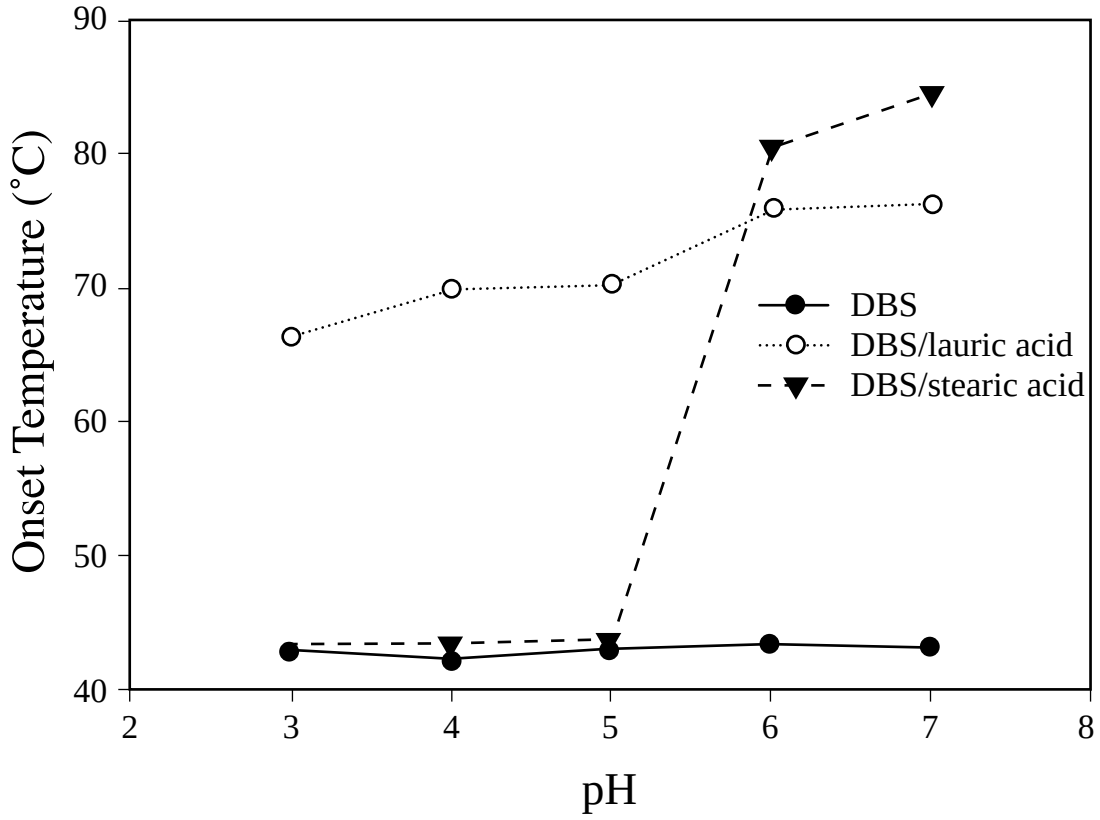

Figure 8. The onset temperatures of precipitation obtained from the RVA profiles of DBS, DBS/lauric acid mixtures and DBS/stearic acid mixtures in acetate buffer solution pH 3-7 (0.1 M).

CONCLUSIONS

The suitable ratio of lauric acid:DBS for the preparation of DBS/lauric acid complexes was 1:50 (w/w). It was found that pH had a tremendous effect on the precipitation of DBS in the presence of FAs. At pH below the pKa, DBS mainly precipitated as free form. However, DBS favorably formed inclusion complex with FAs at a pH above the pKa and precipitated as V_h-type at pH 7. The RVA can be used to evaluate the complex formation between DBS and FAs. The RVA profiles revealed both the precipitation of DBS/FA complexes and DBS alone. The DBS/FA complexes precipitated at a temperature higher than those of DBS alone. The formation and the precipitation of DBS/FA complexes seemed to occur at a higher temperature when the pH of the mixture was increased.

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