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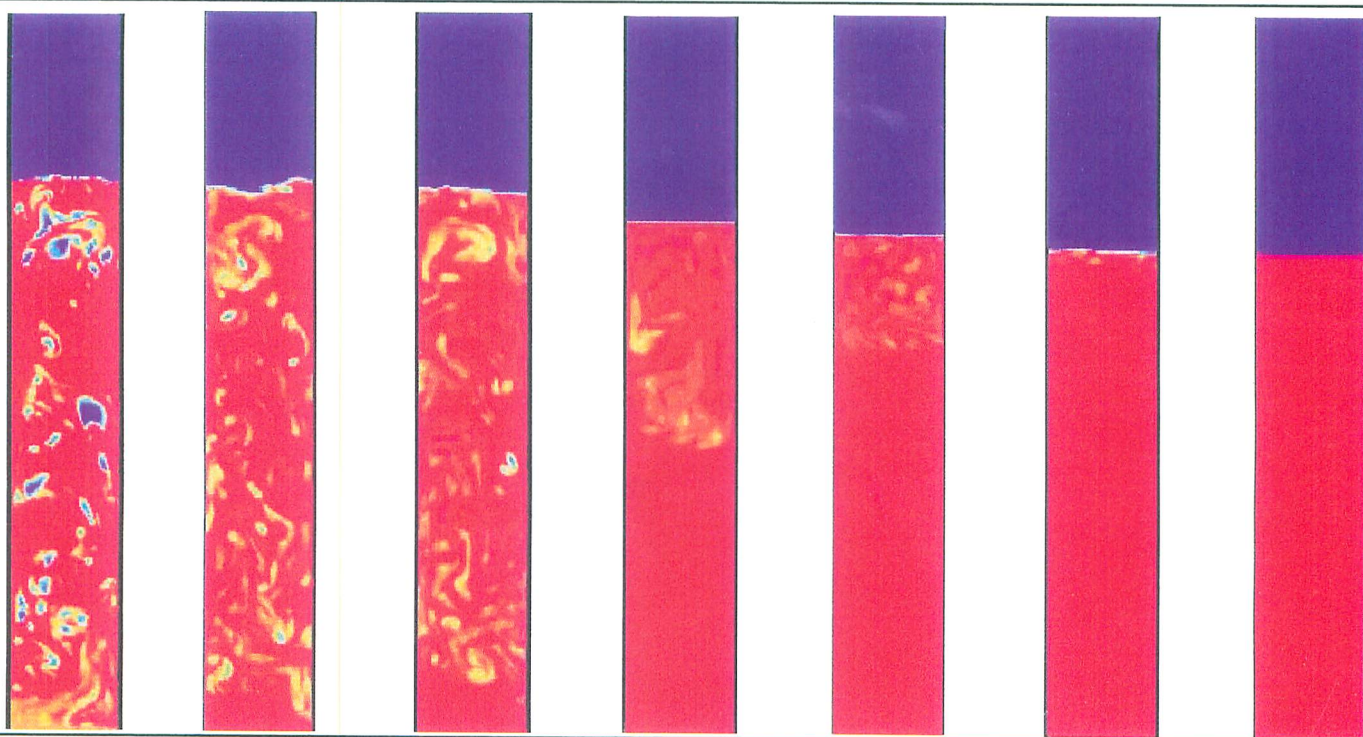
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# Synthesis of a Novel Protein-Based Plastic Using Sub-Critical Water Technology

Wael Abdelmoez and Hiroyuki Yoshida

Center of Excellence Project at the Dept. of Chemical Engineering, Osaka Prefecture University, Osaka 599-8531, Japan

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*This article reports the synthesis and characterization of a novel biodegradable plastic synthesized from the serum albumin of bovine blood (BSA) by using the sub-critical water technology in batch reactors. The optimum synthesis conditions were found to be in the range of 250–275°C for a reaction time of 1 min using 160 g/L BSA as an initial concentration. After a reaction time of only 0.5 min, all water-soluble BSA molecules were completely transformed into water-insoluble solids without hydrolysis or decomposition. A variety of techniques have been used to study the nature of the formed solids. The obtained results revealed that such solids showed many plastic properties and could be processed to produce a biodegradable plastic. The produced solids were recovered, dried, and thermally processed to produce a plastic BSA (PBSA). In this way, it was possible to obtain a material with a variety of mechanical properties that might allow for its application in both the biomedical as well as the traditional biodegradable plastic fields. The average values of the yield strength and modulus of elasticity of the processed samples synthesized at 250°C for 1-min reaction time were 31.2 and 780 MPa, respectively. The biodegradability was confirmed by measuring the methane gas production and weight losses during an anaerobic fermentation of the processed plastic samples. © 2006 American Institute of Chemical Engineers AIChE J, 52: 2607–2617, 2006*

**Keywords:** biodegradable polymers, BSA, protein-based plastics, sub-critical water, batch reactors

## Introduction

Polymeric materials occurring naturally or produced from renewable resources were recognized as extremely useful many years ago. The concept of combining the biodegradability and high availability of natural polymers is attracting scientists from different areas. Nowadays, protein-based materials (from vegetable or animal origin) are proposed as a potential solution to provide new biodegradable plastics.<sup>1</sup> Proteins are very versatile materials, both by source and because of a wide variety of possible modifications. Appropriately, they can vary from being rigid to flexible in structure and have similar mechanical properties as have existing polymers, with features that can be

tailored towards the diverse requirements of a specific application.

Recently, there has been a great deal of interest in producing plastic materials that can be used in the biomedical field and, furthermore, disposed of safely and easily in the environment by biodegradation. The biodegradable plastics can quantitatively be converted either to H<sub>2</sub>O and CO<sub>2</sub> and/or CH<sub>4</sub> by the action of naturally occurring microorganisms. They can be synthesized from abundant agricultural/animal resources such as cellulose,<sup>2</sup> starch,<sup>3</sup> collagen,<sup>4,5</sup> casein,<sup>1</sup> soy protein,<sup>6</sup> polylactic acid,<sup>7</sup> and polyhydroxybutyrate.<sup>8</sup> However, the number of biodegradable plastics in clinical use is rather small and still suffering several weaknesses, such as complexity of synthesis procedures, processing techniques, and a high production cost. A potential solution to overcome these difficulties may rely on the development of new materials, such as new protein-based thermoplastics (from vegetable or animal origin). These new

Correspondence concerning this article should be addressed to H. Yoshida at yoshida@chemeng.osakafu-u.ac.jp.

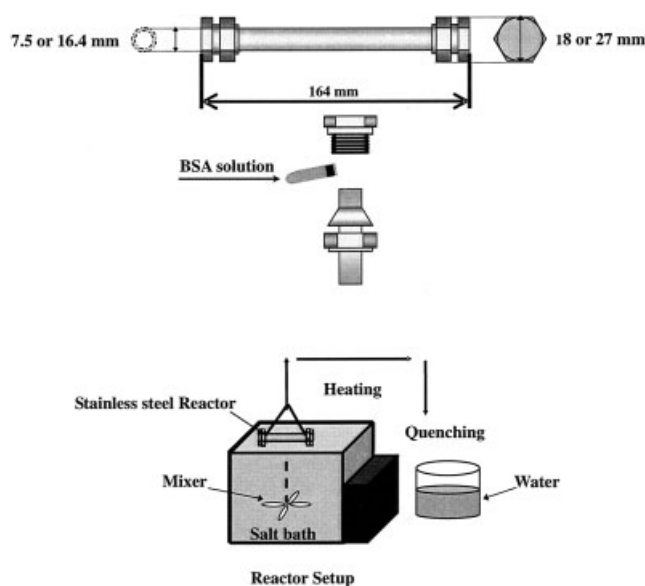
plastics should have a reduced susceptibility to thermal degradation (allowing for its easy processing by melt-based technologies into complex 3-D implants) and convenient degradation behavior.<sup>1</sup> Accordingly, new cost effective technologies are required to be developed to produce novel protein-based biodegradable plastics.

Lately, sub-critical water technology has attracted many researchers for its versatile applications in the field of the environment as well as synthesis of new attractive materials. On heating within the critical point of water (temperature < 374°C, pressure < 22.1 MPa) under enough pressure to maintain the liquid state, water (sub-critical water) was reported to have distinctive properties, such as a low dielectric constant and high ion product.<sup>9,10</sup> Many chemical reactions are catalyzed under sub-critical water conditions without any additives. In our laboratory, Yoshida (one of the present authors) and his team carried out many reactions under the sub-critical water condition.<sup>11-17</sup> Recently, the present authors provided a review for the different applications of the sub-critical water technology.<sup>18</sup>

In this article we present the synthesis and characterization of a novel biodegradable plastic prepared from bovine serum albumin (BSA) using sub-critical water technology. Serum albumin (the word serum refers to the clear fluid portion of the blood) represents the most abundant protein in the serum blood, with a typical concentration of 50 g/L. The word albumin is also used to describe a protein or a group of proteins defined by their solubility in water, as those found in the albumin fraction of wheat and serum albumin of proteins in the whey-milk (whey results when the fat and casein are removed from milk).<sup>19</sup>

BSA is a monomeric highly water-soluble protein of molecular weight of 66 kD. BSA forms soluble aggregates (during the early stages of heat-induced gelation of the protein) of polymerized molecule through disulphide and noncovalent bonds upon heating.<sup>20</sup> Subsequent polymerization results in the formation of a rigid gel network.<sup>21</sup> Ferry<sup>22</sup> explained the gelation in a two-step mechanism. An initiation step involving unfolding or dissociation of the protein molecules, followed by an aggregation step in which association or aggregation reactions occur, resulting in a gel formation under appropriate conditions. For the formation of a highly ordered gel, it is essential that the aggregation step proceed at a slower rate than the unfolding step.<sup>23,24</sup> It is important to notice that under atmospheric condition the BSA gelation resulted only in a gel matrix, that lacks the strength of a strong polymer which has a high degree of polymerization. However, it was reported that hydrostatic pressure induces coagulation of proteins without the more drastic chemical changes observed with heat treatment, such as destruction of covalent bonds,<sup>25</sup> and also can rapidly lead to the aggregation of protein molecules.<sup>26</sup> The changes in the structure and state of aggregation of a certain protein, such as BSA, are dependent upon the magnitude of the applied pressure and the duration of the pressure treatment.<sup>26,27</sup>

According to the above brief discussions, it is expected that by applying a combination of both high pressure and high temperature on BSA molecules that the aggregation step can be effectively enhanced to produce a biodegradable plastic with a strong polymer matrix. Such plastic could be synthesized commercially from the serum albumin of the animal blood to offer a novel biodegradable plastic as well as a new method of waste



**Figure 1.** The reactor configuration and setup used for PBSA synthesis using sub-critical water technology.

blood disposal. Moreover, this study represents the first step towards the understanding of the hydrolysis and decomposition mechanism of water-soluble proteins under the sub-critical water condition.

## Materials and Methods

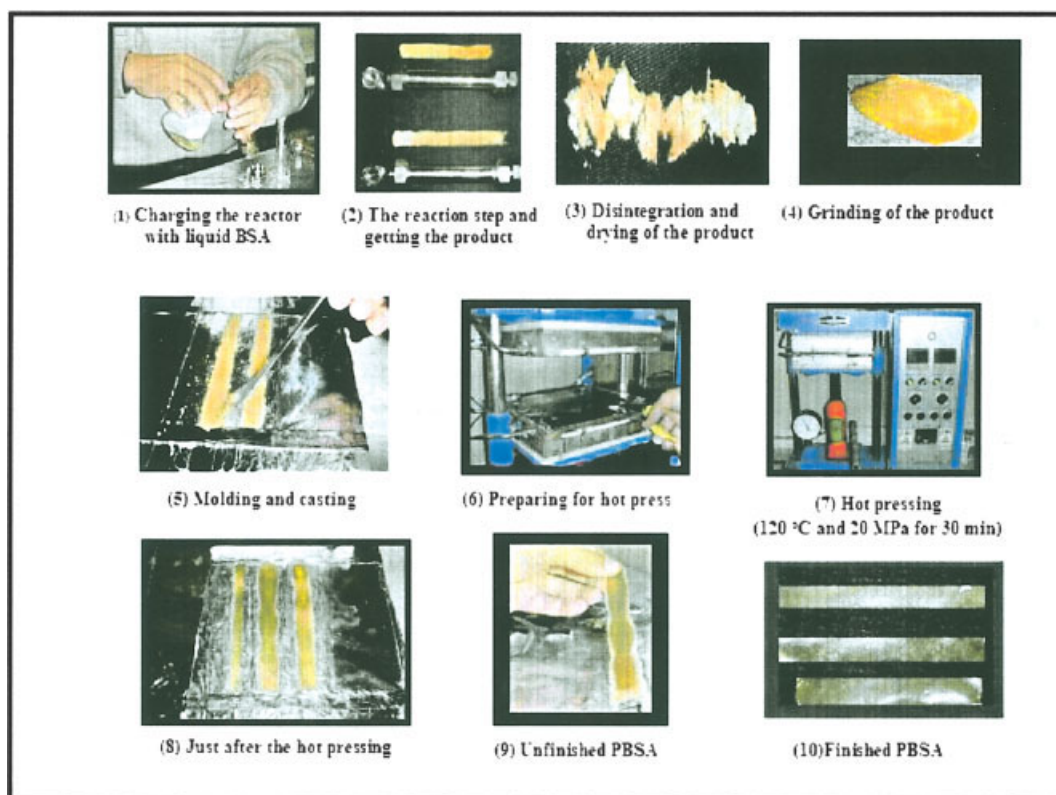
### Materials

Pure BSA (first grade) and other chemicals used in this study were the product of Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The BSA used was found to be homogenous in the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with 5% water content.

### Synthesis of the biodegradable plastic

The reactor configuration and setup used in this study are shown in Figure 1. The synthesis reactions were carried out using two different reactors with different sizes. Stainless steel pipes SUS 316, i.d. 0.0075 m × 0.15 m (with a reactor volume of  $9.0 \times 10^{-6} \text{ m}^3$ ) and i.d. 0.0168 m × 0.15 m (with a reactor volume of  $34 \times 10^{-6} \text{ m}^3$ ) with Swagelok caps were used as small and large reactors, respectively. The solid BSA was completely dissolved into Milli-Q water (160 g/L, unless stated otherwise) and charged into the reactor tube. Then, dissolved air in the space of the reactor and oxygen in the liquid phase were removed by purging argon gas. The reactor was then sealed and immersed in a preheated molten salt bath (Thomas Kagaku Co. Ltd.) containing a mixture of potassium nitrate and sodium nitrate (1:1). The synthesis reactions were carried out in the range of 200–300°C, and the pressure inside the reactor was estimated from the steam table for the sub-critical conditions. After the desired reaction time, the reactor was immediately cooled down by immersing it into a chilled water bath. The product of the reaction was a water-insoluble solid phase and an aqueous phase. The solid product was found to have





**Figure 2. The processing steps of the PBSA synthesized using sub-critical water technology.**

[Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

many plastic properties (as will be described in the Results Section) and represents the newly synthesized biodegradable plastic (plastic bovine serum albumin (hereafter called PBSA)). Both PBSA and the aqueous phase were recovered from the reactor, diluted to 50 mL with Milli-Q water, and centrifuged at  $3,000 \times g$  for 15 min at room temperature. The resulting supernatant was decanted and filtrated through Millipore membrane (with a pore diameter of  $2.2 \times 10^{-7}$  m) (Millipore Co., MA) to remove any traces of the PBSA. Samples were taken from both the solid and aqueous phases for further analysis.

### Processing of the PBSA

The different processing steps of the PBSA are shown in Figure 2. The solid product (raw PBSA) resulting from the reaction of BSA (using the large reactor) under the sub-critical water condition was recovered from the reactor in the form of a long cylindrical bar and dried at 50°C for 24 h. Then, it was grinded to a powder form using a TML-16 grinder (TESCOM Co., Tokyo, Japan). The PBSA, in the powder form, was hand mixed and stirred with 150% water (based on the solid PBSA) until a homogenous slurry mixture was obtained. The final mixture was poured into a test specimen mold made from aluminum foil mounted over a stainless steel plate. Then, the mold was covered with another aluminum-covered stainless steel plate. The stainless steel plates, containing the test specimen in between, were subjected to a hot pressing using a hot pressing machine equipped with a thermostat (to control the temperature of the pressing) and pressure gage (for monitoring the pressure). The hot pressing was processed at 120°C and 20

MPa for 30 min. Then, the mold was removed from the hot pressing machine and cooled down at room temperature. The molded PBSA was removed and adjusted for a definite shape and dimension by using a sharp cutter for further mechanical tests. If the modeled samples showed any air bubbles, the processes were repeated to get optimum mold specifications. In this work duplicated heat-treated samples were used for the mechanical tests.

### Analytical Procedures

#### SDS-PAGE

The protein identification in the filtrate was performed by SDS-PAGE using acrylamide concentration of 4% for stacking and 15% for resolving gels. The BSA band was identified using a standard molecular weight marker under identical electrophoretic conditions.

#### Electron microscopy

A JEOL 6700F field emission scanning electron microscope (SEM) (JEOL Inc, MA) was used to observe the microstructure of the prepared PBSA. Solid dry samples of the PBSA were prepared for the SEM.

#### Water holding capacity of the biodegradable PBSA

Dry solid samples of the PBSA of a known weight (W1) were immersed into 50-ml plastic tubes containing Milli-Q water at 4°C (to avoid biodegradation) for 3 days until reaching

equilibrium maximum water absorption. The remaining water was decanted and the surface of the PBSA samples was gently wiped using tissue paper. The weight of the wet PBSA samples (W2) was determined. The water holding capacity (WHC) was calculated as follows:

$$\text{WHC} = (W2 - W1)/W1$$

### ***FT-IR spectra***

IR spectra for both native and PBSA samples were recorded with an FT/IR-410 Fourier-transform infrared spectrometer (JASCO Co. Ltd., Tokyo, Japan). The solid samples for the IR analysis were compression-molded with KBr powders and subjected to the FT/IR test.

### ***Differential Scanning Calorimetry (DSC)***

Thermal denaturation and bond strength of the PBSA samples were studied using a DSC (DSC 6200, Seiko Instrument Inc., Tokyo, Japan) with a scan rate of 5°C/min following the manufacturer's standard protocol. Data acquisition and analysis software were provided by the instrument manufacturer. Indium ( $\Delta H = 28.5 \text{ J/g}$ ,  $T_m = 159^\circ\text{C}$ ) was used for the calibration of the machine, and an empty pan was used as a reference. The samples were prepared in aluminum DSC pans and sealed hermetically. The weight of the pan and lid was measured and subtracted from the weight of the sample in the sealed pan to get the weight of the sample.

### ***Carbon and nitrogen contents of the native BSA and PBSA***

Carbon and nitrogen contents of the native BSA and PBSA were measured with a CHNS corder Perkin Elmer 2400 (Perkin-Elmer, Inc., MA).

### ***Amino acids concentration in the aqueous phase***

The filtrated aqueous phase resulting from the BSA reactions was diluted 200-fold with Milli-Q water. The amino acids content was measured using an HPLC system (JASCO Co., Osaka, Japan) incorporated with a combination of an ion exclusion column (Shedex Rspak KC-811, JASCO Co., Osaka, Japan) and post labeling method with spectrofluorophotometer (Shedex Rspak KC-G, JASCO Co., Osaka, Japan). Seven different buffer systems with different concentrations, pH values, and flow rates were used based on the manufacturer's standard method. Data acquisition and analysis software were provided by the instrument manufacturer. The retention time of each organic acid was confirmed by injecting a sample in which a known amount of the authentic acid was added as an internal standard.

### ***Mechanical properties of the PBSA***

Both the unprocessed PBSA, which was recovered from the reactor without any further treatment in the form of a cylindrical bar (Figure 2, Photo 2), and processed specimens (Figure 2, Photo 10) were tested in tension at room temperature using an Instron® 5582 test machine (Instron Cor., MA) equipped with a 100 kN static load cell. The gauge length of the test machine was set at 40 mm. The PBSA samples were clamped such that

the interface was equidistant from each clamp. All samples were pulled at a rate of 10 mm/min.

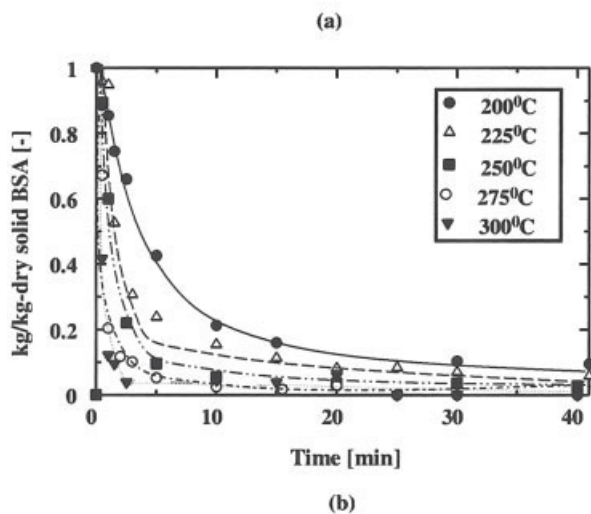
### ***Biodegradability test***

The biodegradability of the processed PBSA was investigated by measuring the production of methane gas during an anaerobic fermentation of the PBSA samples. All fermentation experiments were performed in a 22-mL glass vial (Perkin-Elmer 20-CV) with a butyl rubber and aluminum seal in a batch mode at 37°C. A digester (offered by Yogi Bio-Ecology Center, Yagi-cho, Kyoto, Japan) was used as the fermentation medium. The inocula solution was supplemented with about 1.1 g of the processed PBSA (in the form of a plastic strip) into the vial and sealed carefully under an atmosphere of  $\text{N}_2/\text{CO}_2$  (80/20 vol/vol). Two different batches with one specimen of the PBSA for each were tested for their biodegradability. The first batch was tested for one week, while the other one was tested for one month. The first sample was tested only for its weight losses and morphology changes due to the biodegradation reactions, while the second one was tested for methane ( $\text{CH}_4$ ) production during the biodegradation and at the end of testing period was also tested for weight losses and morphology changes. The concentrations of  $\text{CH}_4$  were measured in the headspace of the vial by taking 0.5 mL samples with a gas-tight syringe. The produced methane gas was measured using a gas chromatograph (Shimadzu GC-8APT). As a control, a vial containing 5-mL of inocula and free from PBSA was used to show the biodegradability of the PBSA containing samples. The control sample was incubated under the same conditions as the test samples.

## **Results and Discussion**

Figure 3a shows photographs of the reaction products at 200°C and 250°C for different reaction intervals in the range of 0.5-20 minutes compared to a photograph of a solution of the water-soluble BSA before the reaction. The product of the reaction was a water-insoluble solid phase and an aqueous phase. The formed solids were found to be completely water insoluble while having many plastic properties (as will be discussed later). These solids resulted from the aggregation and polymerization of the BSA molecules. Thus, the sub-critical water treatment induces the aggregation and polymerization of the BSA molecules. This suggests that sub-critical water treatment can be used to produce a biodegradable plastic in a very short time from a water-soluble protein without using any catalyst and cross-linking agents. The effect of the reaction temperature on the aggregation and polymerization yield of the BSA was studied using the small reactor. Figure 3b shows the time courses of the BSA aggregation and polymerization measured at different temperatures (200-300°C) in the range of 0.5 to 41 min. Within only 0.5 min, almost all the water-soluble BSA molecules were transformed into water-insoluble solids (note that before the reaction the solid content was zero). Moreover, after 0.5 min as the temperature and reaction time increases, the amount of the formed solid decreases.

To prove that all BSA molecules were completely converted into polymerized water-insoluble aggregates within a reaction period of only 0.5 min, the existence of BSA in the aqueous phase at different interval times was analyzed by SDS-PAGE



**Figure 3. (a) Photographs of the reaction products of the BSA reacted at 200 and 225°C for different interval times; (b) the time courses of the BSA aggregation and polymerization at different temperatures.**

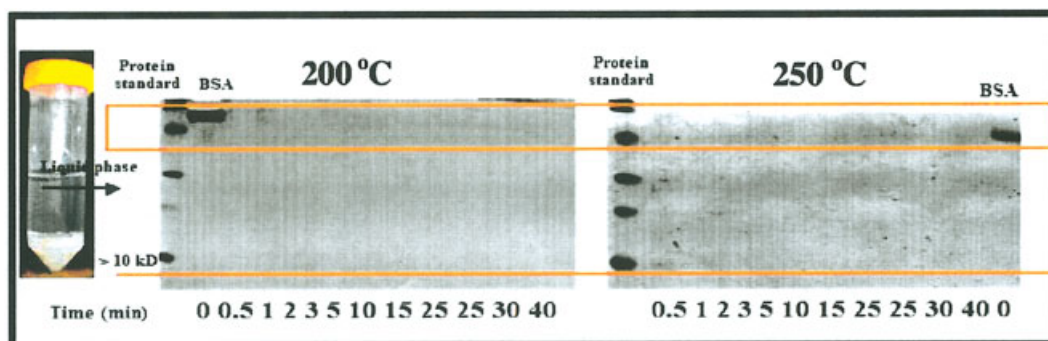
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electrophoresis. Figure 4 shows the electrophoresis patterns of the aqueous phase of the BSA reaction mixture after the sub-critical water reaction at 200°C (Figure 4a) and 225°C (Figure 4b) at different interval times. Before the sub-critical water

reaction, the BSA band clearly existed in both electrophoresis patterns. However, there were no protein bands for all samples at any reaction time in the tested range (0.5–40 min). The disappearance of the BSA band after the sub-critical water reaction indicates that all the BSA molecules were polymerized to form water-insoluble solids within only 0.5 min.

To investigate the nature of such formed solids, a microscopic study was carried out. Figure 5 shows the micrographs from SEM of the native BSA and the solid formed after sub-critical water reaction at 200 and 225°C for a reaction time of 0.5 min. The micrograph of the native BSA (Figure 5a) showed a smooth surface morphology and was easily differentiated from that reacted under the sub-critical water condition. At 200°C, BSA aggregated as a thin strand with interconnected network having numerous pores (Figure 5b). The BSA aggregated much more at 250°C, having a larger cluster size (Figure 5c). These solids resulted from the formation of soluble aggregates of BSA of polymerized molecules through disulphide and noncovalent bonds.<sup>20</sup> As the SEM results revealed, these aggregates grew in size (leading to the water insolubility) by being heated and formed a strong polymeric matrix, which we identified in this study as the PBSA.

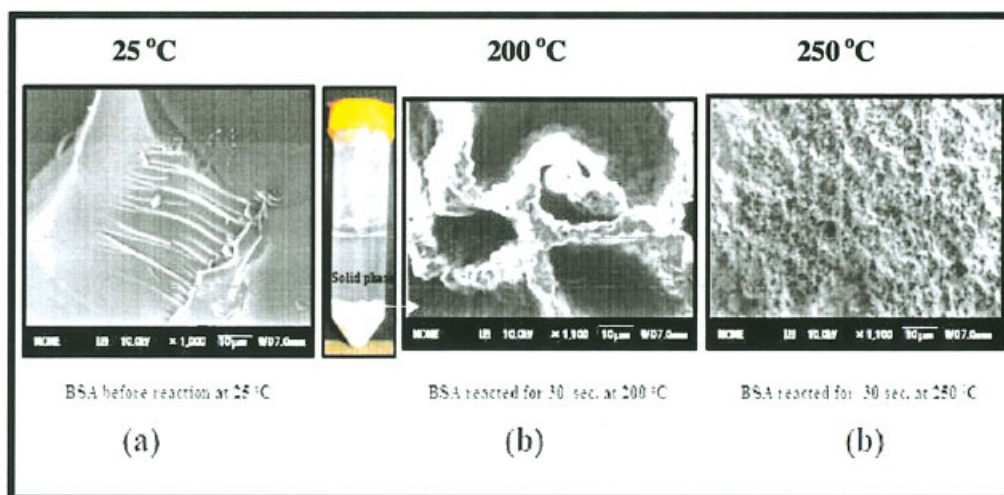
To investigate the nature of the bond formed during the BSA polymerization step, FT-IR spectra of both native and PBSA were obtained. Figure 6 shows the FT-IR spectra of PBSA prepared at 250°C after reaction times of 0.5, 1, and 20 min, respectively, compared to the native BSA. The identical characteristics of the IR spectra of native BSA and PBSA samples revealed that the PBSA prepared under these conditions resulted from the polymerization of identical BSA molecules through disulfide bond formation having the same macrostructure as the native BSA. It is important to note that the newly formed disulfide bonds between the polymerized BSA molecules, which are mainly responsible for the polymerization process, are superimposed with those already existing in the BSA molecules. Accordingly, these new bonds do not come into view in the IR spectrum of the PBSA. By comparing the solid formed due to the reaction of BSA using the sub-critical water treatment at the same temperature but for a longer reaction time (20 min), it is very easy to recognize the dramatic deformation of the protein structure under this condition. The existence of new peaks (815–560 and 710–695 for the -R-O-N-



**Figure 4. The electrophoresis patterns of the aqueous phase of the BSA reaction mixture after sub-critical water reaction at 200 and 225°C at different interval times.**

[Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]





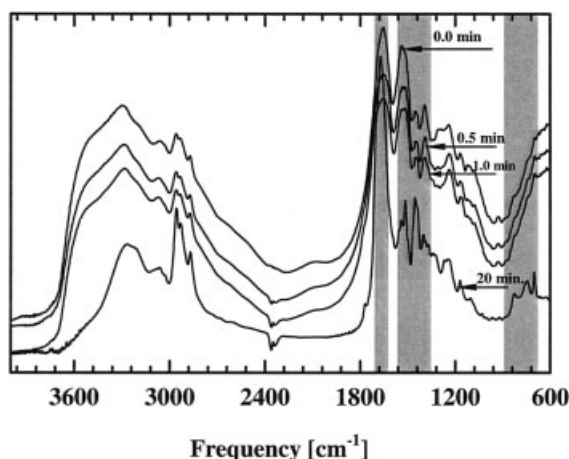
**Figure 5.** The SEM micrograph of the native BSA (a) and the formed PBSA (b) after sub-critical water reaction at 200 and 250°C for a reaction time of 0.5 min.

[Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

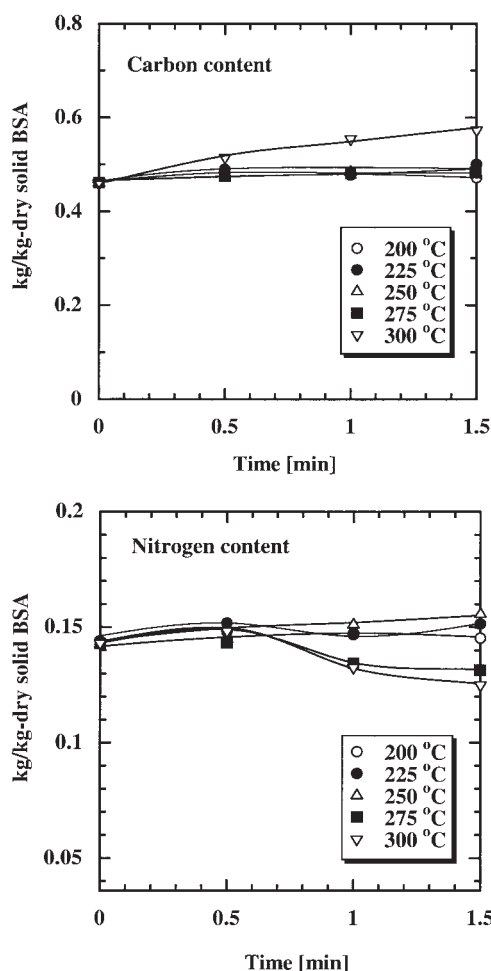
bond and 690-685 for the -Cs-SS-CS- bond) indicates such deformation.

The elemental analysis, carbon and nitrogen, of the solid phase of the BSA reaction gives further information about the nature of the formed PBSA. Figure 7 shows the results before and after sub-critical water treatment in the range of 0.5-1.5 min. The results showed that the carbon and nitrogen contents are almost unchanged after a reaction time of 0.5 min at any tested temperature. After a reaction time of 1.5 min at 275 and 300°C, the carbon content increased while the nitrogen content decreased, indicating the initiation of the hydrolysis process.

To inspect the hydrolysis product of PBSA at different temperatures, the amino acids in the aqueous phase were analyzed. Figure 8 shows the time course of the concentration of amino acids in the aqueous phase produced during the sub-critical water reaction of BSA under different temperatures for different interval times. There were almost no amino acids produced in the temperature ranges of 200-225°C within 2 min

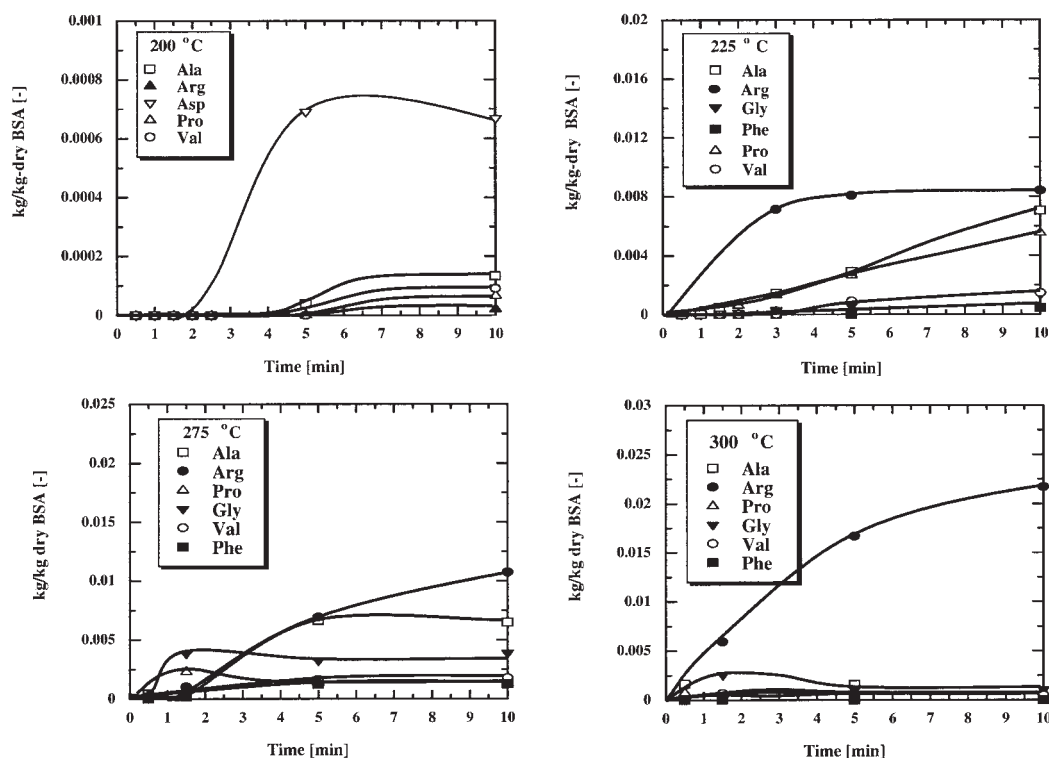


**Figure 6.** IR spectra of the solid BSA before and after sub-critical water reaction at 250°C for reaction times of 0.5, 1, and 20 min.



**Figure 7.** The carbon (a) and nitrogen (b) contents of the solid BSA before and after the sub-critical water reaction prepared at different reaction temperatures in the range of 0.5-1.5 min.





**Figure 8. Amino acids released during the reaction of the BSA under sub-critical water conditions at different temperatures.**

and 275–300°C within 0.5 min. Subsequently, the amount of produced amino acids increased with the increase of reaction time with high concentrations at elevated temperatures. These results suggest the initiation of the hydrolysis of PBSA indicated by the cleavage of PBSA into different amino acids in the aqueous phase. Consequently, it could be concluded that the BSA was transformed to PBSA within only 0.5 min without any significant hydrolysis in the range of 200–300°C.

To study the molecular bond strength of the PBSA, DSC was used. Regarding proteins, the thermally induced process detectable by DSC is structural melting or unfolding of the molecule. The transition of protein from a native to a denatured conformation is accompanied by the rupture of inter- and intra-molecular bonds, and the process has to occur in a cooperative manner to be discerned by DSC.<sup>28</sup> Accordingly, the DSC data were used to measure the bond strength and, in turn, the degree of cross-linking of PBSA. Figure 9 shows the DSC spectra of native BSA (as a control) and PBSA prepared at different temperatures, typically at 200, 250, 275, and 300°C, for a reaction time of 1 min. Native BSA had a transition temperature at 64°C. In the case of PBSA, the transition temperature was 66, 77, 78, and 85.5°C for samples synthesized at 200, 250, 275, and 300°C, respectively. The transition temperature increased by the increase in the temperature of the reaction, indicating that the bond strength and, in turn, the degree of cross-linking increases with the increase in the temperature of the reaction.

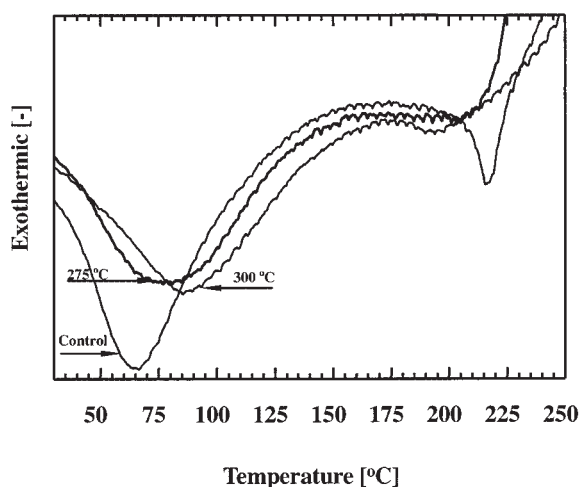
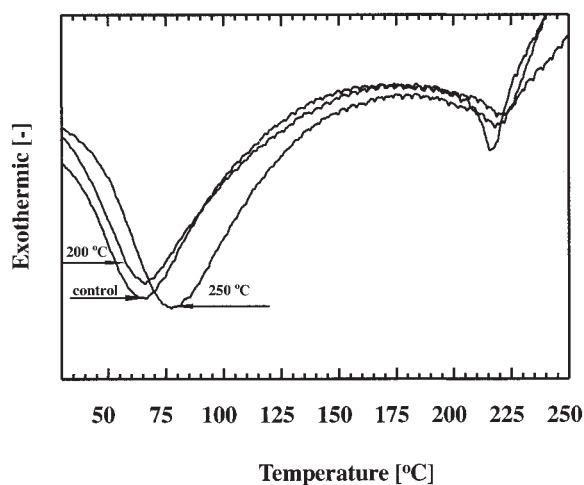
For more confirmation of such results, the water holding capacity (WHC) of PBSA was measured. Figure 10 shows that the WHC of the PBSA decreases with the increase in the temperature of the synthesis reaction of the PBSA. This result

indicates that the sorption ability of water decreases by the increase in the synthesis temperature due to the increase of the degree of cross-linking. This agrees with the result obtained using the DSC mentioned above.

To study the effect of the initial BSA concentration on the yield of the PBSA formation, the synthesis reaction under the sub-critical water condition was carried out using the large reactor with different initial concentrations of BSA. The experiment was carried out at 250°C for a reaction time of 1 min using different initial BSA concentrations in the range of 5–160 g/L. The results showed that at any initial BSA concentration, the aggregation and polymerization processes took place and produced water-insoluble PBSA. However, the yield of PBSA decreased by the increase in the initial BSA concentration (Figure 11).

All the results mentioned above revealed that the BSA reaction, under the sub-critical water condition, passed through two distinguishable steps. First, the BSA formed a polymeric matrix through a protein unfolding and polymerization process. Second, as the reaction proceeded, PBSA was hydrolyzed with fast rates at higher temperatures. To explain this reaction, we had to explore the effect of both temperature and pressure under the sub-critical water conditions on protein molecules.

The chemical interactions that stabilize the native conformation of protein include disulfide bonds and weak non-covalent interactions, such as hydrogen bonds, hydrophobic interactions, ionic interactions, and van der Waals interactions. Although all these bonds are holding the protein molecules together, the overall stability of proteins is considerably low.<sup>29</sup> Because of this lower stability, the native structure of protein is easily affected by environmental conditions, which finally may

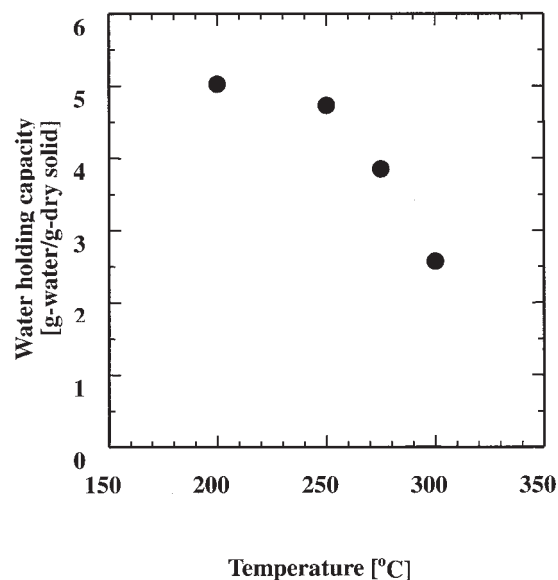


**Figure 9.** Differential scanning calorimeter spectra of the native BSA and PBSA prepared at different temperatures: 200 and 250 (a), and 275 and 300°C (b) for a reaction time of 1 min.

lead to denaturation. The term “denaturation of protein” indicates the phenomenon in which the higher structure of protein is ruptured by environmental changes, while the primary structure is kept without damage. Temperature, pressure, and solvent composition are all factors affecting the stability of proteins.<sup>30</sup>

Concerning temperature, BSA was reported to pass through two structural stages when heat-treated. The first stage is reversible, while the second stage is irreversible.<sup>31–33</sup> Heating up to 65°C (under atmospheric pressure) can be regarded as the first stage, with subsequent heating above that as the second stage.<sup>34</sup> Parallel to the structure deformation, BSA on heating aggregates and, finally, forms a rigid gel network.<sup>20,21</sup>

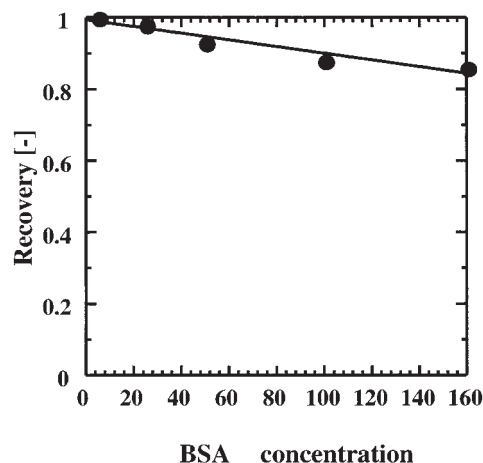
Concerning pressure, the main targets of pressure on protein aggregation are the electrostatic and hydrophobic interactions that maintain the higher structure of protein. Consequently, the pressure induces unfolding and finally leads to the formation of high molecular weight aggregates. These aggregates are stabilized by intermolecular disulfide bonds, which formed either by SH/S-S interchange reactions or by SH oxidation reactions.<sup>35–37</sup>



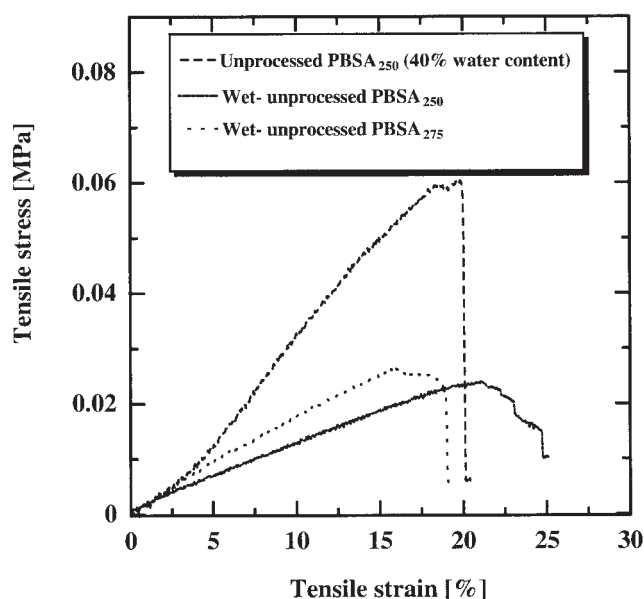
**Figure 10.** The water holding capacity of the PBSA prepared at 200, 250, 275, and 300°C for a reaction time of 1 min.

Based on the above discussion, a simple mechanism could be drawn for that reaction. At the beginning of the reaction (short time), most of the energy was absorbed by the water molecules as a latent heat of vaporization. The generated vapor pressure at a high temperature is responsible for the breaking down of both hydrophobic and electrostatic interactions without affecting the covalent and disulfide bonds, in turn initiating the aggregation step. By increasing the reaction time, the effect of the high temperature becomes predominant and leads to peptide and disulfide bonds breaking. Finally, the hydrolysis and decomposition of the formed aggregated molecules is initiated.

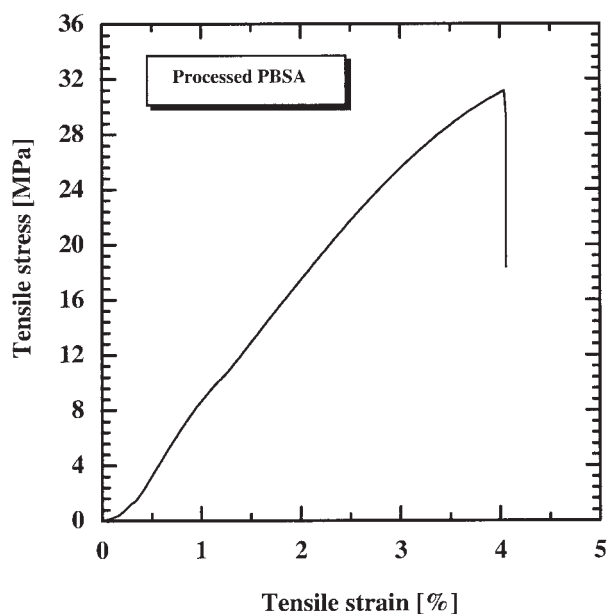
To investigate the plastic and mechanical properties of the PBSA, uniaxial tensile tests were performed on both the unprocessed and processed PBSA samples. For the unprocessed samples, the mechanical properties of wet samples at their



**Figure 11.** The recovery ratio at 250°C for a reaction time of 1 min using different initial concentrations of BSA in the range of 5–160 g/L.



(a)



(b)

**Figure 12. The stress strain curve of the unprocessed (wet and dry) and processed PBSA samples prepared at different temperatures for 1-min reaction time.**

maximum water holding capacity prepared at two different temperatures, 250 and 275°C, for a reaction time of 1 min, and another with 40% water content prepared at 275°C, were compared (Figure 12 a). For the processed PBSA samples, raw PBSA (synthesized under the sub-critical water condition of 250°C for a reaction time of 1 min) was processed and dried for the mechanical test (Figure 12 b). All tested samples showed a stress-strain curve typical to that of brittle materials, which are linear over their full range of strain, eventually terminating in

a fracture without appreciable plastic flow. This indicates that such materials are lacking mobility, which means that they have internal microstructures that block dislocation motion, which in turn indicates that they are brittle rather than ductile. For all samples (wet and dry), the plastic portions were small but it was too small for the dry one. The average yield stresses for the unprocessed wet samples (at the maximum water holding capacity) were 0.026 MPa and 0.024 MPa, and the average modulus of elasticity was about 0.16 and 0.14 MPa for the PBSA samples prepared at 250 and 275°C, respectively. In the case of the 40% water-content samples of the unprocessed PBSA, the values of the yield and ultimate strength were higher than those of the wet samples. The average values of the yield strength and modulus of elasticity were 0.06 and 0.32 MPa, respectively, indicating that the stiffness of the partially dry samples was higher than those completely wet samples. However, for the dried-processed samples, both the yield strength and modulus of elasticity increased dramatically, to 31.2 MPa and 780 MPa, respectively. These results indicate that by optimizing the processing of such material, more enhanced mechanical properties could be achieved. It is also important to note that such observed brittleness could be modified easily by using plasticizers, as will be presented in a future study.

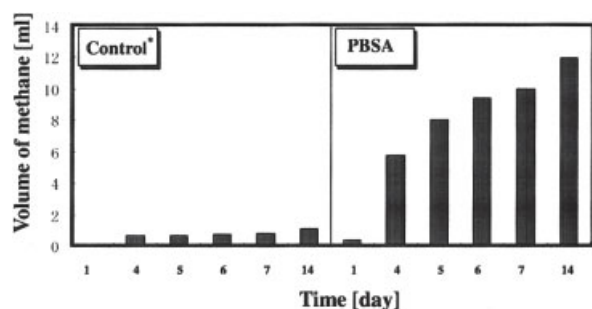
Although PBSA is mainly a protein-based plastic, which is obviously expected to be a biodegradable, we tried to provide clear evidence for its biodegradability. Figure 13a shows the volume of methane released during the biodegradation of a PBSA sample compared with a control sample containing no PBSA. Methane production was discontinued after almost 4 days for the control sample. However, in the sample containing PBSA, methane was increasing continuously up to 14 days, indicating the continuous biodegradability of PBSA. Figure 13b shows a comparison between the PBSA sample before, during, and after the incubation in the fermentation medium. The effect of the microgram attack to the PBSA sample was clearly observed in the color change. The morphology changed due to the biodegradation reaction, with remarkable changes for longer incubation periods as can be seen easily in the photos. Moreover, measuring the difference in weight before and after the degradation reaction gave further proof for the biodegradability of the PBSA samples. The results showed that PBSA samples incubated for 1 week and 1 month lost 15 and 35% of their weight, respectively, due to the biodegradation reactions.

The synthesized biodegradable plastic in this work can be produced from animal blood, offering a new method for blood disposal and, furthermore, a new method for synthesizing a novel biodegradable plastic. Both targets have a great impact on the biomedical and environmental fields.

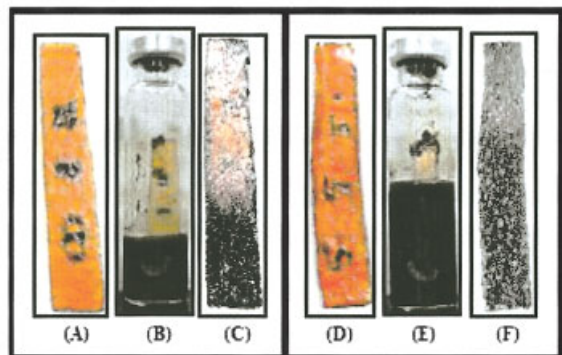
## Conclusions

The obtained results indicated that sub-critical water treatment induces the aggregation and polymerization of BSA molecules. The resultant polymer has a very rigid matrix enhanced by the formation of strong disulfide bonds, and has many plastic properties. The general properties of the obtained polymer allow it to be considered as a novel protein-based biodegradable plastic. The optimum synthesis conditions were found to be in the range of 250-275°C for a reaction time of 1 min using 160 g/L BSA as an initial concentration. These synthesis





(a)



(b)

**Figure 13. Production of methane gas during the anaerobic biodegradation of the PBSA sample compared to a control sample (a) and photographs of the PBSA sample before (A and D), during (B and E), and after (C and F) biodegradation for 1 week (C) and 1 month (E).**

[Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

conditions are considered very mild compared to other plastics synthesis. The processed PBSA samples (without any additives or modifiers) showed a stress-strain curve typical to those of brittle materials, with a high tensile strength of 31.2 MPa. These results indicate that the mechanical characteristics of PBSA resemble those materials showing brittle behavior at room temperature or below, such as polystyrene, polymethylmethacrylate, and many unfilled phenol formaldehyde resins, which have a range of tensile strength of 18–70 MPa, 47–70 MPa, and 35–63 MPa, respectively. One of the most important results from this study is the introduction of the newly developed sub-critical water technology in the field of biomaterial synthesis. The application of such technology could be extended to other proteins for the synthesis of novel biomaterials. Moreover, such a biodegradable plastic could be produced from animal blood, offering a new method for blood disposal and a new method of synthesizing a novel biodegradable plastic.

### Future work

In the very near future, we are going to present a detailed study for the kinetics and mechanism of PBSA synthesis, as

well as a detailed study for the mechanical and thermal properties of both PBSA and composite PBSA.

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