ANTIOXIDATIVE AND SOLUTION PROPERTIES OF NEW CINNAMATE ESTERS OF CARBOXYMETHYLCELLULOSE

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Abstract
New cinnamate esters of O - carboxymethylcellulose (CMC) with low degree of substitution (DS) were prepared by esterification of cinnamic acid. The chemical modification was performed in different reaction media N,N-dimethylformamide (DMF)/4-toluenesulfonic acid (4-TSA) and H_2O/DMF with 4-dimethylaminopyridine (DMAP) as catalyst and N, N-dicyclohexylcarbodiimide (DCCI) as activator of cinnamic acid (CA), at various reaction conditions. The obtained derivatives were characterized by FT-IR and UV spectroscopy. Surface-active properties and antioxidative activity of the cinnamate esters of CMC was evaluated. In spite of the low surface tension-lowering effect, prepared derivatives exhibited excellent emulsifying efficiency. The cinnamate CMC derivatives showed low antioxidant activity.

Keywords: carboxymethylcellulose, cinnamic acid, surface-active properties, antioxidative properties.

1 Introduction

Recently years, increased attention to ecological problems, especially in the field of polymeric materials was observed. Increasingly we aware of the need to replace the now used synthetic polymers by natural, biodegradable surfactants from renewable materials. Considerable attention has been devoted to polysaccharide-based surfactants, since they shown potential use in environmental protection, pharmaceutical, food, textile and other industries. Recently considerable number of studies report on the polymeric surfactants prepared by partial hydrophobization of commercial and non-commercial polysaccharides such as hydroxyethyl cellulose [1, 2] pullulan [3], carboxymethylstarch [4] and beechwood xylan [5]. As a further commercially available anionic cellulose derivative, O - carboxymethylcellulose (CMC) was used for targeted hydrophobic modification in order to prepare the ionic polymeric surfactants. The aim of this research work was esterification of CMC with cinnamic acid, which can lead to preparation of new biopolymers with interesting surface-active and antioxidant properties. Unsubstituted cinnamic acid is a good inhibitor of making excellent antibacterial, antimicrobial, and anticancer effects [6]. Cinnamic acid has not industrial use [7]. But their derivates showed antioxidant properties which are important for the food and pharmaceutical industry. In this contribution preparation of the cinnamate esters of CMC will be presented. Their surface-active and antioxidative properties have been tested.

2 Experimental part

2.1 Materials

CMC, Lovoza T20 (Na+ salt, DS_{CM}=0.5) was from Lovochemie (Lovosice, Czech Republic). Cinnamic acid (CA) was a commercial product from Merck Chemicals Ltd. (Bratislava, Slovak Republic), N,N'-dicyclohexylcarbodiimide (DCCI), 4-dimethylaminopyridine (DMAP), 4-toluenesulfonic acid (4-TSA) and Tween 20 were from Aldrich Chemical Co. (Steinheim, Germany).

Fourier-transform infrared (FT-IR) spectra were obtained on the Nicolet 6700 spectrometer with an ATR extension piece (Smart orbit diamond) with using 128 scans at a resolution of 4 cm^{-1} at the Institute of Chemistry, SAS, Bratislava, Slovak Republic.

UV spectra of CMC derivatives dissolved in water (1 mg/1 ml) were obtained on the SpectroFlex 6600 (WTW Weilheim, Germany).

Antioxidation activity was measured by chemiluminescence's method (photon counting instrument Lumipol 3, manufactured at the Polymer institute SAS Bratislava).

2.2 Synthesis of cinnamate esters of carboxymethylcellulose

(i) CMC (1.0 g) was activated in 22 ml DMF containing 4-TSA (0.6 g) by stirring at 50 °C for 30 min to yield the 'gel suspension' consistence [8]. CA (0.5-2 g) dissolved in 10 ml DMF, DCCI (1.4 g) and DMAP (0.05 g) were added to the gel suspension of CMC. The reaction mixture was stirred at 50 °C for 0.5-1 h and subsequently poured into 4-6 volumes of ethanol. The precipitated derivative was separated by filtration, thoroughly washed with ethanol, and extracted in a Soxhlet apparatus with ethanol for 8 - 20 h to remove the unreacted acylation agent. The product was obtained in the non-ionized form and was transformed to the carboxylate form. The derivative (1.0 g) was stirred in distilled water (50 ml) at room temperature for 6 h, then the pH was adjusted to 7 by slow addition of 0.1 M NaOH. The recovery and purification of CA-CMC product was the same as described above.

(ii) CMC (1.0 g) was solubilized in 35 ml H_2O under stirring at room temperature for 1 h. CA (0.5-1.0 g) dissolved in 20 ml DMF, DCCI (0.35 g) and DMAP (0.05 g) were added to the CMC solution and stirred at 50 °C for 0.5-2 h. The recovery and purification of the esters were the same as described in method (i).

2.3 Testing methods

The emulsifying efficiency was tested on emulsion of the ‘oil in water’ (O/W) type. The emulsion was prepared by mixing 9 ml water containing 0.05 g of the CA-CMC derivative and 1 ml of paraffinic oil dyed with SUDAN IV in the laboratory mixer Heidolph DIAX 600 at 20 500 rpm for 1 min. The stability of the emulsion was estimated at three time intervals after the emulsions had been prepared, i. e. 5 min (h_1), 1 h (h_2) and 24 h (h_3), and express in terms of the height (mm) of the oil and cream layers formed on the surface of the emulsion.

The surface tension of polysaccharide solution in water in the concentration range 0.0391 – 5.0 g/l was determined at 25 °C using the Du Nouy ring apparatus. Surface tension data were plotted against the logarithm of polysaccharide concentration in order to obtain the critical micelle concentration (c.m.c.) and the corresponding surface tension (γ_{min}), as described in previous papers [9].

Antioxidant activity was evaluated from the thermo-oxidative stability which was determined by the chemiluminescence’s method [10] in the temperature range 20–220 °C in oxygen atmosphere under constant heating rate 2.5 °C/min. The derivatives in form of powders were measured in aluminum pans; the initial mass was 4 mg and gas flow introduced above the sample was 3 l/h. Antioxidative effect of cinnamic acid bounded to CMC was tested by measurements of light emission in the visible region of spectrum – chemiluminescence (CL) in the temperature range 20-220 °C in oxygen atmosphere under constant heating rate 2.5 °C/min (chemiluminometer Lumipol 3 – product of the Polymer Institute SAS).

3 Results and discussion

Esterification of CMC with cinnamic acid was carried out in homogeneous (DMF/4-TSA) and heterogeneous conditions (H_2O/DMF). Various reaction conditions were served to prepare water-soluble CMC derivatives. The extent of esterification was observed by FT-IR spectra (not shown). The reaction conditions and characteristic spectral data are shown in Tab.1.

FT-IR spectra of CMC esters showed only shoulders at ~1702, attributed to the cinnamoyl group in accord to literature data [11], only the spectrum of CA-CMC-IV indicating a very low degree of esterification. The shift from a more typical absorbance band at 1730 cm^{-1} is attributed to the double bond conjugated with the aromatic ring structure. However, the presence of ester groups is demonstrated also by the intensity increase of the absorption bands at ~ 2922 cm^{-1} and ~ 2860cm^{-1}, respectively, attributed to the $\nu_{as}(CH_2)$ and $\nu_s(CH_2)$ vibrations of the fatty acyl substituents.

***Table 1** Esterification of CMC with cinnamic acid (CA) in presence of DCCI and DMAP at 50 °C and their characteristic spectral data*

Sample CA-CMC	CMC:CA (mass ratio)	React. time (h)	React. medium	Yield[a] (g/g)	$\nu(CO)$ (cm^{-1})	$\nu(CH_2)$ (cm^{-1})		λ_{max} (280 nm)
I[b]	1:2	0.5	DMF/4-TSA	0.43	1699	2924	2871	+
II[b]	1:1	1	DMF/4-TSA	0.39	-	2923	2869	-
III[b]	2:1	0.5	DMF/4-TSA	0.54	-	2922	2860	-
IV	2:1	0.5	H_2O/DMF	0.33	1702	2922	2855	+
V	1:1	1	H_2O/DMF	0.20	-	2924	2879	+
VI	2:1	2	H_2O/DMF	0.26	-	2921	2860	-

[a] Expressed as g of the recovered derivative per g CMC (on dry mass basis); [b]partially soluble in H_2O; (-) not determined

For further characterization and confirmation of CMC functionalization by cinnamic acid the UV spectroscopy was used. The UV spectra of CA-CMC derivatives exhibit the same absorbance characteristic as the cinnamic acid (not shown). An intense absorbance at ~ 280 nm is characteristic of the vinylene C=C absorbance of the cinnamate group [11, 12]. The unmodified CMC does not show any distinct absorbance in the UV region.

The tensioactive properties of derivatives were tested by studying the emulsifying efficiency in the oil in water system. As can be seen in Table 2, most of the tested derivatives exhibited remarkable emulsifying properties, comparable to the control system - Tween 20 (derivate IV - VI), although they had no pronounced suppressing effect on the surface tension of water (γ_0).

***Table 2** Surface - active properties of the prepared esters and control (γ_{min} - minimal surface tension, c.m.c. - critical micelle concentration, π_{cmc} - effectiveness and emulsifying efficiency)*

Sample CA-CMC	γ_{min} (mN/m)	$\pi_{cmc}{}^{b}$ (mN/m)	c.m.c. (g/l)	Oil/Cream layers[a] (mm/mm)		
				h_1	h_2	h_3
I	72.30	0.50	none	0/40	0/38	0/33
II	-	-	-	0/0	0/1	0/13
III	61.32	11.48	1.25	0/74	0/47	0/43
IV	67.49	5.31	0.68	0/0	0/0	0/27
V	72.68	0.12	none	0/0	0/0	0/9
VI	72.26	0.52	none	0/0	0/0	0/18
Tween 20	-	-	-	0/0	0/0	0/11

[a] height of oil and cream layers formed on the surface of the emulsion after 5 min (h_1), 1 h (h_2) and 24 h (h_3);
[b] maximum reduction of surface tension (or surface pressure $\pi_{cmc} = \gamma_0 - \gamma_{min}$), attained at the c.m.c.;
(-) not determined.

They lowered the γ_{min} from 72.8 only to 61.32-72.68 mN/m. The c.m.c. values ranged between 0.68-1.25 g/l, except of CA-CMC I, V and VI. The effectiveness (π_{cmc}) is defined as degree of reduction of surface pressure attained at the c.m.c., any reduction beyond c.m.c. being considered relatively insignificant. The best effectiveness was achieved with derivative CA-CMC III (11.48 mN/m). These small surface tension changes were consistent with cinnamate groups in CMC molecules that were too short to form ordered structures at the air/water interface.

Selected antioxidative properties for CMC ester (derivate IV) illustrate Figure 1. The beginning of significant increase of CL intensity is shifted for CMC ester to the higher temperature while the overall number of emitted photons in the range 40-220 °C is reduced. But antioxidative activity is not too high, because it is low-substituted derivative.

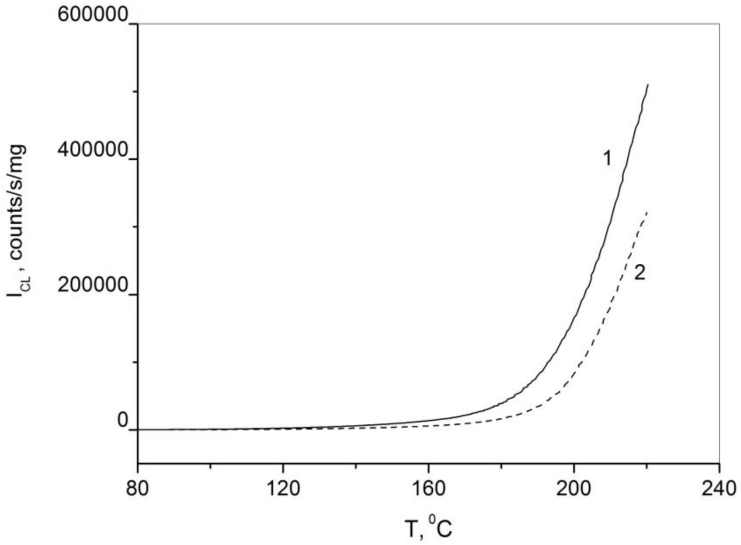

***Fig. 1** The dependence of chemiluminescence intensity (I_{CL}) on temperature (T) in O_2 for carboxymethylcellulose (curve 1) and CA-CMC IV (curve 2) at constant rate of heating v = 2.5 °C /min*

4 Conclusions

New cinnamate esters of the CMC were prepared using cinnamon acid and DCCI as activator and DMAP as catalyst in two different reaction media. The achieved degree of esterification of prepared CA-CMC derivatives was very low, but sufficiently high enough to impart surface active properties of derivatives.

Most of the prepared esters showed low surface tension-lowering effect but same of then form micelles at concentration 0.68 and 1.25 g/l. However they exhibit excellent emulsifying efficiency which is comparable to the commercial synthetic emulsifier Tween 20. Despite the low degree of substitution, some of the derivatives showed antioxidative stability.

The results suggested that the hydrophobized CMC derivatives represent suitable polymeric biosurfactants applicable as emulsifying agents or as antioxidants in pharmacy. The subject of future investigations will be determination of associative and performance properties of prepared derivatives.

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