Biosurfactants Based on Partially Esterified
O-(Carboxymethyl)starch

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Water-soluble O-(carboxymethyl)starch (CMS) has been partially hydrophobized in order to prepare water-soluble products possessing surfactant properties. The modification was performed by a nonconventional esterification method using the vinyl dodecanate (vinyl laurate, VL) as reactant at various reaction conditions. The derivatives were characterized by FTIR spectroscopy, surface tension, emulsification efficiency, and detergent performance. The results suggested that pre-activation of CMS with DMF or DMSO, containing 4-toluenesulfonic acid is essential to enhance the esterification reaction and prepare water-soluble CMS derivatives at low VL—CMS mass ratios. The water-soluble derivatives with low degree of esterification represent novel anionic polymeric biosurfactants exhibiting emulsifying ability for oil/water type emulsions as well as good performance properties (washing power and antiredeposition efficiency).

During the last decades, much effort has been made to extend nonfood applications of starch, because it is an inexpensive biopolymer produced in greater quantities than current market demands [1]. Of the various starch derivatives [2, 3], the anionic carboxymethylstarch (CMS) is commercially available in large quantities and represents a potential source for further modification. It can be used as drilling fluids [4], components of cross-linking formulation in easy-care finishing of textile fabrics [5] or as hydrogels after cross-linking by radiation [6].

As shown in a previous report [7], partial hydrophobization of CMS by etherification with higher alkyl halides (C_{10} and C_{12}) yielded biosurfactants with emulsifying efficiency comparable to that of the commercial surfactant Tween 20. From the practical point of view, hydrophobization of polysaccharides by esterification is an industrially more acceptable process, realized in the commercial production of polyol ester surfactants as well [8]. Most of the current commercial starch esters are of low DS (< 0.1) and are prepared by the anhydride slurry method in aqueous alkaline medium [9]. Recently, some nonconventional esterification methods, such as esterification with mixed anhydrides prepared *in situ* [10] and transesterification with vinyl carboxylic acid esters like vinyl laurate [11], have been developed to avoid the use of acid halides. However, there are no reports concerning ester derivatives of CMS.

The aim of the present work was to prepare water-soluble CMS derivatives containing long-chain ester groups by reaction with the vinyl laurate in homogeneous and heterogeneous systems in order to obtain polymeric surfactants with emulsifying and detergent properties.

EXPERIMENTAL

CMS with DS = 0.3 was a gift from Professor *Th. Heinze* (Center of Excellence for Polysaccharide Research at the Friedrich-Schiller University of Jena, Germany) synthesized as described [12]. N,N-Dimethylformamide (DMF), dimethyl sulfoxide (DMSO), vinyl laurate, (VL) and Tween 20 (oxyethylated sorbitol monolaurate) were commercial products from Aldrich Chemical Co. (Steinheim, Germany). Isopropyl alcohol (IPA) of anal. grade and sodium dodecyl sulfate (SDS) were from Lachema (Brno, Czech Republic). 4-Dimethylaminopyridine (DMAP) was purchased from Merck (Darmstadt, Germany). Water-free 4-toluenesulfonic acid (TSA) was prepared by drying under vacuum at 140 °C.

Fourier-transform infrared (FTIR) spectra were obtained on a NICOLET Magna 750 spectrophotometer with DTGS detector and OMNIC 3.2 software using 128 scans at a resolution of 4 cm^{-1}. The samples (2 mg) were pressed into pellets of KBr (200 mg). The peak molecular mass (M_P) of the samples was de-

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termined by HPGPC as previously described [13] using a commercial instrument (Laboratorní přístroje, Prague, Czech Republic) equipped with two Labio Prague Biospher GM 300 and 1000 exclusion columns (8 mm × 250 mm) and aqueous 0.1 M-NaNO$_3$ as solvent (0.4 cm^3 min^{-1}). The elution was monitored by a RI detector and the columns were calibrated with pullulan standards P10—P800.

Carboxymethylstarch Esters

CMS (1.0 g) was put into 30 cm^3 of DMF (or DMSO, or IPA) and the dispersion was stirred at room temperature for 2 h. Subsequently VL was added in various CMS—VL mass ratios and the reaction mixture was stirred at 20 °C, 40 °C or 50 °C for 3—20 h (see Table 1). The reaction product was precipitated into ethanol, filtered and then purified by Soxhlet extraction with ethanol for 8 h. In some experiments, CMS was activated prior to esterification in DMF or DMSO containing TSA (1 g/30 cm^3) by stirring the dispersion at 50—60 °C for 30 min to yield the "gel suspension" [14].

In order to obtain derivatives having the carboxymethyl substituent in the carboxylate form, the derivative (2 g) was stirred in 100 cm^3 of distilled water at room temperature for 6 h, then the pH of the dispersion was adjusted to 7 by slow addition of 0.5 M-NaOH, and the product was recovered by lyophilization. In the case of only partially water-soluble preparations, these were re-dispersed in distilled water under stirring for 4 h. After separation of the insoluble part by centrifugation, the soluble portion was recovered by lyophilization.

Testing Methods

The surface tension of the aqueous polysaccharide solution in the concentration range 0.015—5 kg m^{-3} was determined at 25 °C using the Du Nouy ring methods as described in detail by *Lunkenheimer* [15]. Surface tension data were plotted against polysaccharide concentration in order to obtain the critical micelle concentration (c.m.c.) and the corresponding surface tension (γ_{min}). Emulsifying efficiency was tested on emulsions of the oil/water (O/W) type. They were prepared by mixing 9 cm^3 of water, containing 0.05 g of the polysaccharide and 1 cm^3 of paraffin oil dyed with SUDAN IV, in a laboratory mixer (Heidolph DIAX 600) at 13 500 min^{-1}. The stability of the emulsion was estimated at three time intervals (5 min, 1 h, and 24 h) after the emulsions had been prepared, and expressed in terms of the heights (h/mm) of the oil and cream layers formed on the surface of the emulsion.

Washing power (WP) was assessed by determining the reflectance of an artificially soiled cotton fabric (SK-standard 800101) after washing in an alkaline surfactant bath. The cotton fabric was immersed for 5 min in the model soil consisting of 30 g dry milk, 18.8 g CCl$_4$, 2.2 g gelatin, 45 g sunflower oil, 15 g black ink, and 262 cm^3 of water. The excess soil was removed by a squeezing mangle and the fabric was air-dried. Samples of the soiled fabric (6.0 cm × 6.0 cm) were washed in a Koltest apparatus. The volume of the alkaline bath containing 2 g/l000 cm^3 of Na$_2$CO$_3$ was 100 cm^3, and the tested derivatives were used in two concentrations (0.5 kg m^{-3} and 2 kg m^{-3}). Washing temperature was 60 °C and washing time 10 min.

Antiredeposition efficiency (ARE) was determined by the method of *Stüpel* [16], using the abovementioned standard cotton fabric. The model soil consisted of 1.0 g carbon black, 0.5 g Spolion 8 (wetting agent), and 100 cm^3 of water. Fabric samples (6.0 cm × 6.0 cm) were washed at 60 °C for 60 min using a bath containing 89 cm^3 of water and 10 cm^3 of soil with and without addition of 1 cm^3 of solution of the tested derivative (0.5 kg m^{-3}). ARE was calculated from the relation ARE = $(R_2 - R_1)/(R_0 - R_1)$, where R_0 is the reflectance of the unsoiled cotton fabric, R_1 is the reflectance of the cotton fabric after scouring in the bath of soil, and R_2 is the reflectance of the fabric after scouring in a bath containing the derivative.

RESULTS AND DISCUSSION

Partial hydrophobization of CMS was carried out by transesterification with VL according to the procedure applied for esterification of cellulose [11]. A series of water-soluble derivatives was synthesized by varying the VL—CMS mass ratio, reaction time, temperature, and media (DMF, DMSO, and IPA), as listed in Table 1. The aim was to prepare water-soluble CMS ester derivatives, that could be expected only with derivatives of low hydrophobization degree, as has been previously found [7] with the long-alkyl chain ethers of CMS.

FTIR spectra were used to characterize the derivatives, because elemental analysis (not shown) revealed nonsignificant differences from the data obtained with the starting CMS. The FTIR spectra (Fig. 1) showed the characteristic ν(C=O) absorption band at (1741 ± 2) cm^{-1} providing a qualitative proof of the introduced ester function. However, it might be overlapped by the carbonyl group vibration at ≈ (1732 ± 2) cm^{-1} of the CMS carboxymethyl substituent, when this is present in the protonated form. Both substituents can be distinguished by measuring the spectra of the salt form. They show (Fig. 2), next to the ester vibration, absorption bands of the ν_{as}(COO$^-$) and ν_s(COO$^-$) vibrations at ≈ (1602 ± 2) cm^{-1} and ≈ (1417 ± 2) cm^{-1}, respectively. However, at very low esterification degree the ester group vibration appeared as a shoulder. The introduction of lauroyl groups is further supported by the increase of the bands at (2927 ± 3) cm^{-1} and (2853 ± 2) cm^{-1}, attributed to ν_{as}(CH$_2$) and ν_s(CH$_2$) vibrations, respectively, of the polymethylene

Table 1. Preparation, Yield, and Characteristics of CMS Derivatives Obtained by Esterification with Vinyl Laurate (VL) at Various Reaction Conditions

Derivative	CMS—VL $m(CMS)/m(VL)$	Reaction medium	Temp. °C	Time h	Yield %[a]	Extent of esterification[b]	Behaviour in water
CMS/1	1 : 1	DMF/TSA	20	20	93	+++	dsp
CMS/2	1 : 3	DMF/TSA	20	20	93	+	s
CMS/3	2 : 1	DMF/TSA	20	20	90	++	g
CMS/4	4 : 1	DMF/TSA	20	20	88	++	md
CMS/5	1 : 3	DMF	20	20	88	−	g
CMS/6	1 : 3	DMF	50	3	88	+	g
CMS/7	1 : 2	DMSO	40	3	73	+	ps
CMS/8	2 : 1	DMSO	40	3	80	−	ps
CMS/9	2 : 1	DMSO	40	6	64	−	ps
CMS/10	1 : 1	DMSO/TSA	50	2	44	+++	s
CMS/11	2 : 1	DMSO/TSA	50	3	53	+++	s
CMS/12	3 : 1	DMSO/TSA	50	3	66	++	s
CMS/13	1 : 1	IPA	20	20	93	−	g
CMS/14	1 : 2	IPA	50	3	96	+	g

a) Expressed as m (g) of the recovered derivative per 1 g CMS; b) estimated from the differential FTIR spectrum in the region 2980—2700 cm^{-1}. dsp – dispersion of swelled particles, s – soluble, md – milky dispersion, g – gel, ps – partially soluble.
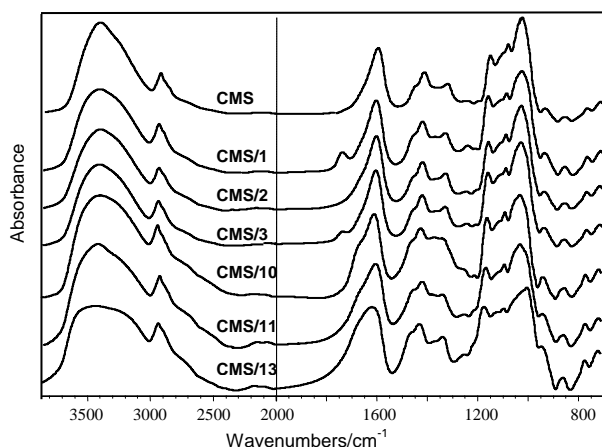
Fig. 1. FTIR spectra (in KBr) of CMS and its lauroyl derivatives.
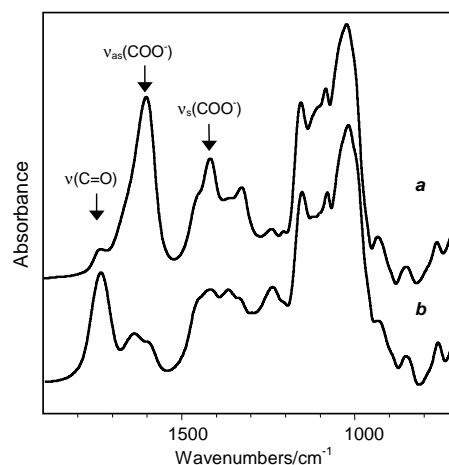
Fig. 2. FTIR spectra of lauroyl derivative CMS/1 in the Na$^+$ form (a) and H$^+$ form (b) of the carboxymethyl substituent.

chain. This is documented by the differential FTIR spectrum of CMS and its derivative (shown for sample CMS/5 in Fig. 3). The differential spectra were further used to compare the extent of esterification achieved under various reaction conditions.

The spectral data of derivatives prepared in DMF and DMSO (Table 1) indicate no or very weak esterification. In these solvents CMS was only partially soluble and the dispersion contained swollen CMS particles. This is due to the presence of the ionic carboxymethyl susbtituents, which impedes the macromolecules from solvation by the aprotic solvents. Therefore, the reaction proceeded under rather heterogeneous conditions. A very low extent of esterification was observed also during the heterogeneous esterification of CMS performed by the "slurry method" in IPA.

The most efficient was the esterification of CMS

activated in the DMSO/TSA medium and lesser one in DMF/TSA. Under these conditions the carboxyl groups were protonated and the polysaccharide was transformed into a suspension of highly swollen particles (gel suspension) [14], enabling rather homogeneous reaction conditions. The advantage of this "pre-activation" is the increased accessibility of starch hydroxyl groups. A disadvantage is that the products were obtained in the H$^+$ form, in which they are swellable and dispersable, but not well dissolvable in water. For further analyses, the derivatives have to be transformed into their ionic forms.

Higher reaction temperatures favoured the esterification reaction, but particular polymer degradation occurred in the TSA-containing reaction medium. Due to increasing amount of degraded, ethanol-nonprecipitable products, the yield of the derivatives was substantially lower (Table 1). Polymer degrada-

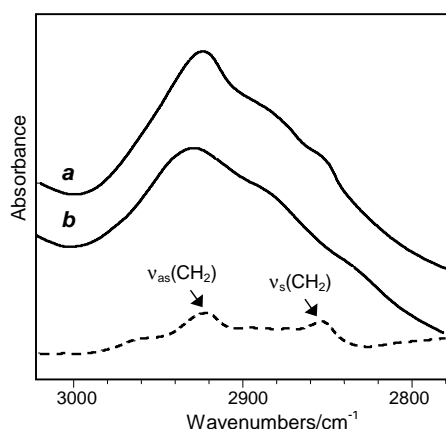**Table 2.** Molar Mass Distribution[a] of the Original and Esterified CMS

Derivative	$M_P \cdot 10^{-3}/(\text{kg mol}^{-1})$			
	Peak I	Peak II	Peak III	Peak IV
CMS	> 1800 (15)[b]	1360 (85)	–	–
CMS/5	–	1650 (90)	385 (10)	–
CMS/2	–	1426 (65)	324 (25)	1.5 (10)
CMS/11	–	1307 (56)	353 (32)	3.5 (12)

a) Measured on GM Biospher columns calibrated with pullulan standards. *b*) The value in brackets represents the relative proportion/% calculated from the molecular peak (M_P) areas of the elution curve.

Table 3. Surface Tension ($\gamma_{\min}$), Critical Micelle Concentration (c.m.c.), and Emulsifying Efficiency[a] of CMS, its Lauroyl Derivatives, and Controls

Derivative	$\gamma_{\min}$	c.m.c.	$h(\text{Oil})/h(\text{Cream})$		
	mN m^{-1}	kg m^{-3}	h_1	h_2	h_3
CMS	72.0	None	0/1	2/10	5/3
CMS/1	52.2	0.63	0/0	0/0	0/44
CMS/2	–	–	0/0	0/0	0/68
CMS/4	51.4	0.60	0/0	0/0	0/39
CMS/5	51.3	0.65	0/0	0/0	0.5/18
CMS/6	51.2	0.59	0/0	0/0	0/45
CMS/9	–	–	0/0	0/19	1/10
CMS/10	60.8	0.27	0/0	0/0	0/10
CMS/11	–	–	0/1	0/4	0/9
CMS/12	64.0	0.15	0/0	0/11	0/12
CMS/13	–	–	0/0	0/0	5/50
CMSVI [b]	53.0	0.62	0/0	0/0	0/10
Tween 20	–	–	0/0	0/0	0/2

a) Expressed in terms of the 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*) Lauryl ether of CMS [7].

Fig. 3. Differential FTIR spectrum (dashed line) of CMS/5 (*a*) and CMS (*b*).

tion was indicated also by corresponding changes of the molar mass distribution (Table 2). However, the tensioactive properties of the derivatives should not be negatively affected by the depolymerization [17].

The surface-active properties of the lauroylated CMS derivatives were characterized by surface tension ($\gamma_{\min}$), critical micelle concentration (c.m.c.), and emulsifying efficiency. Recently, in the case of poly-meric surfactants the term critical polymer concentration (c.p.c.) [18] has been introduced, which corresponds to the c.m.c. of classical surfactants [19]. As shown in Table 3, the esterified derivatives had no pronounced suppressing effect on the surface tension of water. The $\gamma_{\min}$ values decreased from 72.8 to $\approx$ 51—64 mN m^{-1} and the c.m.c. ranged between 0.65 kg m^{-3} and 0.15 kg m^{-3}. Similar effects were reported also for other biopolymeric surfactants, *e.g.* carboxymethylcellulose (CMC) grafted with butyl or octyl amines [20], C_n-alkylated CMC [21], alginate-derived surfactants with C-8 to C-16 alkyl chains, introduced by reductive amination of the 2,3-dialdehydic alginate [22], C_n-esterified CMC [17], and the lauryl CMS ethers [7]. In spite of the low extent of esterification, the derivatives displayed considerable emulsifying ability. Most of the tested derivatives gave emulsions of the oil/water type exhibiting high stability, as no oil was separated after 24 h from the emulsion. In comparison to the synthetic emulsifier Tween 20, from the CMS ester emulsions larger but stable cream layers were formed after 24 h. Exceptions are the derivatives CMS/10—CMS/12, prepared in the DMSO—TSA reaction medium, which behaved similarly as Tween 20.

Table 4. Washing Power (WP) and Antiredeposition Efficiency (ARE) of the Original and Esterified CMS and Controls

Derivative	WP/%[a]		ARE
	ρ_1	ρ_2	%
CMS/1	13.0	–	23.9
CMS/2	23.9	25.4	–
CMS/4	13.0	–	23.9
CMS/5	48.2	36.5	23.7
CMS/6	15.5	29.0	39.2
CMS/9	6.1	5.8	20.0
CMS/11	15.7	14.2	–
CMS/12	10.2	10.8	12.1
CMS/14	16.5	–	21.0
CMS	–	–	16.5
CMC	–	–	21.7
SDS	–	8.1	–

a) Measured at polymer concentrations: $\rho_1 = 0.5$ kg m^{-3} and $\rho_2 = 2$ kg m^{-3}. SDS – sodium dodecyl sulfate.

The different effect of the CMS derivatives on the character of the O/W emulsions is not explained. However, except of the hydrophilic/hydrophobic balance governed by the type, amount, and distribution of the hydrophobic substituent, the molecular structure and hydrodynamic properties of the polymeric surfactants play a significant role in emulsion stability [23, 24] as well as foamability [25, 26].

The detergent performance of some of the surface-active CMS derivatives was tested by the washing power (WP) at two polymer concentrations $\rho_1 = 0.5$ kg m^{-3} and $\rho_2 = 2.0$ kg m^{-3} using SDS as standard, and by the antiredepositive efficiency (ARE). The results summarized in Table 4 suggest that the WP of some samples was higher in comparison to the standard, even if tested at a lower polymer concentration. The antiredepositive efficiency (ARE) of the lauroylated CMS samples ranged from 12.1 % to 39.2 %, and was comparable to or even higher than that displayed by the nonmodified CMS and CMC, often used as co-builders in detergents [27]. The ARE values indicate acceptable performance properties of the CMS derivatives.

CONCLUSION

The partial hydrophobization of CMS with vinyl laurate to low esterification degree yielded water-soluble surface-active polysaccharide esters. The results suggested that the derivatives can be prepared under mild reaction conditions, *e.g.* at shortened reaction time and elevated temperature or at longer reaction time at room temperature, using appropriate reaction media. The used unconventional esterification method might substitute the classical, hazardous and environmentally unfriendly acyl chloride method. Due to the low degree of esterification, the derivatives can be assumed to have maintained biodegradability.

The partially hydrophobized CMS derivatives are potential polymeric biosurfactants, which are exploitable in various technical applications. They produce emulsions of the oil/water type with excellent stability, comparable to that of the emulsion prepared by the commercial emulsifier Tween 20. The derivatives showed good washing power and acceptable antiredeposition efficiency, both characterizing moderate detergent-performance properties.

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