POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2003, Vol. 12/53, No 3, pp. 25–29

XANTHAN GUM-OVALBUMIN COMPLEXES FROM ELECTROSYNTHESIS AND COACERVATION

Cheng-yi Lii[1], Sou-chieh Liaw[1], Piotr Tomasik[2]

[1] Institute of Chemistry, Academia Sinica, Nankang, Taipei, Taiwan; [2] Department of Chemistry, Agricultural University, Kraków, Poland

Key words: coacervation, electrosynthesis, ovalbumin, polysaccharide-protein complexes, xanthan gum

Electrosynthesis as well as coacervation at pH 3.2 were employed for preparation of complexes of xanthan gum with ovalbumin. Complexes contained by ~10% of ovalbumin more than blends from which the complexes were prepared. Preparation method had a minor effect on the properties of the prepared complexes. Complexes from coacervation were slightly more thermally stable than complexes prepared electrosynthetically.

Xanthan gum and ovalbumin were combined with one another to form a complex of the stoichiometric ratio close to 1:1. Excessive amount of ovalbumin, if present in the reaction mixture, sorbed on this complex. The obtained products were weak electrostatic complexes with involvement of non-polar interactions.

INTRODUCTION

Anionic polysaccharides, among them xanthan gum with its one carboxylic group of β-D-glucuronic acid moiety, is composed of the cellulose backbone being pentasaccharide in one mer [Merck Index, 1989]. It can, at least potentially, form ionic and/or covalent complexes with proteins and such circumstance may be either helpful or disturbing in attempted procedures of food texturisation [Dickinson & Pawlowsky, 1997, 1998; Syrbe *et al.*, 1998]. Among numerous papers on complexes of anionic plant gums with proteins [Schmitt *et al.*, 1998], there are only two patents describing complexes of xanthan gum with ovalbumin [Chen & Soucie, 1986; Chen *et al.*, 1989], the protein on which attention is focused in this report. The gum-ovalbumin ratio investigated in patents varied from 1:4 to 1:10, this is, always ovalbumin was taken in essential excess. In every report on xanthan gum-protein complexes electrostatic interactions were claimed as involved in complexation.

In our former papers [Dejewska *et al.*, 1995; Zaleska *et al.*, 1999, 2000, 2001a, b], electrosynthesis of polysaccharide--protein complexes were described. In several cases covalent complexes were formed instead of electrostatic complexes usually resulting from coacervation. Thus, one could assume that specific conditions of electrosynthesis could be helpful in preparation of xanthan gum-ovalbumin covalent complexes. In this paper complexes prepared from the 2:1, 1:1, and 1:2 aqueous blends of components by electrosynthesis and coacervation are described.

MATERIALS AND METHODS

MATERIALS: Xanthan gum (reagent grade) and ovalbumin (98%) were purchased from Sigma (St. Louis, MO, USA).

METHODS

Electrosynthesis. A 400 cm^3 beaker equipped with two Pt-foil electrodes situated at the distance of 2.5 cm from one another was applied as an electrolytic cell. It was connected to a power supply (model P-3003D, Taiwan). The cell beaker was filled with 250 cm^3 of aqueous solution containing 0.125 g of xanthan gum and 0.0625, 0.125, and 0.250 g of ovalbumin, respectively. The polymer solution was completely dissolved in distilled water on mild heating (~40°C) and stirring and cooled to room temperature. The pH was adjusted to 9.0 with 0.1 M NaOH. The electrosynthesis was conducted for 2 h at a constant potential of 12 V. Initial current was 0.01 A. After this period, current intensity recorded by galvanometer included in a circuit did not change anymore. A layer of white, gelatinous product was collected from the surface of anode, centrifuged to remove liquid, and dried in vacuum at room temperature.

Coacervation. Aqueous 2:1, 1:1, and 1:2 (w/w) blends of xanthan gum and ovalbumin were brought on agitation to pH 2.3 with 0.25 M hydrochloric acid. Resulting precipitates were centrifuged (2000 rpm) and dried in vacuum at room temperature.

Combustion analysis. Combustion analysis was carried out with the Perkin – Elmer 2400 CHN Elemental Analyser (Norwalk, CT, USA). Analyses were duplicated. The results between particular estimations varied by up to 0.25% of recorded values.

Author's address for correspondence: Piotr Tomasik, Katedra Chemii, Akademia Rolnicza, al. Mickiewicza 21, 31-120 Kraków; e-mail: rrtomasi@cyf-kr.edu.pl

Solubility tests. The solubility at 25°C of starting materials and the products in water, 5% hydrochloric acid, in 5% Na_2CO_3, 0.1–1 M NaOH, dimethylsulfoxide, and 7 M aqueous urea was examined.

IR spectra. The infrared spectra of xanthan gum, ovalbumin, and their coacervates were run in KBr discs using Perkin – Elmer FTIR spectrophotometer PARAGON 1000 (Norwalk, CT, USA), in the frequency range of 4000–500 cm^{-1}. Differential spectra of xanthan gum-ovalbumin complexes and pure xanthan gum or ovalbumin were also recorded.

Thermal analysis. The thermal characteristics [thermogravimetry (TGA) and differential thermogravimetry (DTGA)] of pure xanthan gum, ovalbumin, and xanthan gum-ovalbumin complexes were determined under nitrogen using a DUPONT – TGA 951 system (Wilmington, DE, USA) apparatus scanned from 25–500°C at 10°C/min. Corrundum was used as a reference.

RESULTS AND DISCUSSION

Electrosynthesis provided complexes, which regardless initial composition of the blend, were richer in ovalbumin. Higher content of ovalbumin in the blend was beneficial for overall yield of the complex but the composition-yield relationship was not linear. The yield of the complex from the 1:1 blend was the highest (Table 1). The current applied (Table 1) automatically rose in time totally by 0.02 to 0.03 A, to stabilize after two hours signalizing the end of the process.

Both methods delivered complexes richer in ovalbumin by 11–8 % than the initial blend. Coacervation at pH of 2.3 resulted in the products richer in ovalbumin by 1–2.5% than corresponding complexes prepared by electrosyntheis. Usually, electrosynthesis provided higher yield of complexes than coacervation, however, the yield of complexes from the blend richer in xanthan gum was higher when coacervation was applied. This difference can be rationalised in terms of the rate of the complex formation. On coacervation, formation of the complex was fast whereas on electrosynthesis the rate of the complexation was governed by diffusion of depolariser and complexons to anode. Electroreaction of depolariser in the bath resulted in a local increase in pH around cathode and local decrease in pH around anode. Thus, electrosynthesis was also, in fact, coacervation.

The solubility tests for xanthan gum, ovalbumin, and products prepared either by coacervation or electrosynthesis, particularly solubility in 7 M aqueous urea, revealed that Coulombic and non-polar interactions were involved. All products were soluble in the urea solution. Thus, electrosynthesis did not provide formation of covalent bonds between those partners.

Infrared absorption spectra of so prepared complexes were identical with the spectra of complexes formed in electrolytic cell. Complexes prepared from electrolysis of different compositions differed from one another in relative intensities of the bands due to different component predominating in the complex (Figure 1).

Differential FTIR spectra of the 1:1 xanthan gum--ovalbumin coacervate (Figures 2 and 3) showed that the spectra of complexes resembled a simple combination of the spectra of the components. However, shifts of bands

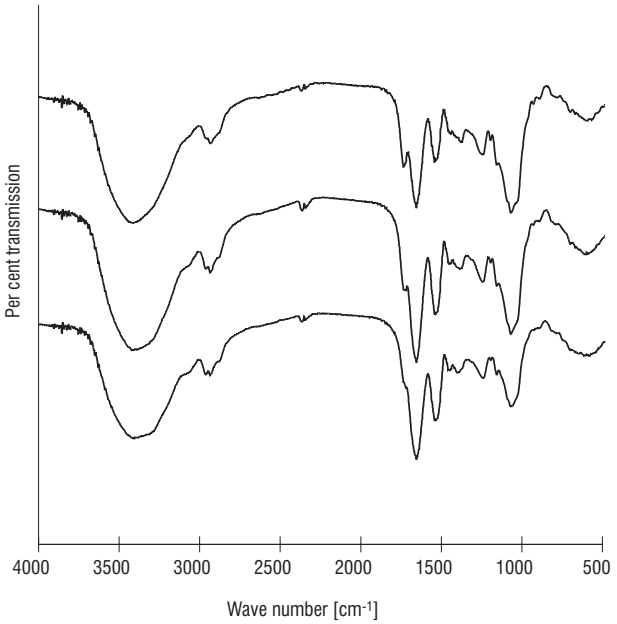

FIGURE 1. IR spectra of xanthan gum-ovalbumin complexes prepared by electrosynthesis from the blends of components of the following composition: 2:1 (upper spectrum), 1:1 (central spectrum), and 1:2 (bottom spectrum). Corresponding spectra of complexes prepared by coacervation were identical.

TABLE 1. Composition and yield of xanthan gum-ovalbumin complexes from electrosyntheis and coacervation.

Composition				Current change [A]	Yield[c] [%]
Initial electrolyte		Resulting complex[a]			
Xanthan gum: Ovalbumin	Ovalbumin [%]	Nitrogen [%]	Ovalbumin[b] [%]		
2:1	33	6.13	43.26	0.01→0.03	71.2
		6.25	*44.11*		*58.7*
1:1	50	8.29	58.50	0.01→0.04	84.4
		8.63	*60.90*		*79.0*
1:2	67	10.78	76.08	0.02→0.04	76.7
		10.62	*74.95*		*78.3*

[a] Upper values relate to complexes from electrosynthesis and lower data in italics relate to complexes from normal coacervation. [b] Calculated from nitrogen content in ovalbumin (%N = 14.17). [c] Weight calculated in respect to the total weight of both polymers with standard deviations 0.4–4.7% in the case of electrosynthesis and 0.6–4.7% in the case of simple coacervation.

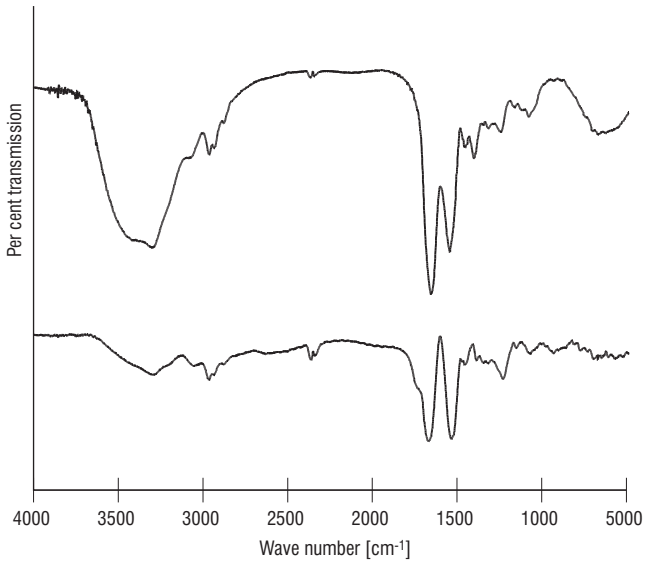

FIGURE 2. Differential IR spectrum of the xanthan gum-ovalbumin complex prepared by electrosynthesis from the 1:1 mixture of components from which the spectrum of xanthan gum was subtracted (below). The spectrum of ovalbumin (above) is quoted for comparison. The spectrum for the corresponding complex prepared by coacervation was identical.

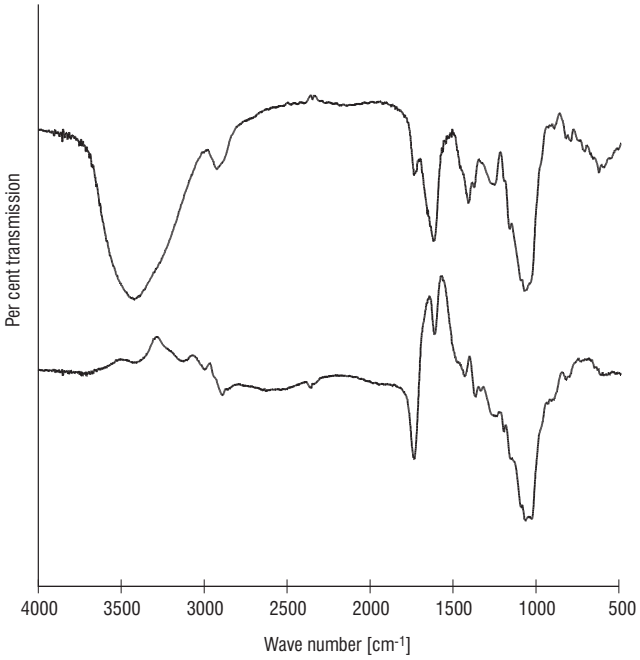

FIGURE 3. Differential spectrum of the xanthan gum-ovalbumin complex prepared by electrosynthesis from the 1:1 mixture of components from which the spectrum of ovalbumin was subtracted (below). The spectrum of xanthan gum (above) is quoted for comparison. The spectrum for analogous complex prepared by coacervation was identical.

representing vibrations of the carboxylic groups of xanthan gum and amide groups of ovalbumin (Table 2) could be observed. These shifts usually did not exceed 5 cm^{-1}. Fairly remarkable, by 9 cm^{-1}, decrease in a wave number of the carboxylic group was observed in the spectrum of the 1:1 complex. It could suggest that this component ratio might be close to the stoichiometric ratio and in both attempted 2:1 and 1:2 complexes the 1:1 complex could be enveloped by the component being in excess. Xanthan gum complexed to ovalbumin being weakly ionized as evidenced by a weak, immobile band of the stretching vibrations of the ionized

TABLE 2. Infrared spectra of xanthan gum, ovalbumin, and their complexes.

Band positions, ν [cm^{-1}] [a]					Band assignement
Xanthan gum	Ovalbumin	Complexes [b,c]			
		2:1	1:1	1:2	
3416vs		3416vs	3416vs	3416vs	ν_{OH} intramol. H-bond
	3292vs				ν_{NH} intramol. H-bond
	3072w	sh	sh	sh	ν_{OH} polymeric association
	2956w	2956w	2956vw	2956vw	ν_{CH}
2919vw		2928vw	2928vw	2928vw	ν_{CH}
1728m		1733m	1719m	sh	ν_{COOH}
	1652vs	1652vs	1652vs	1652vsw	$\nu_{C=O}$, δ_{OH}
1614s					$\nu_{C=O}$, δ_{OH}
	1543s	1538s	1538s	1533s	$\nu_{=C=N}$, δ_{NH}
	1448vw	1448vw	1448vw	1448vw	δCH
1404m					dCH
	1395w	sh	1395w	1395w	δCH
1371w		1371vw	sh	sh	ν_{COO^-}
	1314vw				?
1248m		sh	sh	sh	δ_{CH}
	1238vw	1238vw	1238vw	1238vw	δ_{NH}
	1162vw	1190vw	1190vw	1190vw	ν_{CN}?
1157m		1152m	1152m	1152m	$\nu_{CO\ (glucose\ units)}$
	1071w				δ_{COH}
1067vs		1067vs	1067vs	1067vs	$\nu_{C\text{-}O}$, $\nu_{C\text{-}C}$, δ_{COOH}, C1-H

[a] vs, s, m, w, vw denote band intensity: very strong, strong, medium, weak, and very weak, respectively. [b] The indicated proportions relate to the xanthan gum: ovalbumin ratio. [c] sh denotes a shoulder.

TABLE 3. Differential thermogravimetric (DTGA) analysis of xanthan gum, ovalbumin, and their complexes.

Temperatures of thermal effects [°C]					Effect
Xanthan gum	Ovalbumin	Complexes[a,b]			
		2:1	1:1	1:2	
51	64	53	63	44	Loss of adsorbed water
		59	*49*	*53*	
250sh	224	226	224	235sh	Melting
		224	*231*	*242sh*	
281.5	294.5	276	276	287	Decomposition
		279	*283*	*288*	
		320sh	320sh	320sh	?
		320sh	*320sh*	*320sh*	
	359				?

[a] The proportions relate to the xanthan gum:ovalbumin ratio. [b] Upper values describe the thermal effect in electrosynthesised complexes and lower values in italics are related to complexes from coacervation.

carboxylic groups. Probably, non-polar interactions played essential role in the complex formation. Stretching vibrations of the C-O bonds in glucose units also moved up by 5 cm^{-1}.

The thermal analysis gave the most straightforward evidence for the complex formation and effect of the preparation method upon the properties of complexes (Table 3, Figures 4 and 5).

Figure 4 presents thermogravimetric (TGA) curves of decomposition of xanthan gum and ovalbumin. Slow decomposition of ovalbumin began around 200°C and accelerated around 245°C. At this temperature began a vigorous decomposition of xanthan gum. Xanthan gum held more water than ovalbumin, 15 and 8%, respectively. Simultaneously, ovalbumin held water more strongly than xanthan gum. The peaks of the evolution of absorbed water appeared in thermograms at 64 and 51°C, respectively.

Products obtained electrosynthetically as well as by coacervation showed practically identical thermogravimetric (TGA) curves. Regardless their composition their relatively fast decomposition began at 200°C and proceeded slightly more readily for complexes with excess of xanthan gum, whereas the complexes with excess of ovalbumin decomposed more slowly (Figure 5).

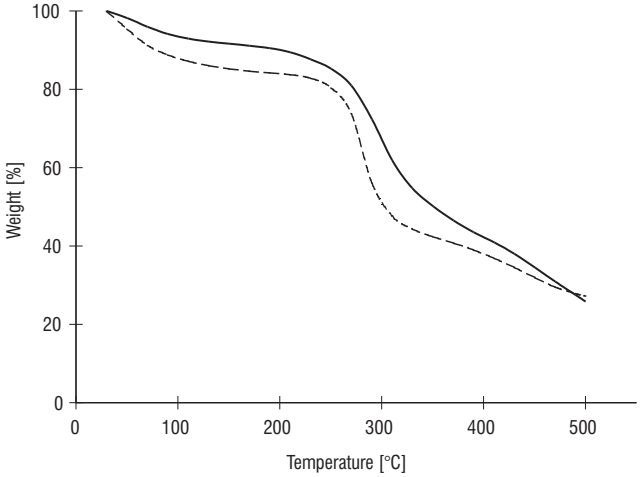

FIGURE 4. Thermogravimetric (TGA) curves for ovalbumin (solid line) and xanthan gum (broken line).

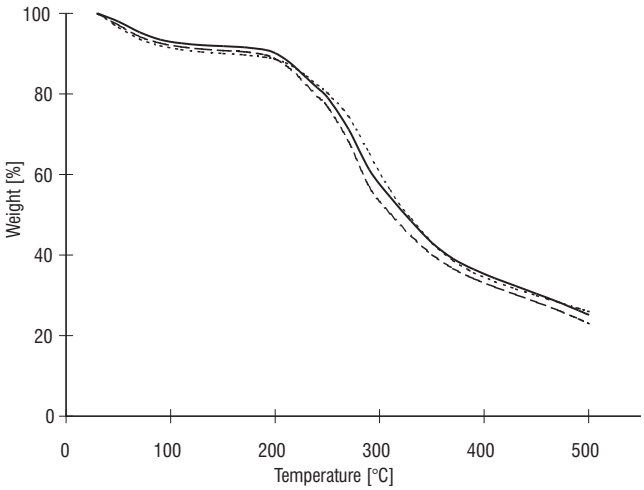

FIGURE 5. Comparison of the course of thermal decomposition of the xanthan gum-ovalbumin complexes presented by means of thermogravimetric curves. Complexes were prepared by coacervation from the blends of the composition of 2:1 (broken line), 1:1 (solid line), and 1:2 (pointed line).

The thermal stability of complexes decreased. It was lower than that of components alone, however, it increased with higher proportion of more thermally stable ovalbumin in the complex. The thermal stability of complexes from coacervation was slightly higher than that of complexes prepared electrosynthetically. The main peak of decomposition of ovalbumin was followed by another small peak with maximum at 350°C. In thermograms of every complex, the main decomposition peak was followed by a shoulder, intensity of which depended on the level of ovalbumin in the complex. These shoulders point to the existence of another, more thermally stable fractions of complexes.

CONCLUSIONS

Electrosynthesis and coacervation appeared to be equivalent to one another providing identical products. The benefit from the use of electrosynthesis comes from higher yields of resulting complexes; on the other hand coacervtion was faster and technically more facile. The presented study showed that covalent complexes of xanthan gum and ovalbumin could not be prepared.

REFERENCES

1. Chen W.-S., Henry G.A., Gaud S.M., Miller M.S., Kaiser J.M., Balmadecca E.A., Morgan R.G., Baer C.C., Borwankar R.P., Hellgeth L.C., Strandholm J.J., Hassenheuttl G.L., Kerwin P.J., Chen C.C., Kratochvil J.F., Lloyd W.L., Fat substitutes from microfragmented ionic polysaccharide – protein complexes. Eur. Patent Appl., 0,340,035 (1989).
2. Chen W.-S., Soucie W.G., Edible fibrous serum milk – xanthan gum complexes. US Patent Appl., 4,563,360 (1986).
3. Dejewska A., Mazurkiewicz, J., Tomasik P., Zaleska H., Electrochemical synthesis of polysaccharide – protein complexes. Part I. Preliminary studies on apple pectin – albumin complexes. Starch/Staerke, 1995, 47, 219–223.
4. Dickinson E., Pawlowsky K., Effect of ι-carrageenan on flocculation, creaming, and rheology of protein-stabilized emulsions. J. Agric. Food Chem., 1997, 45, 3799–3806.
5. Dickinson E., Pawlowsky K., Influence of κ-carrageenan on the properties of protein – stabilized emulsions. Food Hydrocoll., 1998, 12, 417–423.
6. The Merck Index, 1989, Xith Edition (ed. Budavari S.), Merck & Co., Inc., Rahway, N.Y., p. 1586.
7. Schmitt C., Sanchez C., Desobry-Banon S., Hardy J., Structure and technofunctional properties of protein – polysaccharide complexes. A review. Crit. Rev. Food Sci. Nutr., 1998, 38, 689–733.
8. Syrbe A., Bauer W.J., Klostermeyer H., Polymer science concept in dairy systems. An overview of milk protein and food hydrocolloid interaction. Int. Dairy J., 1998, 8, 179–193.
9. Zaleska H., Mazurkiewicz J., Tomasik P., Bączkowicz M., Electrosynthesis of apple pectin – casein complexes. Nahrung, 1999, 43, 278–283.
10. Zaleska H., Ring S., Tomasik P., Apple pectin complexes with whey protein isolate. Food Hydrocoll., 2000, 14, 377–382.
11. Zaleska H., Ring S., Tomasik P., Electrosynthesis of potato starch – whey protein isolate complexes. Carbohydr. Polym., 2001a, 45, 89–94.
12. Zaleska H., Ring S., Tomasik P., Complexes of potato starch with casein. Int. J. Food Chem. Technol., 2001b, 36, 509–513.

Received April 2002. Revision received May 2002 and accepted February 2003.

KOMPLEKSY GUMY KSANTANOWEJ Z OWOALBUMINĄ OTRZYMANE ELEKTROSYNTETYCZNIE I PRZEZ KOACERWACJĘ

Cheng-yi Lii[1], Sou-chieh Liaw[1], Piotr Tomasik[2]

[1] Institute of Chemistry, Academia Sinica, Nankang, Taipei, Taiwan; [2] Katedra Chemii, Akademia Rolnicza, Kraków

Otrzymano kompleksy gumy ksantanowej z owoalbuminą za pomocą elektrosyntezy z alkalicznych roztworów wodnych obu składników pobranych w proporcjach 2:1, 1:1 i 1:2 oraz przez koacerwację przy pH 3,2. Kompleksy otrzymane obiema metodami były niemal identyczne z tym, że wydajność kompleksów w przypadku elektrosyntezy była o ok. 3% wyższa a kompleksy otrzymano przez koacerwację były nieco bardziej trwałe w czasie ich termolizy. Kompleksy zawierały o około 10% albuminy więcej niż wyjściowe mieszaniny składników, z których otrzymywano te kompleksy. Prawdopodobnie właściwy kompleks ma stechiometrię zbliżoną do 1:1. Produkty otrzymane z mieszanin zawierające nadmiar jednego ze składników składają się z właściwego kompleksu 1:1, na którym zaadsorbował się ten składnik, który w nadmiarze znajdował się w mieszaninie reakcyjnej. Kompleksy są słabe i mają naturę elektrostatyczną z udziałem oddziaływań niepolarnych.