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Carboxymethyl cellulose–gelatin complexes

Cheng-yi Lii[a,*], Piotr Tomasik[b], Hanna Zaleska[b], Sou-chieh Liaw[a], Vivian M.-F. Lai[c]

[a]*Institute of Chemistry, Academia Sinica, Nankang, Taipei 11529, Taiwan, ROC*
[b]*Department of Chemistry, University of Agriculture, Mickiewicz Avenue 21, 31120 Cracow, Poland*
[c]*Department of Food and Nutrition, Providence University, Shalu, Taichung 43301, Taiwan, ROC*

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Abstract

Carboxymethyl cellulose (CMC) complexes with gelatin (G) were prepared electrochemically (12 V, on chromium–nickel stainless steel electrodes) from aqueous blends of initial CMC/G ratio = 1:0.5, 1:1 and 1:2, at pH 9, 10 and 11. The resulting complexes always contained lesser G than in the electrolytic solution. The 1:1 complexes resulted from the CMC/G = 1:2 solution. The complexes were slightly more thermally stable than plain CMC and G but their glassy transition took place at a slightly lower temperature. Solubility tests, thermogravimetric, differential thermal analysis, and FTIR spectroscopy suggested that apart from hydrogen bonds and dispersion forces also strong interactions between carboxyl groups of CMC and peptide moieties of G were involved in the formation of CMC–G complexes.

Keywords: Electrosynthesis; Polysaccharide–protein complexes

1. Introduction

Anionic polysaccharides and proteins are capable of the complex formation with involvement of electrostatic interactions, provided both components are ionised at the same time (Booty & Bungenberg de Jong, 1956; Hocking, 1992; Parker, Boulanguer, & Kravchenko, 1992; Roeper & Koch, 1990; Tolstoguzov, 1986, 1997; Tomasik & Schilling, 1998). The stoichiometry of such complexes, a factor seriously influencing their functional properties, is difficult to control even if the number of potential reaction sites in complexing partners could be estimated. Because of variable, reaction condition-dependent conformations of both components, several potential reaction sites may be unavailable for bonding. However, when the molecular weight of the original polymers increases, entropy becomes less important (Struminskii & Slominskii, 1956. Resulting complexes may be, in fact, a physical, non-stoichiometric mixture of the polymers.

Commonly, polysaccharide–protein complexes can be prepared by free and forced coacervation (Samant, Singhal, Kulkarni, & Regge, 1993; Tolstoguzov, 1986, 1997; Tomasik & Schilling, 1998). Among them only free coacervation provides non-forcing conditions for the formation of stoichiometric products, but even in this case, due to inclusion and sorption on the surface of separating complexes, products might be contaminated with excessive amounts of parent components.

Recently, an electrochemical method has been used for preparation of several polysaccharide–protein complexes, including pectin–egg albumin (Dejewska, Mazurkiewicz, Tomasik, & Zaleska, 1995), pectin–casein (Zaleska, Mazurkiewicz, Tomasik, & Baczlkowicz, 1999), pectin–whey protein isolate (Zaleska, Ring, & Tomasik, 2001a), potato starch–whey protein isolate (Zaleska, Ring, & Tomasik, 2000), and potato starch–casein (Zaleska, Ring, & Tomasik, 2001b), complexes, protein containing colloids (Barisci, Hodgson, Liu, Wallace, & Harpe, 1999), heparin containing composites (Zhou, Too, & Wallace, 1999), and glycitolated proteins (Cayot, Roullier, & Tainturier, 1999). Electrosynthesis appeared to be a simple, reproducible method for the preparation of stoichiometric polysaccharide–protein complexes, which separated on the anode in a natural, non-forced, and autocontrolled manner. Physical properties of the resultant, novel complexes were essentially different from those of their parent biopolymers. For example, the solubility of these complexes in water was generally much lower than those of the parent polysaccharide or protein. Thus, for example, such complexes could be potentially considered as edible films and coatings (Donhowe & Fennema, 1994), emulsion stabilisers (Dickinson, 1995) and biodegradable, environmentally benign construction

* Corresponding author.
E-mail address: cylii@chem.sinica.edu.tw (C.-y. Lii).

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Table 1
Yields and proximate compositions of CMC–G complexes

Initial blends			Current change, (A)	Complexes formed		
CMC/G ratio	pH	G (%)		Yield (g) (%)[a]	Nitrogen (%)	G (%)[b]
1:0.5	9	33	0.05 → 0.11	0.234 (31.2)	6.36	39.3
	10	33	0.06 → 0.13	0.230 (30.7)	5.96	36.8
	11	33	0.07 → 0.12	0.297 (39.6)	4.59	28.3
1:1	9	50	0.05 → 0.06	0.469 (46.9)	7.60	46.9
	10	50	0.06 → 0.08	0.449 (44.9)	6.72	41.5
	11	50	0.07 → 0.09	0.424 (42.4)	6.60	40.7
1:2	9	67	0.06 → 0.04	0.620 (41.4)	8.23	50.8
	10	67	0.06 → 0.04	0.598 (39.9)	8.77	54.1
	11	67	0.07 → 0.05	0.541 (36.1)	8.18	50.5
G plain		100			16.20	

[a] Weight percentage of yields were calculated on the basis of total parent biopolymer weight with standard deviations of 0.1–2.5%.
[b] Data were calculated from the corresponding nitrogen contents by comparing with that of G.

materials, for instance, biodegradable plastics (Hocking, 1992).

Recently, carboxymethyl cellulose (CMC) evoked considerable interest as a texturing additive for foodstuffs (Ganz, 1974; Samant et al., 1993). Interactions of CMC with proteins in solution have been thoroughly studied (Delben & Stefancich, 1997, 1998; Stefancich & Delben, 1997). Much less is known about physical properties of these complexes and possibilities of their applications. Because gelatin (G) is well known as a gel-making additive to food (Johnston-Banks, 1990; Poppe, 1997) it was decided to prepare complexes of CMC with G. Recently developed (Dejewska et al., 1995; Zaleska et al., 1999; Zaleska et al., 2000, 2001a,b) electrosynthesis was applied for this purpose. In this report novel complexes from CMC and G are described. Results proved that electrosynthesis is a suitable method for the preparation of a wide range of polysaccharide–protein complexes.

2. Materials and methods

2.1. Materials

CMC for chromatography, degree of carboxymethylation of 1.0 ± 0.15, particle size 45–180 μm over 40% as well as bovine bone G of the B-type (isoelectric point 6.08) with 12% of moisture, viscosity of 46 ± 3 MPa, residue after ignition (as sulphate) of 2% were purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan).

2.2. Methods

Electrosynthesis. An electrolytic cell was constructed by using a manually regulated dc power supply (model P-3003D, Taiwan) and a 200 cm^3 beaker equipped with two chromium–nickel H18N9 stainless steel 16.5 cm^2 electrodes positioned at a distance of 2.5 cm from one another. The beaker was filled with an aqueous solution (100 ml) containing 0.50 g of CMC and either 0.25, 0.75 or 1.00 g of G. These proportions corresponded to the CMC/G ratio of 1:0.5, 1:1 and 1:2 (w/w) and total polymer concentration of 0.75, 1.0 and 1.5% w/v, respectively. The polymer solution was completely dissolved in distilled water with mild heating (~35 °C) and stirring and then cooled to room temperature. Subsequently, the reaction mixture was brought to pH 9.0, 10.0 and 11.0 with 0.01–0.1 M aqueous NaOH. The electrosynthesis was conducted at room temperature and 12 V with an initial current intensity of 0.05–0.1 A. Current intensity depended on the initial pH of the solution and decreased with time. Commonly, the change in current intensity was negligible during the first 60 min. A layer of white, gelatinous complex was collected from the surface of anode after 2 h. This reaction period was considered as sufficient to carry the reaction to its end because change in the current intensity ceased. Such a period was also sufficient for electrosyntheses of apple pectin–albumin complexes (Dejewska et al., 1995). Complexes collected from the anode were rinsed with water and dried under vacuum at room temperature. All complexes were produced in triplicate.

Elemental analysis. Elemental analysis for nitrogen was carried out by the by semi-micro Dumas method (Bobranski, 1956). Measurements were duplicated with estimated standard 0.5% error.

Solubility tests. Qualitative solubility tests were made in 5% aq. Na_2CO_3 and 5% hydrochloric acid (both tests up to 100 °C), and in dimethyl sulfoxide, 7 M aqueous urea and 2 M aqueous guanidinium thiocyanate at room temperature.

Infrared spectroscopy. Infrared spectra of the complexes were recorded with a Mattson FTIR 3000 spectrophotometer (Mattson Instruments, Inc., Madison, Wisconsin, USA). Discs were made of CMC, G or complex (~3 mg) thoroughly grounded with KBr (800 mg). Transmission was measured in the range of 4000–650 cm^{-1}.

Table 2
Results of TG analysis of CMC and G

Sample	Thermal behaviour during TG analysis		
	T (°C)	WL (%)[a]	T_p (°C)[b]
CMC	20–160	12.8	101
	160–240	1.9	
	240–315	39.0	286
	315–500	9.2	
		Total: 62.9	
G	20–160	11.5	95
	160–240	3.7	
	240–310	22.7	291 and 312
	310–352	20.2	325
		Total: 79.8	

[a] Percentages of weight loss during the specified temperature ranges.
[b] Peak temperatures on differential thermogravimetric (DTG) plots.

Thermogravimetric analysis (TG and DTG). Thermogravimetric measurements were carried out using a Paulik − Paulik − Erdey 1500Q instrument (Budapest, Hungary). 200–220 mg samples were heated from 20 to 500 °C in the air in a ceramic crucible. The heating rate was 5 °C/min. Corundum of 8 μm in diameter was used as the standard.

Atomic force microscopy (AFM). Microscopic investigation utilised an atomic force microscope (The Park Scientific Instrument, CA, USA) with LS AutoProbe cantilevers. The prepared complex was redissolved in an aqueous solution of NaOH (pH 11.6) at room temperature and at a complex concentration of 20 μg/ml. A portion of the complex solution (~4 μl) was placed on a slide glass, followed by dehydration in air for 1 h. The AFM image of the dried complex films was then recorded under a constant force in the range of 7.1–7.8 nN.

3. Results and discussion

Complexes separating on electrosynthesis were gelatinous but they could be dried to solid, amorphous powders. They dissolved neither in 5% aq. Na_2CO_3 nor in 5% HCl at up to 100 °C. Insolubility in dimethyl sulphoxide, 7 M aqueous urea, as well as 2 M aqueous guanidinium thiocyanate at room temperature suggested that genuine complexes with strong bonds between components were formed.

The resulting complexes always contained less G than intended from the composition of the initial reaction mixture (Table 1). The 1:1 complex resulted from the solution of the composition CMC/G = 1:2. The initial composition of the electrolyte had an effect on the changes in the current as well as the yield and composition of the resulting complexes. Thus, using electrolytes with CMC/G = 2:1 and 1:1 ratios current intensity increased in time, possibly due to a decrease in the viscosity of the solution. Using the 1:2 composition ratio the current intensity gradually decreased with time (Table 1). In the latter case it could be due to a complex formation-induced decrease in the concentration of the charge carriers as well as due to obstruction in the access of the charge carriers to the electrodes. Indeed, the yield of the complex deposited around the anode was the highest and its gelatinous layer was the most compact. However, the viscosity of CMC solutions changes at this pH (Stefancich & Delben, 1997). Table 1 also shows that the yield of complexes achieved its maximum (42.5–47%) when the electrolyte had a 1:1 composition. However, the 1:1 CMC/G complex resulted from the electrolyte had 1:2 composition. Lower pH usually favoured enrichment of the complexes in G. The pH was of minor importance for the yield of complex products. Generally, the small effect of pH on the complex formation resulted from the fact that local pH at electrode and in a double electrical layer, which might decide the complex formation, was automatically adjusted by transport and discharge of the components at the electrodes.

Table 2 shows the course of thermal decomposition of CMC and G in the air.

CMC and G contained almost 13 and 11.5 wt% of water that was more strongly held by CMC as shown by the temperature of the water loss. Dry CMC decomposed in one step with the loss of 39% of its weight within the range of 240–315 °C with a differential thermogravimetry (DTG) peak minimum at 286 °C. G decomposed in three steps (DTG minima at 291, 312 and 325 °C, respectively) loosing in this range totally almost 23% of its weight. These data are supplemented by thermograms reproduced in Fig. 1.

Table 3 collects details of the thermogravimetric analysis (TG and DTG) of complexes. One might see that after loosing water these complexes decomposed at a temperature lower by at least 17 °C than did their components. Also, the higher the content of G in the complexes, the lower was the weight loss caused by decomposition. Thus, the observed decrease in the temperature of the thermal effect might suggest that the point of the phase transition of the complex was shifted to a lower temperature but the complexation of G to CMC resulted in the thermal stabilisation of CMC and G.

Inspection of Fig. 1 shows that complexes decomposed more monotonously than their plain components (TG-curve). DTG peaks of complexes showed additional thermal effects demonstrated by shoulders on their high-temperature sides. Analysis of the TG patterns might suggest that the shoulders reflected the presence of a small amount of CMC, which was adsorbed on the CMC/G complex. However, also a dual mode of interaction of CMC with G, e.g. with involvement of strong chemical interactions and only with hydrogen bonds and dispersion forces might be taken into account. In no case could these shoulders be related to excessive or non-bound G because related peaks of G were absent in the thermograms of complexes. The differential thermal analysis (DTA) proved the fully amorphous

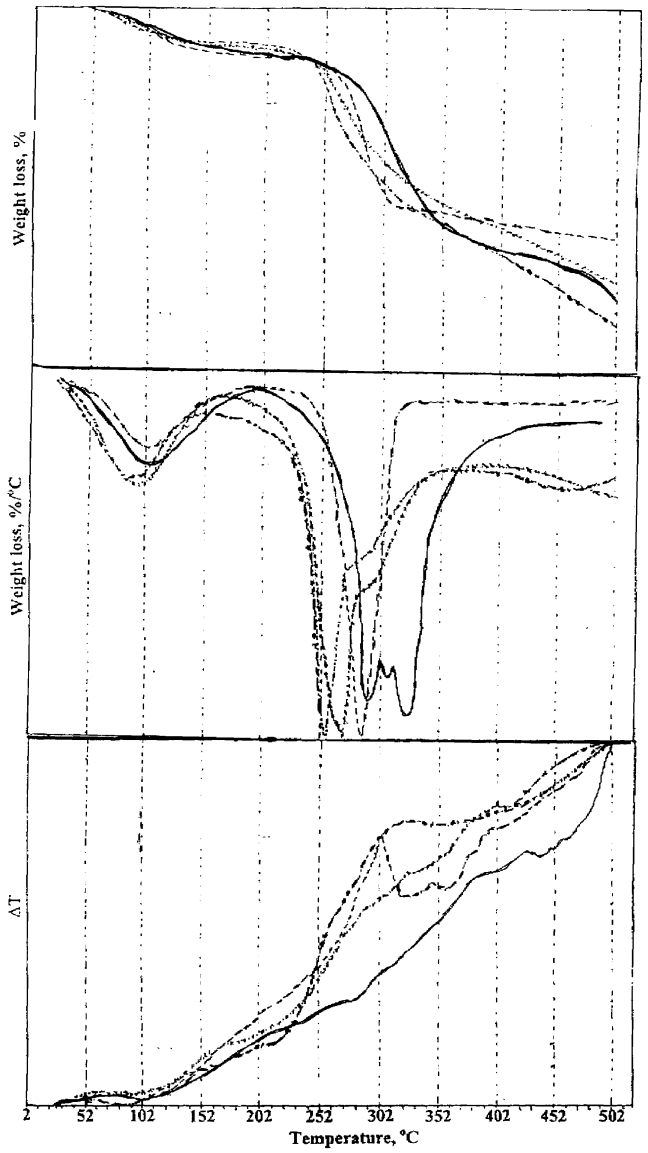

Fig. 1. Thermograms: TG (upper), DTG (center), and DTA (bottom) curves for CMC (dashed lines), G (solid lines), and 1:1 CMC–G complexes prepared at pH 9 (dotted lines) and 10 (dashed-pointed lines).

character of the complexes. No peaks could be observed in the DTA curves.

The hypothesis on the manner of complexation of CMC with G made is supported by the analysis of IR spectral data. Table 4 reports spectral band positions and their assignments.

Typically, for the plain CMC a very strong absorbance band at 3439 cm^{-1} was assigned to the vibrations of the intra-molecular hydrogen bond (ν_{OH}). The shoulder at 2920 cm^{-1} and weak peak at 1377 cm^{-1} were assigned to stretching and bending vibrations of the C–H bonds (ν_{CH} and δ_{CH}, respectively). Subsequently, the strong peak at 1605 cm^{-1} and very weak peak at 1267 cm^{-1} belonged to stretching vibrations in the carboxylic group (ν_{COOH}) and bending vibrations in the hydroxyl group (δ_{OH}), respectively. One of two strong peaks at 1072 and 1020 cm^{-1} reflected bending vibrations of the ether (glycosidic) linkage (δ_{C-O-C}). In the spectrum of G a very strong band at 3459 cm^{-1} indicated the presence of the intra-molecular hydrogen bond of the stretching vibrations of the N–H bond (ν_{NH}); the shoulder at 2967 cm^{-1} and weak peak at 1454 cm^{-1} belonged to ν_{CH} and δ_{CH}, respectively. The strong peak at 1645 cm^{-1} was assigned to the stretching vibrations of the carbonyl group ($\nu_{C=O}$) of amide. The medium peak at 1548 cm^{-1} belonged to the bending modes if the N–H bond (δ_{N-H}); and very weak peaks at 1244 and 1082 cm^{-1} were assigned to the bending modes in the C–N bond (δ_{C-N}). Table 5 lists IR spectral bands of complexes. Most of the IR characteristics of complexes were qualitatively and quantitatively different from those for plain CMC and G.

One might see from the comparison of the data in Tables 4 and 5 that stretching vibrations of the O–H and N=H bonds (ν_{OH} and ν_{NH}, respectively) in the spectra of complexes moved to lower wave numbers. Thus, it was likely that the energy of intra-molecular interactions involving these groups decreased because formation of the complexes required conformational changes of CMC and G to provide complexation. These changes were also reflected by shifts of ν_{CH} by +8 to +10 cm^{-1}. A weak band at ~1726 cm^{-1} assigned to ν_{CO} observed in the spectra of complexes could not be recognised in the spectra of CMC and G. This peak might be considered as an evidence for a random involvement of the carbonyl group of CMC in the formation of a chemical bond with G. Such interactions utilised the ionised carboxylate moiety, COO^-, and related peak of asymmetric stretching vibrations in ionised carboxylic group (ν_{COO^-},asym) indeed vanished in the spectra of complexes. The bands of the stretching vibrations of the amide carbonyl group ($\nu_{CO-amide}$) in the spectrum of G were shifted by ~15 cm^{-1} to the higher wave number suggesting that this group was also involved in the complexation with CMC. The bands at 1547–1549 cm^{-1} related to δ_{NH} in G and the bands at 1452 cm^{-1} related to δ_{CH} in G retained their positions in the spectra of complexes. Also peaks of δ_{CH} in CMC and other very weak peaks in the region down up to 1200 cm^{-1} behaved similarly. However, the pattern of the spectra of complexes in the region between 1200 and 1030 cm^{-1} was entirely different from the pattern of the spectra of CMC and G. It could be clearly seen in Figs. 2 and 3, which are differential spectra of complexes, CMC and G.

Thus, again, in spite of suggestions that such components might form coacervates and/or micelar systems (Delben & Stefanchich, 1997, 1998; Samant et al., 1993; Stefancich & Delben, 1997) stronger chemical bonds might be involved when the reaction was forced by a potential gradient. Such phenomena were observed in earlier electrosyntheses of polysaccharide–protein complexes (Dejewska et al., 1995; Zaleska et al., 1999; Zaleska et al., 2000, 2001a,b). It is likely that

Table 3
Results of TG analysis of CMC–G complexes prepared at different pH values

Initial CMC/G ratio	pH 9			pH 10			pH 11		
	T (°C)	WL (%)[a]	T_p (°C)[b]	T (°C)	WL (%)	T_p (°C)	T (°C)	WL (%)	T_p (°C)
1:0.5	20–160	10.7	100	20–160	9.6	110	20–160	9.6	98
	160–230	3.2		160–225	5.4		160–230	2.8	
	230–320	39.1	270	225–310	42.5	272	230–310	35.9	270
	320–500	32.1		310–350	6.6		310–350	9.1	
		(85.1)[c]		350–500	14.8		350–500	24.9	
					(79.0)			(82.2)	
1:1	20–160	11.4	97	20–160	9.3	88	20–160	9.4	96
	160–225	2.8		160–230	3.6		160–230	3.2	
	225–310	37.4	262	230–310	36.9	256	230–310	33.9	260
	310–350	7.6		310–350	8.6		310–350	8.0	
	350–500	26.1		350–500	27.6		350–500	24.7	
		(85.3)			(86.0)			(79.1)	
1:2	20–160	7.1	98	20–160	10.7	102	20–160	10.3	92
	160–230	2.2		160–230	3.0		160–230	2.9	
	230–310	24.3	269	230–310	30.5	270	230–310	30.6	270
	310–350	6.6		310–350	8.4		310–350	8.5	
	350–500	18.6		350–500	22.9		350–500	23.8	
		(58.8)			(75.5)			(76.0)	

[a] Percentages of weight loss during the specified temperature ranges.
[b] Peak temperatures on DTG plots.
[c] Data in parenthesis were the overall weight loss (WL) values at up to 500 °C.

at the electrode at certain local pH a hydrolysis of proteins could occur.

Fig. 4a showed the AFM image of the G film. It was a coarse conglomerate of fibrils. Conglomerates formed strips of 2–3 μm width. The image of CMC film (Fig. 4b) showed strips of spruce branches distinctly different from the image of G. The strips had a 24 μm width and mangled ends. The 1:1 complexes prepared at pH 9 and 10 (Fig. 4c and d) seen under AFM were more like one another but they were different from images of CMC and G, although they resembled the image of G rather than CMC. Strips with mangled ends could be seen but they were not as sharp as these of G. Widths of the strips of complexes reached at least 5 μm. Strips showed a subtle structure built of approximately 0.5 μm thick, long fibrils. Nevertheless, the surface of the strips seemed to be free of coarse material seen on the surface of G.

In our previous studies on electrosyntheses of complexes

Table 4
IR-spectra characteristics of CMC and G samples (the band assignments after Pavia, Lampman and Kriz (1996) and Zundel, Boehner, Fritsch, Mertz and Vogt (1984))

CMC		G	
Band position[a] (cm^{-1})	Band assignment	Band position[a] (cm^{-1})	Band assignment
3439 vs	ν_{OH} intra-molecular H-bond	3459 vs	ν_{NH} intra-molecular H-bond
2920 sh	ν_{CH}	2967 sh	ν_{CH}
1605 s	ν_{COO^-}, asym	1645 s	$\nu_{C=O\ amide}$
1420 m	δ_{CH2}, CH_2 scission	1548 m	δ_{NH}
1377 vw	δ_{CH}, ν_{COO^-}, sym	1454 w	δ_{CH}; CH_2 scission
1327 m	ν_{C-O}	1402 w	ν_{COO^-}, sym
1267 vw	δ_{OH}	1337 vw	ν_{C-O}
1208 vw		1244 vw	δ_{NH}
1072 s	δ_{C-O-C}	1204 vw	δ_{OH}
1020 s		1165 vw	
901 m		1082 vw	δ_{C-N}
665 m		1032 vw	
607 m		669 m	
575 m		559 m	

[a] Band intensity: vs—very strong; s—strong; m—medium; w—weak; vw—very weak; sh—shoulder.

Table 5
IR spectral characteristics of various CMC–G complexes (for the complexes prepared at pH 10 and 11, only the bands at different positions by more than 4 cm^{-1} from those in the spectrum of the complex at pH 9 are quoted)

Initial bath composition								
$CMC_i/G_i = 1:0.5$			$CMC_i/G_i = 1:1$			$CMC_i/G_i = 1:2$		
pH 9	pH 10	pH 11	pH9	pH10	pH11	PH9	pH10	pH 11
3408 vs[a]			3390 vs	3410 vs	3410 vs	3410 vs	3397 vs	
2928 sh			2930 sh			2934 sh		
1728 vw			1726 vw					
1659 s			1659 s			1657 vs		
1549 m			1549 m			1547 m		
1452 vw			1451 w			1452 w		
1373 vw			1375 vw			1377 vw		
1337 vw			1337 vw			1337 w		
1240 w			1242 w			1242 w		
1204 vw			1206 vw			1204 vw		
1154 vw			1155 vw			1155 vw		
1111 vw			1113 vw					
1065 vs	1069 vs		1065 vs			1065 s		
1026 vw		1032 vw	1026 vw			1028 vw	No band	
897 vw			897 vw			897v w		
662 m			662 m	657 m		660 m		

[a] Wavenumbers (cm^{-1}) and intensities of IR absorbance bands. Notations for band intensities are the same as in Table 4.

of pectin and starch with albumin, casein and whey protein isolate (Dejewska et al., 1995; Zaleska et al., 1999; Zaleska et al., 2000, 2001a,b), the complexes precipitated on the surface of anode and they dumped current completely within 4 min intervals. In this study the current was not dumped during the complex formation although complexes also separated on the anode and enveloped it. Evidently, electrolyte was included in the matrix of gelatinous complex being responsible for its conductivity. For this reason CMC–G complexes in a gelatinous form can potentially be considered as an electrode gels for electrocardiograms and pastes for ultrasonographic examinations. We anticipate, that the electrosynthesis would be suitable for preparation of microcapsules.

4. Conclusions

Electrosynthesis is a suitable method for synthesis of

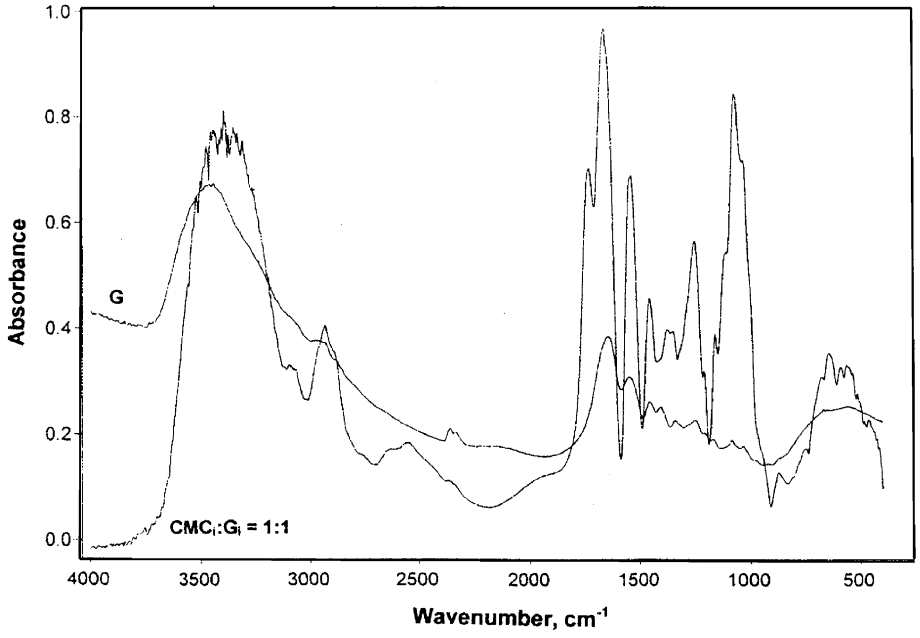

Fig. 2. Differential IR spectrum of the CMC–G = 1:1 complex from which the spectrum of CMC was subtracted. Spectrum of G is given for comparison.

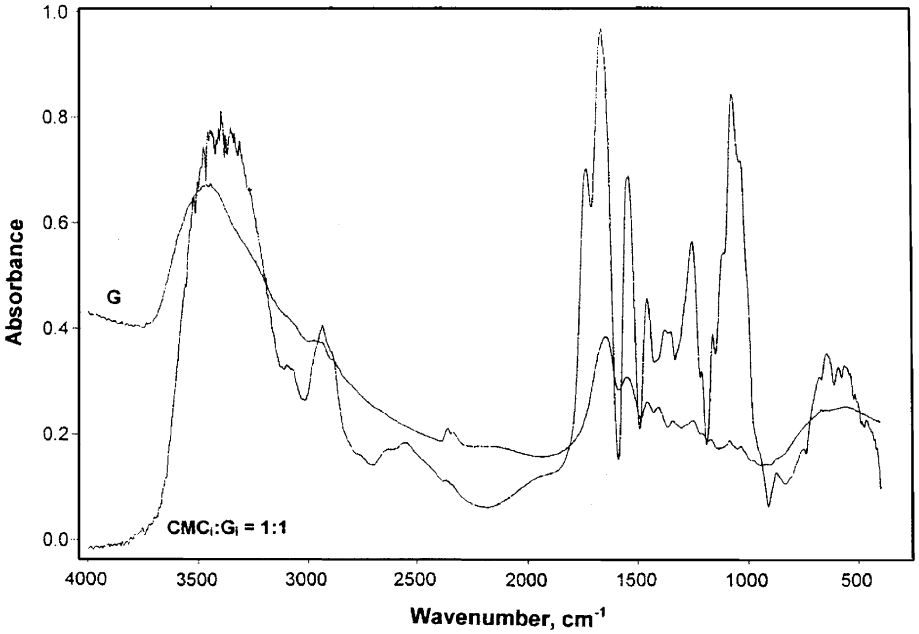

Fig. 3. Differential IR spectrum of the CMC–G = 1:1 complex from which the spectrum of G was subtracted. Spectrum of CMC is given for comparison.

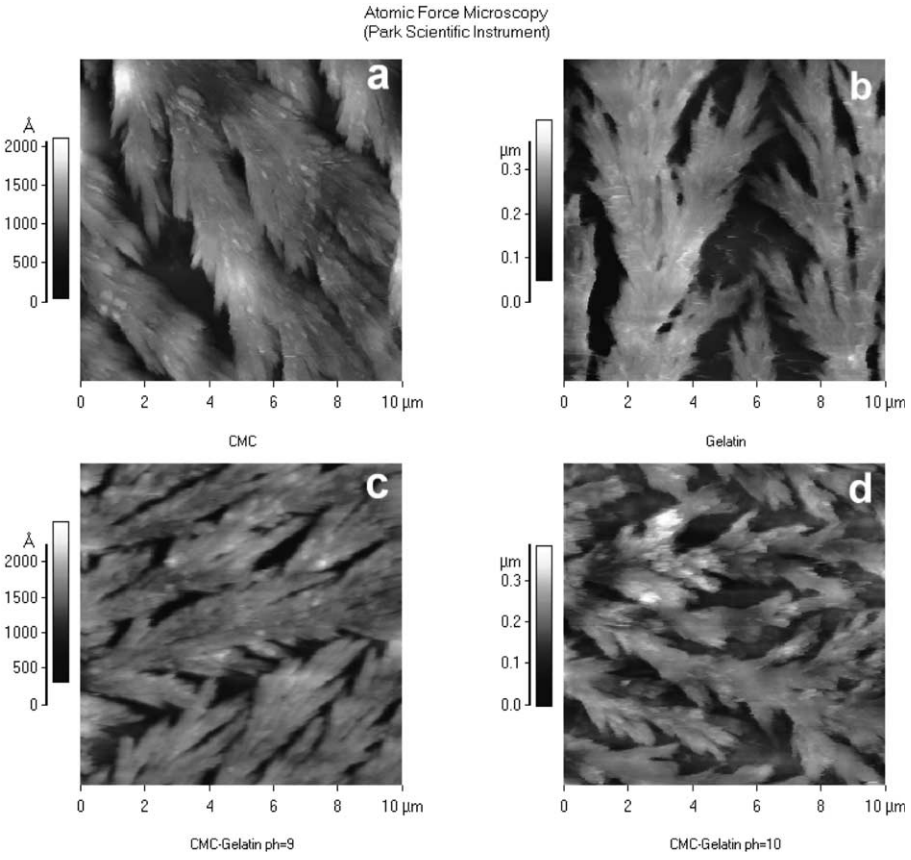

Fig. 4. AFM image of G (a), CMC (b), and CMC–G complexes prepared at pH 9 (c) and 10 (d).

G–CMC complexes. In the complex both components are bound with covalent bond.

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