Synthesis of Bioactive 2,2-Dichloropropionic Acid Esters of Carboxymethyl Cellulose and Investigations on Their Release Behavior

MATTHIAS SCHNABELRAUCH, GÜNTER GESCHWEND, and DIETER KLEMM, *Sektion Chemie der Friedrich-Schiller- Universität Jena, Humboldtstrasse 10, DDR-6900 Jena, German Democratic Republic*

Synopsis

Polymer bound bioactive agents are prepared by linking 2,2-dichloropropionic acid to the hydroxyl groups of carboxymethyl cellulose (CMC) and enzymatically cleavable CMC networks, and to the carboxyl groups of CMC via enzymatically cleavable spacer units using a simple as well as effective CMC activation procedure. The hydrolytic release of the bioactive agent is mainly dependent on the hydrophilicity of the CMC esters. In the case of polymers containing enzymatically cleavable structural units the release can be accelerated by addition of esterases.

INTRODUCTION

Carboxymethyl cellulose (CMC) has gained growing interest as hydrophilic polymer support of a variety of bioactive compounds.[1,2] During the last decade numerous papers have been published dealing with covalent attachment of drugs, enzymes, or biocides to the carboxyl groups of CMC.[3-5] The resulting polymer–agent bonds are mostly of ester or amido type. In a previous paper we reported on the fixation of biocidal halocarboxylic acids via spacers to the carboxyl groups of CMC.[6] In comparison with the corresponding derivatives based on cellulose, these polymeric biocides show a controlled and also complete release of the bioactive agents. Less attention has been paid to the utilization of free hydroxyl groups of CMC as agent binding moieties.

In connection with studies on functionalization of cellulose and its derivatives as well as on controlled release active agents, our interests were directed to preparing polymers containing hydrolytically and enzymatically cleavable structural units suitable for bearing the bioactive component. In the present article we describe the coupling of such components containing carboxylic groups directly to unsubstituted hydroxyl groups of CMC and enzymatically cleavable CMC networks, or to the carboxyl groups of CMC via enzymatically cleavable spacer units. Furthermore, we investigated the different hydrolytic release behavior of these new CMC derivatives. The bioactive agent we used was 2,2-dichloropropionic acid. This well-known biocide belongs to the important class of halocarboxylic acids distinguished by a wide spectrum of biological activity ranging from microbiocidal and fungicidal effects to plant-growth stimulation.

Journal of Applied Polymer Science, Vol. 39, 621–628 (1990)
 CCC 0021-8995/90/030621-08$04.00

EXPERIMENTAL

Materials

Sodium carboxymethyl cellulose[7] (Na–CMC, DS 0.9) was prepared from cellulose powder (linters cellulose, hydrolytically degraded, Cuoxam DP 152). Reagent grade 2,2-dichloropropionic acid was vacuum distilled before it was used. Its conversion into the acid chloride[8] and the preparation of 2(2,2-dichloropropionyloxy)ethanol[6] (**5**) were performed by conventional methods. The reaction of the latter compound (**5**) with α-bromo-α-ethoxy-acetylchloride yielded 1-(α-bromo-α-ethoxy-acetoxy)-2-(2,2-dichloropropionyloxy)ethane (DBE).[9] The crosslinking agent 1,2-bis(α-bromo-α-ethoxy-acetoxy)ethane (BBE) was obtained by bromination of 1,2-bis(α-ethoxy-acetoxy)ethane in boiling CCl_4 as described earlier.[9]

Other reagents and solvents used in synthesis were of analytical grade or purified by appropriate procedures.[5]

Preparation of CMC–2,2-Dichloropropionates (2a–c)

Na–CMC was activated in three different ways: (i) preswelling in pyridine at 20°C for 24 h (method 1, CMC-ester **2a**), (ii) stepwise solvent exchange methanol–acetone–dioxane–pyridine (method 2, product **2b**), (iii) precipitation of Na–CMC from an aqueous solution into DMF followed by removal of water by distillation in vacuum (method 3; **2c**). After pretreatment, 5 g of Na–CMC were allowed to react with 7.4 g of 2,2-dichloropropionyl chloride in 150 mL of pyridine (method 1 and 2), or in 120 mL of DMF in the presence of 30 mL of pyridine (method 3) at 75°C for 10 h. The reaction mixtures were poured into MeOH, washed three times with MeOH, Soxhlet-extracted with ether (10 h), and dried (25°C, 2 kPa).

Content of 2,2-dichloropropionyl groups (DS/wt %): 0.11/5.6 (product **2a**); 0.17/8.4 (**2b**); 0.79/29.9 (**2c**). IR (KBr; cm^{-1}): 3500 (Cell—OH), 1760 (COO—Cell), 1625 (COONa).

Preparation of Enzymatically Cleavable CMC Networks (CN a–c)

The synthesis of the enzymatically cleavable CMC networks was carried out by *O*-alkylation of CMC with BBE in CH_2Cl_2, glycerol or DMF as described in detail in a previous paper.[10] Characteristics of these networks were summarized in Table I.

TABLE I
Description of Enzymatically Cleavable CMC Networks, Synthesized in CH_2Cl_2 (**CNa**), Glycerol (**CNb**), and DMF (**CNc**)

No.	Water-soluble part (wt %)	DS_{COONa}	DS^{a}_{ester}	WRV (wt %)
CNa	20	0.55	0.37	2200
CNb	5	0.51	0.40	2700
CNc	38	0.48	0.44	1600

[a] DS of crosslinking ester groups estimated by a modified uranyl method.[11]

TABLE II
Analytical Data of the Prepared CMC-Network Bound Agents

No.	Starting network	Agent content		WRV (wt %)
		DS_{agent} [a]	(wt %)	
4a	**CNa**	0.09	4.6	1800
4b[b]	**CNa**	0.18	8.8	1200
4c	**CNb**	0.10	5.0	2050
4d[b]	**CNb**	0.14	6.9	1540
4e	**CNc**	0.12	5.9	850
4f		0.16	8.1	950

[a] DS of 2,2-dichloropropionyl groups.
[b] Prepared by using 2.0 eq 2,2-dichloropropionyl chloride for each free OH group.

Preparation of CMC-Network Bound Agents (4a–f)

Pretreatment of CMC networks was performed according to the activation method 3 for Na–CMC (preswelling of the network in water followed by addition of DMF and removal of water). The activated networks (2.0 g) were suspended in a mixture of 50 mL of DMF and 30 mL of pyridine, and esterified with 2,2-dichloropropionyl chloride (1 eq for each free OH group) at 50°C for 30 h. The reaction products (**4a–e**) were isolated and purified as described above for the esters **2a–c**. In an alternative way, a CMC–2,2-dichloropropionate (DS_{COONa} 0.9; DS_{agent} 0.17) was crosslinked with BBE (1.5 mol per mol free COONa group) in CH_2Cl_2 in the presence of triethylamine (product **4f**). Analytical data can be found in Table II.

IR (KBr; cm^{-1}): 3500 (Cell—OH), 1760, 1735 (COOC), 1625 (COONa).

Preparation of 2,2-Dichloropropionyloxyethoxycarbonylethoxymethyl Esters of CMC (5a–b)

Na–CMC activated as mentioned above (method 3), and finally DMF was exchanged for 150 mL of CH_2Cl_2. After addition of triethylamine (4 g) and 0.8 eq (product **5a**), resp. 1.7 eq (**5b**) BDE for each carboxyl group, the reaction mixture was stirred at 20°C for 50 h.

The polymers were precipitated by pouring into methanol, washed with ethanol/water (80/20 v/v), Soxhlet-extracted, and dried as described for the other derivatives.

Content of 2,2-dichloropropionyl groups (DS/wt % agent): 0.14/7.5 (**5a**), 0.27/13.0 (**5b**). DS_{COONa}: 0.75 (**5a**), 0.62 (**5b**). IR (KBr, cm^{-1}): 3500 (Cell—OH), 1760, 1735 (COOC), 1625 (COONa).

Characterization of the CMC Esters

Degrees of substitution (DS) of CMC esters were determined on the basis of elemental analysis and/or a modified uranyl method.[11] Infrared (IR) spectra were measured by the KBr disk technique using a Carl Zeiss Jena spectrophotometer UR 20. Water retention values (WRV) were estimated by adapting the method by Jayme.[12]

Investigations on Release Behavior of the CMC Esters

The CMC ester (0.5 g) was suspended in 200 mL of water. The suspension was adjusted to pH 7 using a 0.01*M* NaOH and a 0.01*M* acetic acid. Then a buffer solution containing 0.6 g of sodium chloride, 0.33 g of boric acid, and 0.06 g of sodium tetraborate in 10 mL of water was added. Enzymatic cleavage was started by the addition of 0.1 g of lyophilized normal horse serum (from VEB Pharmazeutisches Kombinat Germed, Impfstoffwerk Dessau-Tornau, GDR, 0.03 U/mg, substrate ethyl butyrate) in 10 mL of water. All experiments were carried out in closed vessels at 30°C with constant stirring. To estimate the released agent in appropriate time intervals, samples of 5 mL solution were taken. The released 2,2-dichloropropionic acid was esterified quantitatively with diazomethane and the formed methyl ester was determined by gas chromatography.[6]

RESULTS AND DISCUSSION

Synthesis of the CMC Ester Bound Agents

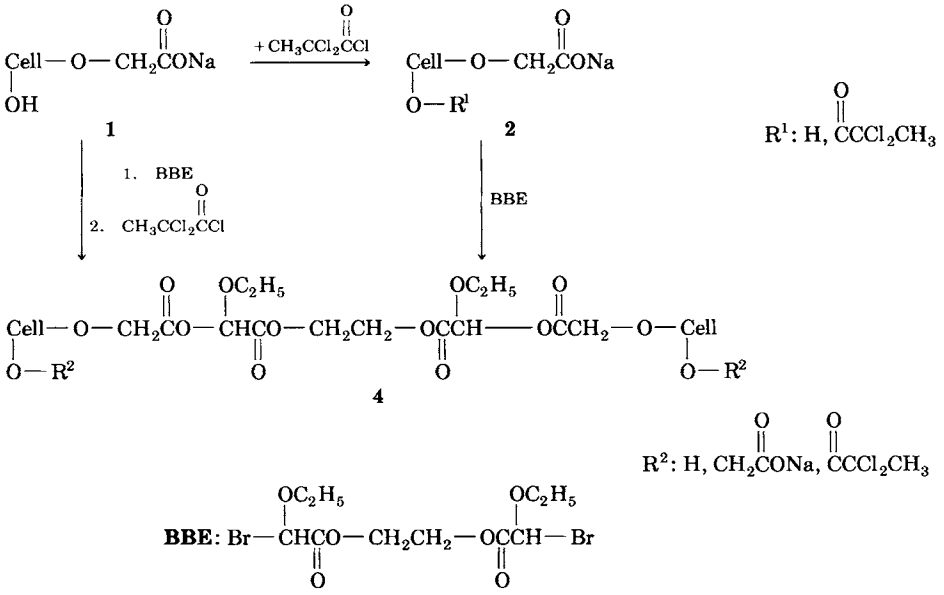
Esterification of free hydroxyl groups of cellulose and its derivatives is strongly dependent on an activating pretreatment of the cellulosic material. To obtain CMC-2,2-dichloropropionates (**2**) with middle or high degree of esterification, as well as to develop a simply practicable synthetic procedure, we examined three different methods of CMC activation followed by the acylation of free OH groups of CMC with 2,2-dichloropropionyl chloride (2 mol per mol OH group). Preswelling in pyridine and stepwise solvent exchange (in order methanol–acetone–dioxane–pyridine) are conventional activation methods in cellulose chemistry. But the acylation of CMC (DS 0.9) after pretreatment according to these procedures led only to CMC esters with DS = 0.11, resp. 0.17. For this reason, we worked out a more effective pretreatment of CMC consisting of the precipitation of an aqueous CMC solution in DMF and the subsequent removal of water from the suspension by distillation under reduced pressure. Acylation of highly swollen CMC gel obtained in this way yielded, under comparable conditions, CMC esters with considerable elevated DS of 0.79. The synthesized CMC–2,2-dichloropropionates are white, powdery solids, strongly swellable in water or DMF, but insoluble in common organic solvents. The products have IR spectra with characteristic carboxyl bands at about 1760 and 1625 cm^{-1} due to the 2,2-dichloropropionyl and the carboxymethyl groups. The solvent exchange water–DMF connected with the reprecipitation of CMC seems to cause an extensive decrease of the supermolecular order of CMC, resulting in an improved accessibility of the hydroxyl groups to attacking reagents. A further advantage of this activation is its application to other CMC derivatives and also to other modification reactions of CMC as it will be shown in the following sections.

Recently we succeeded in preparing enzymatically cleavable CMC networks applicable as biodegradable carrier materials. Now we report on the binding of 2,2-dichloropropionic acid to the hydroxyl groups of these networks synthesized by *O*-alkylation of Na–CMC with 1,2-bis(α-bromo-α-ethoxyacetoxy)ethane (BBE) heterogeneously in CH_2Cl_2 (**CNa**) and DMF (**CNc**),

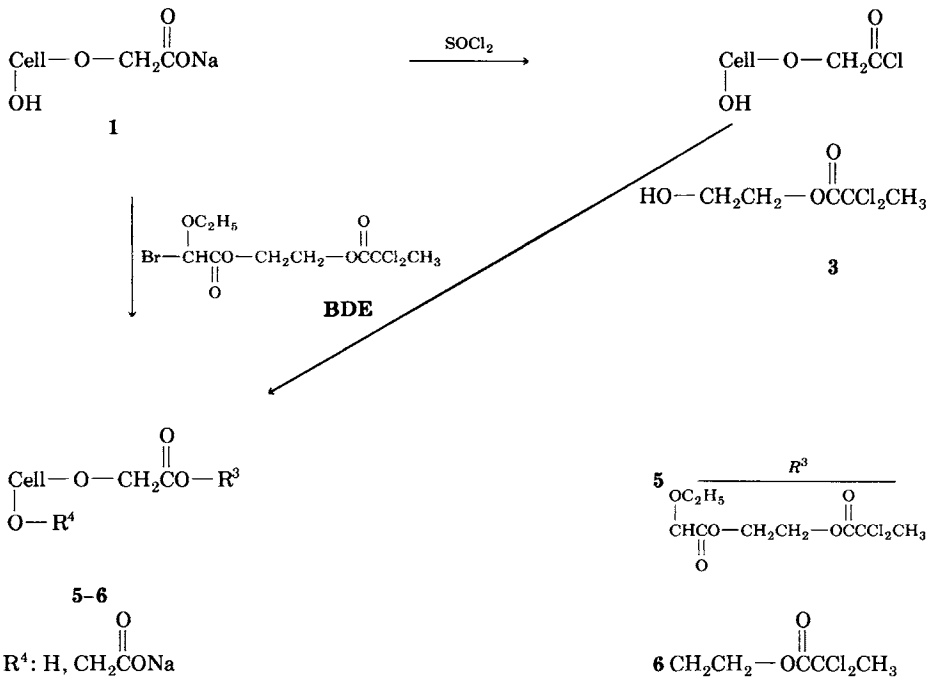
and homogeneously in glycerol (**CNb**). The resulting swelling CMC-networks differ in the degree of crosslinking and the contents of free carboxylic groups (Table I). In order to activate the network for esterification, we used the solvent exchange water–DMF as described above. After acylation at 50°C for 30 h we received CMC network bound agents with DS_{agent} = 0.09–0.12 (4–6% dichloropropionic acid by weight) by reaction with 1.0 mol of 2,2-dichloropropionyl chloride per mol of free OH group, as DS = 0.14–0.18 (7–9% agent by weight) by addition of 2.0 mol equivalents of acylating reagent (Table II). In an alternative synthetic procedure we started with CMC-2,2-dichloropropionate (**2b**, DS_{agent} = 0.17) followed by *O*-alkylation with BBE leading to a CMC network bound agent with quite similar characteristics (**4f**, Table II). Compared with the unmodified networks all the products show a marked decrease of their water retention values. In addition to the two mentioned carbonyl bands (1760, 1625 cm^{-1}) in the IR spectra, a third appears at 1735 cm^{-1}, indicating the presence of the crosslinking unit.

The application of suitable spacer units also permits the use of free carboxyl groups for the covalent fixation of 2,2-dichloropropionic acid. Based on a previous work dealing with the preparation of 2,2-dichloropropionyloxyethyl esters of CMC (**6**), we have now linked the same agent to the carboxyl groups of CMC via enzymatically cleavable ethoxycarbonyl ethoxymethyl ester moieties.

As the first step 2(2,2-dichloropropionyl) ethanol was allowed to react with α-bromo-α-ethoxyacetyl chloride resulting in the agent-spacer linkage. *O*-alkylation of Na–CMC activated by solvent exchange in the order water–DMF–dichloromethane with BDE at 20°C for 50 h in the presence of triethylamine yielded CMC esters (**5a–b**) with DS_{agent} = 0.14–0.27 (7–13%, 2,2-dichloropropionic acid by weight) according to the used amount of BDE



Scheme 1. Binding of 2,2-dichloropropionic acid to the OH groups of CMC and enzymatically cleavable CMC networks.



Scheme 2. Binding of 2,2-dichloropropionic acid to the carboxyl groups of CMC via spacer units.

(0.8, resp. 1.7 mol per mol of free carboxyl groups). The three carbonyl bands (1760, 1735, 1625 cm^{-1}) appearing in the IR spectra indicate the attached spacer bound 2,2-dichloropropionyl units, except from carboxyl groups of the CMC.

Investigations on Release Behavior of the Synthesized CMC Esters

To obtain preliminary information on release behavior of the prepared bioactive polymers in relation to their chemical structure, we examined the enzymatic and hydrolytic cleavage of the CMC esters at 30°C and pH 7.0 in water. Figure 1 illustrates the time course of cleavage of esters **2b**, **4b**, and **4f**, in which 2,2-dichloropropionic acid is directly bound to the hydroxyl groups of CMC or CMC network.

It can be recognized that the release of bioactive agent from the CMC–2,2-dichloropropionate (**2b**) with DS_{agent} = 0.17 is complete and relatively rapid during a period of approximately 400 h. CMC esters with higher content of bioactive agent (for example, **2c**, DS_{agent} = 0.79) have the same release behavior. In this connection, it should be mentioned that 2,2-dichloropropionates of cellulose exhibit a strongly delayed and also incomplete release with increasing content of agent.[2] The different behavior suggests the influence of hydrophilic structural units on the release of polymer bound agents in aqueous media.

This fact is confirmed by the hydrolysis course of the CMC-network bound agents (**4b, f**) as shown in Figure 1. In the absence of esterase after the

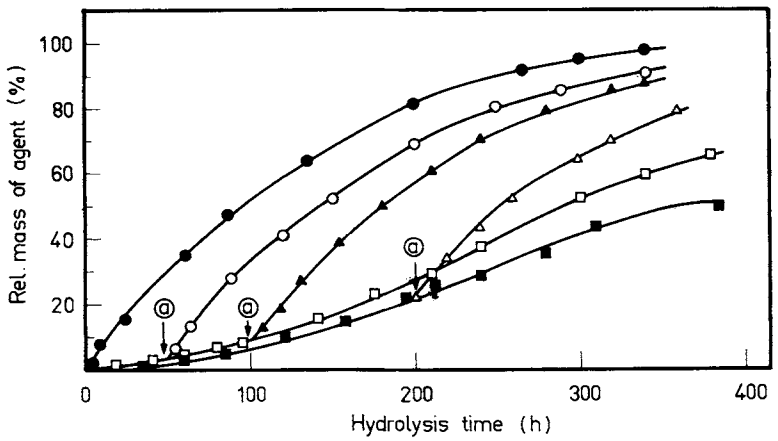


Fig. 1. Release of 2,2-dichloropropionic acid from the CMC esters **2b** (●), **4b**, and **4f** at 30°C and pH 7.0 in water with [(○,▲ **4b**); (△) **4f**] and without [(□) **4b**; (■) **4f**] addition of esterase (a = addition of esterase).

hydrolysis time of 400 h less than 70% of 2,2-dichloropropionic acid was released from the CMC networks. The lower the water retention values and with that the swellability and the accessibility of the networks, the slower the hydrolysis, as it is observed by comparing the samples **4b** and **4f**. Addition of esterase after a desired duration of hydrolysis brings about splitting of the crosslinked ethoxycarbonyl ethoxymethyl ester groups of CMC within 2–4 h, connected with the equivalent reformation of carboxyl groups. Then the hydrolytic behavior of the resulting CMC–2,2-dichloropropionates corresponds to the course which is typical of these polymers as it was described above.

In Figure 2 the release curves of CMC ester **5b** are plotted, in which the bioactive agent is linked to the carboxyl groups of CMC via enzymatically cleavable ethoxycarbonyl ethoxymethyl spacer units. In contrast to the re-

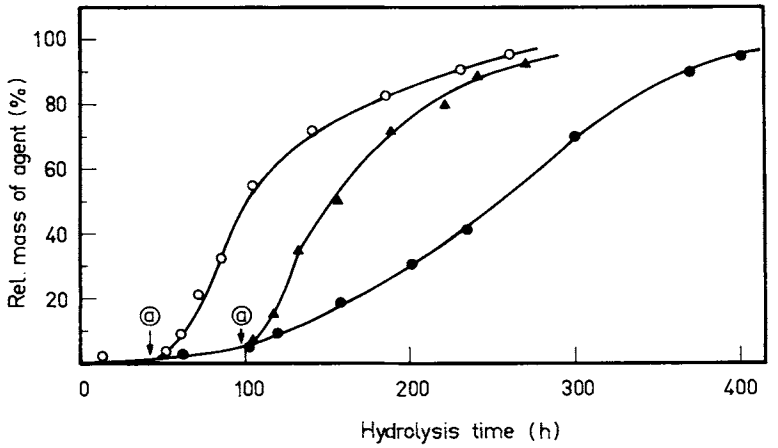


Fig. 2. Release of 2,2-dichloropropionic acid from the CMC ester **5b** at 30°C and pH 7.0 in water with (○,▲) and without (●) addition of esterase (a = addition of esterase).

lease profile of the CMC–2,2-dichloropropionate in the beginning of hydrolysis, a period of induction is observed effecting a relatively slow release of agent. The proceeding hydrolysis is combined with the formation of free carboxyl groups resulting in advanced hydrophilicity of the polymer. For this reason, the rate of hydrolysis rises, and complete release of 2,2-dichloropropionic acid is reached after 400 h. These facts are in good agreement with the results of our previous investigations on hydrolytic behavior of 2,2-dichloropropionyloxy–ethylester of CMC (**6**).[6] In the case of esterase addition, especially in the starting period, a strongly increased release is achieved indicating the rapid enzymatic cleavage of the CMC–spacer linkage. The further splitting of the formed low molecular fragments containing the bioactive agent occurs with considerable higher rate of reaction.

CONCLUSIONS

Bioactive CMC esters can be prepared by attaching 2,2-dichloropropionic acid to the hydroxyl groups of CMC and enzymatically cleavable CMC networks, and to the carboxyl groups of CMC via enzymatically cleavable spacers using a simple as well as very effective method of CMC activation. This procedure is applicable to various acylation or alkylation reactions of CMC providing derivatives with appropriate DS. The synthesized CMC esters show various hydrolysis profiles.

The attachment of the bioactive agent to both the hydroxyl and the carboxyl groups of CMC via hydrolytically cleavable ester bonds, and the utilization of enzymatically cleavable crosslinking units permit us to vary the content of free carboxylic groups of CMC and with it the hydrophilicity of the polymer in a wide range.

The direct fixation of 2,2-dichloropropionic acid to the cellulosic hydroxyl groups results in CMC derivatives with a more retarded release during the hydrolysis in the presence of esterase, compared with CMC esters, in which the bioactive agent is linked to the carboxyl groups of CMC via enzymatically cleavable spacer units. In the latter case after addition of esterase in the enzymatic splitting process low molecular 2,2-dichloropropionic acid esters are formed very rapidly. Such low molecular esters have a higher rate of hydrolysis than the polymeric CMC esters. In this way various release profiles can be obtained dependent on the type of agent linkage.

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Received December 12, 1988
Accepted December 27, 1988