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# Electrosynthesis of potato starch–whey protein isolate complexes

H. Zaleska[a], S. Ring[b], P. Tomasik[a,*]

[a]*Department of Chemistry, University of Agriculture, Mickiewicz Ave., 21, 31 120 Cracow, Poland*
[b]*Food Biopolymer Section, Institute of Food Research, Colney Lane, Norwich NR4 7UA, UK*

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**Abstract**

Potato starch–whey protein isolate complexes were prepared electrochemically. The kinetics of complex formation was examined and the complexes characterized by chemical and physical methods. Their composition was examined through the analysis of carbohydrate and nitrogen content. The physical characteristics were examined by FTIR and theromgravimetry. © 2001 Elsevier Science Ltd. All rights reserved.

*Keywords*: Electrosynthesis; Polysaccharide–protein complexes; Polysaccharide–protein films; Potato starch; Whey proteins

## 1. Introduction

Biopolymer mixtures and complexes have application in the food industry, as packaging materials and pharmaceutical coatings. There is a continuing need to modify the properties of these materials for enhanced and novel applications (Aggour, 1993; Arevalonino, Sandoval, Galan, Imam, Gordon & Greene, 1996; Arvanitoyannis, Psomiadov, Nakayama, Aiba & Yamamoto, 1997; Bystricky, Malavikova & Sticzay, 1990, 1991; Chandra & Rustgi, 1997; Dalev & Simeonova, 1995; Dennenberg, Bothast & Abbott, 1978; Fishman & Coffin, 1998; Ghorpade, Bhatnagar & Hanna, 1997; Golachowski & Leszczynski, 1997; Imam, Gould, Gordon, Kinney, Ramsey & Tosteson, 1992; Jane, Lim, Paetav, Spence & Wand, 1994; Korolczuk, Breton-Dollet, Tissier & Maingonnat, 1996; Lee, Pometto, Fratzke & Bailey, 1991; Tolstoguzov, 1986; Tomasik & Schilling, 1998; Van Soest, Hulleman, Dewit & Viegenthart, 1996; Zaleska, Mazurkiewicz, Tomasik & Baczkowicz, 1999; Zbikowska, Dziuba & Szerszunowicz, 1998). Properties of these complexes or blends strongly depend on the components and parameters of preparation. In the synthetic polymer area an established approach for modifying material characteristics is to make use of polymer blends. For the most part these are phase-separated systems, which contain domains of one polymer dispersed in a matrix of the other. Less frequently there is an attractive interaction between chemically dissimilar polymers providing single-phase materials. In comparison to synthetic polymer blends there is less information available on biopolymer blends. Immiscibility has been demonstrated for polysaccharide mixtures. On the other hand attractive interactions between proteins and anionic polysaccharides has been demonstrated several times (Tolstoguzov, 1986). In this article we investigate the electrochemical synthesis as a novel method (Dejewska, Mazurkiewicz, Tomasik & Zaleska, 1995; Zaleska et al., 1999; Zaleska, Ring & Tomasik, 2000) for the fabrication of anionic polysaccharide–protein complexes. Such a method provides a continuous, facile preparation of the complexes of attempted properties, because several parameters of the reaction could thoroughly be adjusted and controlled.

The polysaccharide chosen for the study was potato starch, which consists of amylose and amylopectin, with typical amylose contents being in the range 20–25% (w/w). Both polymers are based on $1 \rightarrow 4$ linked α-D-glucose. While potato amylose is essentially linear with only limited branching, potato amylopectin is substantially branched with a polymodal distribution of constituent chains. Another characteristic of potato amylopectin, which is particularly relevant to the present study, is its limited phosphorylation, which gives it polyelectrolyte characteristics. Its random phosphato moieties permit proteins to form complexes with it. Whey protein was taken as the possible component of the complex. Its gelation ability (Mulvihill & Kinsella, 1987), water holding, and foaming ability (Sikorski, 1997) make this protein composition a valuable food component.

* Corresponding author. Tel.: +48-126338826; fax: +48-126336245.
*E-mail address:* rrtomasi@cyf-kr.edu.pl (P. Tomasik).


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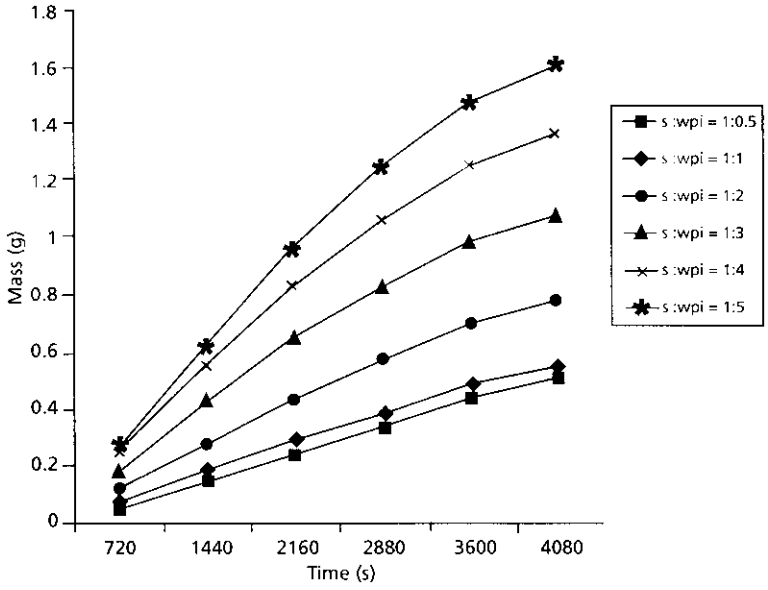


Fig. 1. Amount of complex collected from the anode with respect to time.

## 2. Materials and methods

### 2.1. Materials

Granular potato starch was obtained from Pila Enterprise, Poland. A water (100 ml) suspension containing 0.5 g of potato starch was heated for 5 min from 60 to 80°C in an electrolytic cell fitted with a magnetic stirrer. The whey protein isolate (WPI) was obtained from Eurial, Herbignac, France. This is a WPI that has been de-fatted by filtering through ceramic filters. It contains ~85% of protein.

### 2.2. Complex formation

An electrolytic cell, 100 ml was equipped with two chromnickel H18N9 stainless steel 15 $cm^2$ electrodes at a 2.5 cm separation. The cell was filled with an aqueous solution of starch (0.5% (w/w)) and WPI (0.25–2.5% (w/w)). A 12 V potential was applied across the cell. The initial 0.1 A ± 10% current intensity was significantly reduced within consecutive 4 min periods because of the passivation of the anode with the complex. The complex in the form of a gel covered the anode and it was mechanically removed after each 4 min period. The product was dried in an oven at 40–50°C. The dry product was insoluble in water and 5% hydrochloric acid up to 100°C, and insoluble in DMSO, 7 M urea, and 2 M guanidinium thiocyanate at room temperature.

### 2.3. Film formation

Formation of mixed films was attempted by drying aqueous solutions of pectin (2% (w/w)) and casein (2% (w/w)) as well as blends of these solutions at 40°C under proportions 1:0.5; 1:1; 1:2; 1:3; 1:4; and 1:5.

### 2.4. Calorimetry

Samples were conditioned at different relative humidities (RH) by storage over saturated salt solutions [LiCl, RH = 11.3%; $CH_3COOK$, RH = 23.1%; $MgCl_2{\cdot}6H_2O$, RH = 33.1%; $K_2CO_3$, RH = 43.2%; $Mg(NO_3)_2{\cdot}6H_2O$, RH = 54,4%; NaCl, RH = 75.5%; $(NH_4)_2SO_4$, RH = 81% (Greenspan, 1977)]. Water content of the conditioned materials was obtained by vacuum drying over $P_2O_5$. Samples (~10 mg) were sealed in pre-weighed aluminium pans in an argon atmosphere. DSC experiments were performed using a Perkin–Elmer DSC 7 fitted with robotic autosampler and an intracooler. The samples were cooled at 25°C/min to −20°C and heated at 10°C/min to 75°C. The last stage calorimetric signal was logged by the computer. The calorimeter was calibrated from the melting of indium and the heat capacity of sapphire.

### 2.5. IR spectroscopy

The samples were examined in KBr discs containing 3 mg of either complex or its components in 300 mg of KBr. The spectra were obtained on Mattson 3000 FTIR (Pye Unicam, Cambridge, UK) spectrophotometer.

### 2.6. Sample composition

The protein content of the complexes was estimated from the nitrogen content, which was determined according to the Dumas semi-micro method (Bobranski, 1956). The neutral sugar content of the materials was obtained after the Saeman hydrolysis of the polysaccharide followed by reduction of the monosaccharides released and their determination by gas chromatography of their alditol acetates as described (Blakeney, Harris, Henry & Stone, 1983; Englyst & Cummings, 1984).

Table 1
Rate constant of the complex formation depending on the composition of the reaction blend

| Blend composition | Protein content in the reaction mixture (%) | Rate constant, $k \times 10^{-4}$ ($s^{-1}$) |
|---|---|---|
| 1:0.5 | 33 | 2.34 |
| 1:1 | 50 | 1.75 |
| 1:2 | 67 | 1.68 |
| 1:3 | 75 | 1.82 |
| 1:4 | 80 | 1.87 |
| 1:5 | 83 | 1.86 |

Table 2
Protein content, %, in the complexes depending on the original blend composition

| Blend composition | Protein content[a] (%) | |
|---|---|---|
| | In original blend | In the complex |
| 1:0.5 | 33 | ~31 |
| 1:1 | 50 | ~44 |
| 1:2 | 67 | ~64 |
| 1:3 | 75 | 73 |
| 1:4 | 80 | 74 |
| 1:5 | 83 | 78 |

[a] This value is calculated based on the nitrogen content found in plain WPI (14.31%)

### 2.7. Thermogravimetry (TG), differential thermogravimetry (DTG) and differential thermal analysis (DTA)

The analyses were performed on 200–220 mg samples. They were heated in the open in corundum crucibles from room temperature to 500°C. The 5°C/min rate of temperature increase was applied. Alumina, $\phi = 8$ mm was used as the standard. The instrument of Paulik-Paulik-Erdey Q-1500-D, Budapest, Hungary, was used.

## 3. Results and discussion

### 3.1. Production of starch–protein complexes

The formation of product at the anode was investigated as a function of the biopolymer composition in aqueous solution. The starch–WPI ratio varied from 1:0.5 to 1:5. Fig. 1 shows the time dependence for the formation of product for the WPI–starch mixtures. The amount of product collected at the anode increased with increasing protein content in the bathing solution. The time dependent behaviour could be described by the kinetic equation $\ln c = k_1 t + \text{const}$ (Table 1). The rate of the complex formation was practically independent of the blend composition except the blend with the 1:0.5 starch–protein ratio where the reaction rate was slightly higher. The composition of the product was dependent on the composition of the bathing solution. For a starch–protein ratio of 1:0.5 the protein content of the product, estimated on the basis of the nitrogen content in the product collected at the anode, was in the region of 30–40% (w/w) (Fig. 2, Table 2). An increase in the protein content in the solution was paralleled by an increase in the protein content in the product. The comparison of the figures in Table 2 revealed that protein from solution almost completely co-separated with starch on the anode. Practically, no particular ratio of both biopolymers in complexes had a preference. The content ranged from ~30% for the 1:0.5 ratio to ~80% for the 1:5 ratio. The composition of the product showed a small dependence on time in the range of 500–4000 s. The protein content was falling with increasing time. This effect was more apparent with decreasing protein content in the bathing mixture. During this time the pH of the bathing solution changed (Fig. 3) to the extent depending on the composition of the bathing solution reaching ~12 at the end of the experiment. The rate of the increase was dependent on the protein content. The intial pH ranged from 9.5 to 11 as the starch–WPI ratio fell from 1:5 to 1 to 0.5.

On the basis of the above considerations one might

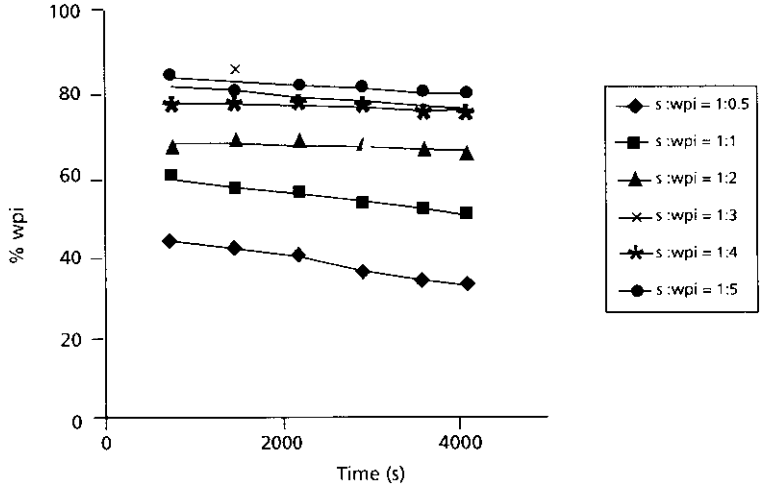


Fig. 2. Protein content in complexes dependent on the reaction time and composition of the reaction blend.

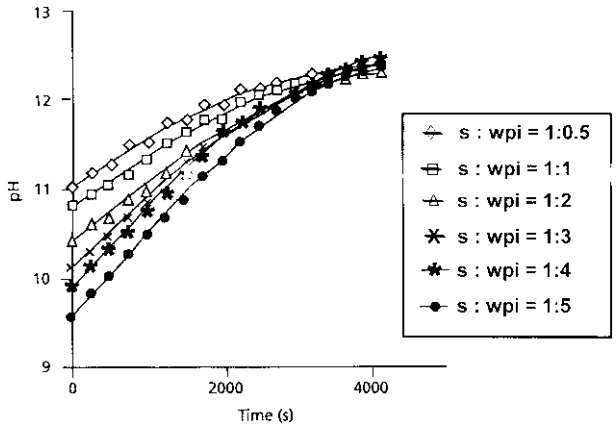


Fig. 3. The pH changes in the reaction blend proceeding with the reaction time and blend composition.

assume that the separation of the starch–WPI complexes on the anode could involve two steps. First, the faster step, which proceeded at a low concentration of protein, initiating the formation of the complex kernel, and the second step, which was relatively steady with time and protein concentration due to the separation of further amounts of protein on that kernel.

### 3.2. Characterization of complexes

At these terminal pH's (>12) the proteins and the amylopectin fraction of potato starch should be negatively charged. After drying the products were insoluble in 90% DMSO, 7 M urea and 2 M guanidinium thiocyanate. As these solutions are powerful solvents for both polysaccharides and proteins, a strong interaction between the starch and the protein was indicated. One interaction that should be considered in this context, was the ionic interaction between the negatively charged polysaccharide and basic amino acid residues of the protein (Korolczuk et al., 1996).

Sugar analysis (Table 3) of complexes fitted theoretical values fairly well in terms of the total sugar content. Small quantities of L-arabinose and D-xylose seemed to be an artefact. Unlike D-galactose, content of these sugars decreased with increase in the content of protein, therefore, L-arabinose and D-xylose could not be contaminants of protein.

Table 3
Neutral sugar composition of starch–WPI complexes (the notation of complexes is based on initial composition of the starch–protein blends from which complexes was synthesized. − = less than 1 μg/mg)

| Complex | Rha | Fuc | Ara | Xyl | Man | Gal | Glc | Total sugars,% |
|---|---|---|---|---|---|---|---|---|
| Starch | 1.6 | − | 1.2 | 1 | 1.7 | | 911.4 | 92 |
| 1:0.5 | − | − | 15 | 6 | − | 8 | 411 | 44 |
| 1:1 | − | − | 7 | − | − | 11 | 322 | 40 |
| 1:2 | − | − | − | − | − | 15 | 202 | 22 |
| 1:3 | − | − | − | − | − | 18 | 163 | 18 |
| 1:4 | − | − | − | − | − | 19 | 120 | 14 |
| 1:5 | − | − | − | − | − | 20 | 120 | 14 |

The starch and protein spectra within the range of 400–4000 $cm^{-1}$ differed from each other, but the spectra of complexes presented almost precise superposition of the spectra of both components (Fig. 4, Table 4). Relatively distinct changes could be observed around 3412, 2930,

Table 4
IR spectra of starch–WPI complexes ($\nu$, $cm^{-1}$)

| Complex composition[a] | | | | | |
|---|---|---|---|---|---|
| 1:0.5 | 1:1 | 1:2 | 1:3 | 1:4 | 1:5 |
| 3412 vs | 3416 vs | 3410 vs | 3406 vs | 3418 vs | 3422 vs |
| | 3018 sh | 3020 sh | 3096 sh | 3075 sh | 3086 sh |
| 2926 w | 2928 w | 2936 w | 2934 w | 2959 w | 2928 w |
| 1649 s | 1657 s | 1657 s | 1657 vw | 1647 vs | 1647 vs |
| 1543 w | 1545 w | 1543 s | 1535 s | 1533 vs | 1535 vs |
| 1404 vw | 1402 vw | 1400 vw | 1398 vw | 1398 w | 1398 w |
| 1242 vw | 1242 vw | 1238 vw | 1238 vw | 1238 vw | 1240 vw |
| 1155 vw | 1153 vw | 1153 vw | 1153 vw | 1153 vw | 1153 vw |
| 1022 s | 1022 s | 1024 w | 1026 w | 1030 w | 1028 w |
| 930 vw | 930 vw | 930 vw | 932 vw | 932 vw | 934 vw |

[a] The notation of the complexes is based on the initial composition of the blend from which complex was prepared. The band intensity notaion is as follows: vs—very strong, s—strong, w—weak, vw—very weak, sh—shoulder.

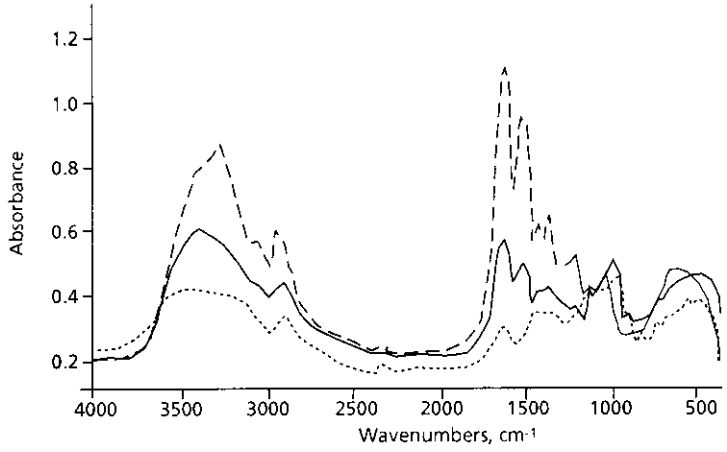


Fig. 4. IR spectra of starch (……), WPI (- - -) and starch–WPI 1:1 complex (—).

Table 5
IR spectra and band assignments for starch and WPI

| Compound | Band position,[a] $\nu$ ($cm^{-1}$) | Band assignment |
|---|---|---|
| *Starch* | 3430 vs | $\nu_{OH}$ intramolecular hydrogen bond |
| | 2928 w | $\nu_{CH}$ |
| | 1655 w | $\nu_{C=O}$ |
| | 1373 w | $\delta_{CH}$ |
| | 1165 s | $\delta_{OH}$ |
| | 1084 s | $\delta_{COC}$ |
| | 980 vs | $\delta_{C-O}$ |
| *WPI* | 3300 vs | $\nu_{NH}$ polymeric association |
| | 3090 sh | $\nu_{OH}$ polymeric association |
| | 2963 w | $\nu_{CH}$ |
| | 1645 vs | $\nu_{C=O}$ |
| | 1535 vs | $\delta_{NH}$ |
| | 1449 vw | $\delta_{CH}$ |
| | 1397 vw | $\delta_{CH}$ |
| | 1240 vw | $\delta_{C-N}$ |
| | 1076 vw | $\delta_{C-N}$ |

[a] The notation of band intensities: vs—very strong, s—strong, w—weak, vw—very weak, sh–shoulder.

and 1450 $cm^{-1}$. The band assignments in the spectra of starch and WPI given in Table 5 indicated that the electrode processes led to the complex formation. The latter involved co-separation of inclusion complexes with participation of the peptide bonds in protein and possibly phosphato groups of polysaccharide. It was supported by the solubility tests. The shifts of the bands, particularly those at 2930 and 1450 $cm^{-1}$ might reflect conformational changes of the chains.

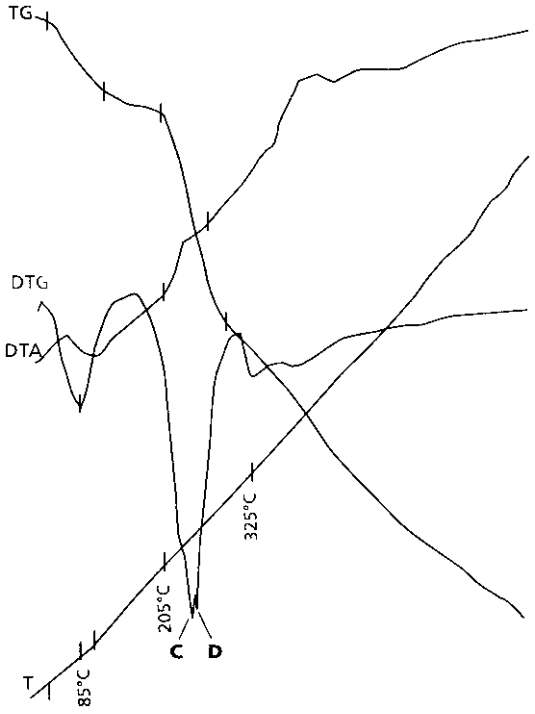


Fig. 5. Thermogram of the starch–WPI 1:2 complex.

Table 6
Thermogravimetric characteristics of starch–WPI complexes

| Compound | Characteristics[a] (°C) |
|---|---|
| *Starch* | TG[b]: 55 to 140 (11.5%), to 225 (12%),<br>DTG: 95, 285 (D-peak)<br>DTA: 120, 190, 235, 270s, 285s, 360, 380 |
| *WPI* | TG[b]: 30 to 100 (8%), to 170 (11.6%), to 275 (46.5%), to 365 (60%)<br>DTG: 75, 210, 240 (C-peak), 395<br>DTA: 80, 170, 185, 265, 280, 300, 320 |
| *Complexes*[c] | |
| 1:0.5 | TG[b]: 50 to 115 (9.5%), 180–280 (30.0%)<br>DTG: 80, 245 (C-peak), 260 (D-peak)<br>DTA: 90, 180, 250, 260, 280, 305, 315, 330, 340 |
| 1:1 | TG[b]: 55 to 135 (10.5%), to 220 to 305 (27.5%)<br>DTG: 95, 245 (C-peak), 260 (D-peak)<br>DTA: 115, 190, 220, 270, 345, 350, 355, 360, 365, 385 |
| 1:2 | TG[b]: 45 to 125 (10.5%), 205 to 300 (30.5%)<br>DTG: 85, 245 (C-peak), 260 (D-peak)<br>DTA: 105, 205, 255, 295, 327, 348, 362, 375, 385 |
| 1:3 | TG[b]: 60 to 145 (11%), 225 to 310 (30.6%)<br>DTG: 100, 245 (C-peak), 270 (D-peak)<br>DTA: 115, 165, 220, 260, 285, 295, 317, 388, 395, 405, 415 |
| 1:4 | TG[b]: 75 to 145 (9%), 215 to 310 (31.0%)<br>DTG: 95, 260 (C-peak), 275 (D-peak)<br>DTA: 115, 180, 198, 235, 267, 280, 295, 300, 328, 345, 355, 365 |
| 1:5 | TG[b]: 75 to 150 (9.5%), 225 to 345 (36.5%)<br>DTG: 105, 275 (C-peak), 285 (D-peak)<br>DTA: 130, 180, 230, 275, 320, 345, 385, 400, 427 |

[a] All peaks are endothermic.
[b] The weight loss in parentheses relates to the total weight loss from the beginning of the TG analysis.
[c] The notation of complexes is based on the initial content of the starch–WPI blend from which the complex was synthesized.

The theory of synthesis of protein–polysaccharide complexes confirmed the TG analysis. In the thermograms of complexes, the characteristic patterns of starch (peak "D" on the DTG curve) and protein (peak "C" on the DTG curve) could be recognized (Fig. 5). Both peaks were shifted to a certain extent relative to their original positions, reflecting the protein–polysaccharide interaction in complexes (Table 6).

In the thermogram of the 1:0.5 complex the C-peak on the DTG curve was barely recognizable as a shoulder of the much stronger D-peak corresponding to starch. As the protein content in the complex increased, the C-peak increased and the D-peak decreased until the latter became a shoulder of the C-peak in the thermogram of the 1:5 complex. The TG curves indicated that the air-dried complexes contained less water than the air-dried protein and starch. The curves also showed that in each complex the weight loss proceeded on a slightly different way. It suggested that water might take an active part in the construction of the network structure. Generally, the thermal stability of the complexes was lower than the stability of the plain components taken separately, and the changes in

the thermal stability were not proportional to the protein content.

Biopolymer films were very brittle. Measurements over the experimental temperature range of −10 to 75°C at 10–20% (w/w) water content showed no calorimetric glass transitions. Presumably, electrochemical production of the complex has resulted in a more rigid material, which had insufficient chain mobility to give a marked calorimetric glass transition.

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