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Short communication

Hydroxyl radical-induced crosslinking and radiation-initiated hydrogel formation in dilute aqueous solutions of carboxymethylcellulose

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ABSTRACT

Ionizing radiation causes chain scission of polysaccharides in the absence of crosslinking agents. It has been demonstrated before that degradation of carboxyalkylated polysaccharides may be prevented, despite presence of strong electrostatic repulsing forces between chains, at very high polymer concentration in water (paste-like state) when physical proximity promotes recombination of radiation-generated polymer radicals. In such conditions, crosslinking dominates over chain scission and covalent, macroscopic gels can be formed. In an approach proposed in this work, neutralizing the charges on carboxymethylcellulose (CMC) by lowering the pH results in retracting the electrostatic repulsion between chain segments and thus allows for substantial reduction of polymer concentration required to achieve gelation due to domination of crosslinking reactions. Electron-beam irradiation of aqueous solutions of low pH containing 0.5–2% CMC results in hydrogel formation with 70% yield, while both concentration and dose determine their swelling properties. Time-resolved studies by laser flash photolysis clearly indicate strong pH influence on decay kinetics of CMC radicals.

1. Introduction

Permanent hydrogels based on three-dimensional networks of covalently bound hydrophilic polymer chains, able to swell in aqueous media till equilibrium, have numerous applications, for instance as soft contact lenses, wound dressings and superabsorbents (Funke, Okay, & Joos-Müller, 1998; Gulrez, Al-Assaf, & Phillips, 2011; Hoffman, 2001, 2002; Mathur, Moorjani, & Scranton, 1996; Peppas, 1986; Tanaka, 2003). Among the available methods for synthesizing hydrogels based on synthetic polymers, radiation technique plays an important role, being since long employed on a commercial scale (Rosiak, Rucinska-Rybus, & Pekala, 1988; Rosiak, 1991). Irradiation of aqueous polymer solution leads to the formation of polymer radicals, which recombine to form covalent bond between the polymer chains. As a result, permanent three-dimensional network is formed, which is hydrophilic and swellable but insoluble (Rosiak & Ulanski, 1999). This method is simple and does not require any added chemicals (initiators, crosslinking agents).

While the radiation method works well for many synthetic polymers, its application for synthesizing hydrogels based on polysaccharides (being very promising materials) is more problematic. Generally, irradiation of polysaccharides induces their degradation, resulting in chains of reduced molecular weight, and no crosslinked structures are formed (Czechowska-Biskup et al., 2007; Ershov, 1998; Ulanski & von Sonntag, 2000; Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005; Zegota & von Sonntag, 1977). Some methods have been elaborated to crosslink polysaccharides by ionizing radiation by using an additive to promote crosslinking process, such as an alkyne gas (Al-Assaf, Phillips, Williams, & Plessis, 2007; Phillips, Plessis, Al-Assaf, & Williams, 2003) or carbon tetrachloride (Ramnani, Chaudhari, Patil, & Sabharwal, 2004). These methods require additives and specific reaction conditions, therefore the processing is rather complex.

Radiation-initiated crosslinking of polysaccharides without additives by a straightforward method – irradiation proceeded in

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so called 'paste-like' state – has been developed in the laboratory of Yoshii (Fei, Wach, Mitomo, Yoshii, & Kume, 2000). Starting from carboxymethylcellulose (CMC) of various degree of substitution (DS), i.e., the average number of functional side groups per one repeating unit of D-glucose, through other derivatives of cellulose, starch, chitin and other water-soluble polysaccharides, the method became widely utilized for formation of polysaccharide hydrogels (Nagasawa, Yagi, Kume, & Yoshii, 2004; Wach, Mitomo, Yoshii, & Kume, 2002; Wach, Mitomo, Nagasawa, & Yoshii, 2003a,b; Zhao, Mitomo, Nagasawa, Yoshii, & Kume, 2003). The essential factor for hydrogel formation was high concentration of the polymer in aqueous solution, when physical proximity of chains facilitated crosslinking. Concentrations over 10%, up to even 60%, until the mixture maintains homogeneity, are commonly used. While this method is effective and can be scaled up, the necessity of preparing the substrate in the form of a highly viscous or solid paste and degassing it (such mixture usually contains air bubbles, which are difficult to remove) before irradiation makes it labor and time consuming.

In the present communication hydrogel formation by radiation-initiated crosslinking of carboxymethylcellulose chains in aqueous solution of low concentration is reported. This innovative approach, conversely to the known methods being applied either in R&D and in the industry, does not require laborious procedures, such as formulation of a paste-like state, nor utilization of additives facilitating crosslinking. Results on gel formation are supported by spectral and kinetic data on recombination of carboxymethylcellulose radicals in oxygen-free acidic aqueous solutions.

Possible applications of polysaccharide hydrogels, for instance based on CMC, include superabsorbents for agriculture and industry, and biomaterials, such as personal care absorbents or components of hydrogel dressings (Yoshii, Kume, & Murakami, 2005). Therefore, radiation technique of polysaccharide manufacturing may be preferred because a product can be sterilized simultaneously. Foremost advantages of polysaccharide hydrogels over synthetic polymers gels are the origin of substrates (sustainable resources) and product biodegradability.

2. Materials and methods

Sodium salt carboxymethylcellulose, CMC (Daicel Co., Ltd., Japan) of the degree of substitution 2.34, and $M_w = 7.4 \times 10^5$ mol g^{-1} was used as received. Solutions were made up with ultrapure water (Milli-Q, Millipore), pH was adjusted with $HClO_4$. Prior to irradiation solutions were saturated with N_2O in order to eliminate oxygen and convert hydrated electrons into $^{\bullet}OH$ radicals (Schuler, Patterson, & Janata, 1980).

Irradiation was conducted using a 6 MeV electron accelerator (ELU-6, Eksma, Russia), yielding an average dose rate of 5 kGy/min. Gel fraction (GF = mass of insoluble part per mass of initial sample) and equilibrium degree of swelling of hydrogels (EDS = mass of absorbed water divided per mass of dry gel) were evaluated in water by a method described elsewhere (Wach, Mitomo, Nagasawa, & Yoshii, 2003a,b).

Laser flash photolysis was used to study the kinetics of macroradicals decay, due to its simplicity comparing to the classical pulse radiolysis technique. An excimer laser (Lambda Physik) operated at 248 nm (KrF) with a pulse duration of 20 ns at an energy of 55 mJ/pulse was used. Aqueous solutions of CMC were filtered through a Sartorius 0.45 μm membrane and saturated with N_2O to remove oxygen. H_2O_2 (10 mM) was used as the source of hydroxyl radicals in kinetic experiments (Wach, Kudoh, Zhai, Muroya, & Katsumura, 2005). Time-resolved absorbance was followed at $\lambda = 320$ nm, as a representative wavelength to follow the decay of the sum of CMC-derived macroradicals.

3. Results and discussion

Radiolysis of water yields reactive transient species: hydroxyl radicals, hydrogen atoms and hydrated electrons. Hydroxyl radicals react with polysaccharides in dilute aqueous solution at a relatively high rate. Second-order rate constant for reaction of CMC ($M_w = 5.3 \times 10^5$ kDa) with $^{\bullet}OH$, dependent on the pH that governs the ionic state of the carboxylic moieties and the conformation of macromolecules, was determined to be $0.5–1.0 \times 10^9$ dm^3 mol^{-1} s^{-1} (where mol refers to mol of repeating units) (Wach et al., 2005). At low pH in N_2O-saturated solutions a part of hydrated electrons is converted to $^{\bullet}OH$ radicals, while the rest reacts with protons to yield hydrogen atoms (Schuler et al., 1980; von Sonntag, 1987). Both hydroxyl radicals and hydrogen atoms abstract hydrogen from carbon atoms in either D-glucose repeating unit or a side chain. This leads to the formation of polysaccharide-based radicals.

Among several radicals found on carboxymethylated polysaccharides irradiated in solution, the most stable ones present on side chains, i.e. on alpha carbon with respect to carboxymethyl group, are essential for recombination reactions that may occur in a favorable environment (Saiki et al., 2011; Wach et al., 2004, 2005). Polysaccharide-based radicals typically display a broad, featureless absorption spectrum in UV. Their decay, which may involve recombination, can be followed by pulse radiolysis or laser flash photolysis with time-resolved spectrophotometric detection.

Electrostatic repulsion of ionized carboxylic groups is the major barrier for recombination of CMC radicals. Therefore recombination at neutral or basic pH is very slow, and thus inefficient in competition with single-radical-initiated scission of glycosidic bonds. This competition and other side reactions may lead to a complex kinetic pattern, involving mixed-order kinetics followed by slow first-order decay (Wach et al., 2004). Since at such conditions the yield of scission exceeds the yield of crosslinking, the observed macroscopic result of irradiation is a low-viscosity solution of degraded polymer chains.

Protonation of the carboxyl groups, by removing the barrier of electrostatic repulsion between macromolecules, may change the dominating mechanism and kinetics of radicals decay. Indeed, as shown in Fig. 1a, the time-dependent absorbance of the radicals varies with pH. A very slowly decaying signal is recorded within several millisecond time-range at neutral pH. By lowering the pH, resulting in increasing protonation of carboxylic groups – pK_a of carboxyl groups of CMC is above 3.5 (Hoogendam et al., 1998) – an increase of the termination rate is observed. Second-order kinetics takes part in overall decay at lower pH and eventually at pH 1 the kinetics follows strictly second order. Solid line representing second-order reaction fits well to the experimental data (see the straight line in Fig. 1b). Calculated $2k = 4.8 \times 10^7$ dm mol^{-1} s^{-1} is relatively low when compared to radicals on flexible, uncharged polymer chains. It is known that recombination rate of radicals on polyelectrolyte depends strongly on the protonation state, and decreases significantly with even small number of ionized units (Görlich & Schnabel, 1973). This is a representation of bimolecular reactions, which can be either disproportionation or recombination. Since formation of macroscopic gel in the cuvette was perceptible after the photolysis, it evidenced a significant yield of the latter reaction.

In order to find out if the fast and efficient recombination of CMC radicals at low pH can lead to hydrogel formation even at low polymer concentrations, CMC solutions of 0.5, 1 and 2% wt/vol (approximately 15, 30 and 60 mM of repeating units, respectively; it should be noted that at such low concentrations CMC does not form a physical gel in acidic media) were adjusted to pH 2 with $HClO_4$, saturated with N_2O, sealed in glass ampoules and subjected to irradiation by electron beam at various doses. Formation of a gel

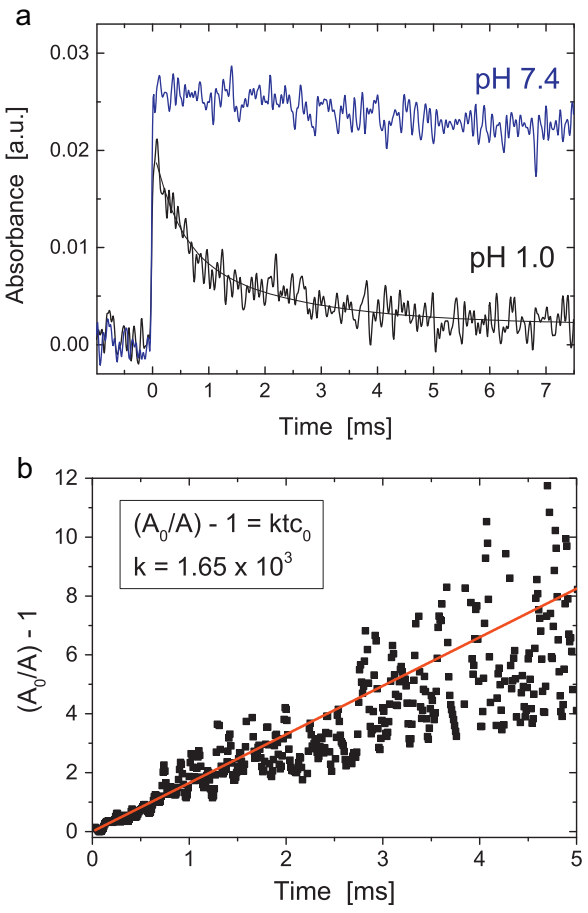

Fig. 1. Laser flash photolysis of N_2O saturated aqueous CMC solutions (20 mM) containing 10 mM of hydrogen peroxide. Decay of carboxymethylcellulose-derived radicals: (a) decay of transients recorded at 320 nm in solutions of different pH, and (b) a second-order kinetics observed at pH 1.0 (A_0, A – absorbance at maximum, and at time t). Initial concentration of radicals, c_0 equals to 3.4×10^{-5} mol dm^{-3}.

has been observed for all tested CMC concentrations at relatively low doses. This indicates that, in contrast to irradiation of dilute CMC solutions at neutral pH, and, what is interesting, in contrast to irradiation of many neutral polysaccharides in dilute solutions, here the crosslinking is fast enough to efficiently compete with degradation. Present experiments confirmed the possibility of CMC radical recombination, which resulted in insoluble network formation. These results bear some similarity to observations reported for synthetic polyelectrolytes, e.g., in deoxygenated aqueous solution of poly(acrylic acid) upon irradiation scission dominates in alkaline and neutral solutions and the prevalence of crosslinking becomes evident only at pH < 2 where the polyacid is nearly fully protonated (Ulanski, Bothe, Hildenbrand, Rosiak, & von Sonntag, 1996).

After extraction of the soluble part, swelling of the hydrogel was evaluated and the remaining insoluble fraction was obtained. Autoclaving of the obtained gels for 20 min at 121 °C was also conducted, confirming stable, covalent character of the network – no dissolving was observed. Amount of the gel, GF, is presented as a function of absorbed dose in Fig. 2. Insoluble fraction can already be found for CMC irradiated with a dose as low as 1 kGy. Literature data indicate that GF of irradiated highly concentrated CMC solutions of natural pH rises gradually at doses over the gelation dose, D_g, which for CMC of the DS 2.2 and the concentrations range 20–60% is in the range of 5–15 kGy. The maximum GF of 70–95% is reached at doses as high as 80–100 kGy (Wach, Mitomo, Yoshii, & Kume, 2001). For lower concentrations of CMC, i.e. 10%, the D_g was

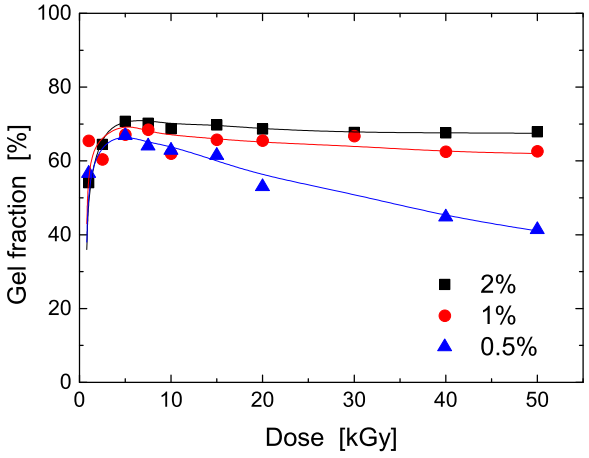

Fig. 2. Gel fraction as a function of dose in EB-irradiated CMC, N_2O-saturated solutions of pH 2 at low polymer concentrations.

about 40 kGy and the maximum GF was below 50%. The tendency found for dilute acidic CMC solutions resembles that characteristic for cellulose derivatives without ionizable substituents when irradiated at a high dose rate. GF of concentrated solutions (over 20%) of hydroxypropylcellulose, hydroxyethylcellulose or methylcellulose irradiated by electron beam increases relatively fast over the D_g and then remains relatively constant (Wach et al., 2002, 2003b). Nevertheless, the D_g for those derivatives was in the range of 4.7–150 kGy (typically over 10 kGy), which is much higher than found in the current studies on CMC (from below 1 to 2 kGy). Maximum GF for CMC solutions of concentrations 0.5–2% irradiated at low pH is achieved at about 5 kGy and remains constants up to 50 kGy (the maximum dose applied in the current investigations), except for the lowest concentration of 0.5%, for which it decreases gradually – apparently under these experimental conditions degradation prevails over crosslinking at higher doses.

The formed CMC hydrogels display swelling characteristics typical for radiation-crosslinked networks of hydrophilic polymers (Fig. 3). Equilibrium degree of swelling of gels in water is the largest, i.e., over 1000 g/g, just over the D_g, and decreases rapidly with absorbed dose. The network becomes more firm and strongly bound when crosslinking density increases with dose. This is obviously due to electrostatic repulsion that is a driving force to stretch the network up to its maximum expansion capacity determined by the crosslinking density.

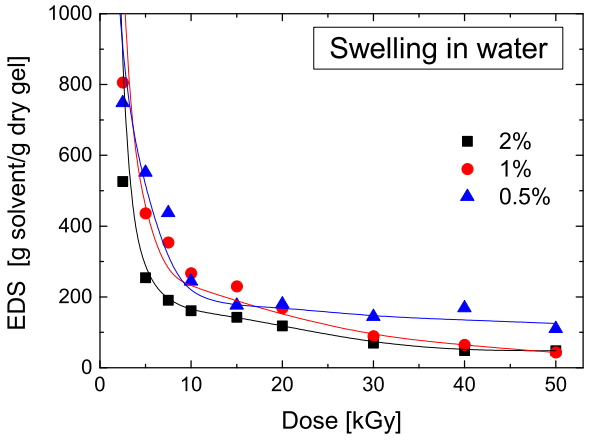

Fig. 3. Equilibrium degree of swelling (EDS) of CMC hydrogels in water as a function of dose. Hydrogels formed by EB-irradiation of CMC N_2O-saturated solutions of pH 2 at low polymer concentrations.

4. Conclusions

Electron-beam irradiation of dilute, deoxygenated aqueous solutions of carboxymethylcellulose at low pH leads, via OH-radical-induced generation and recombination of macroradicals, to the formation of permanent, covalently crosslinked macroscopic hydrogels. As demonstrated by radical decay studies using laser flash photolysis, neutralization of charges on CMC chains and the resulting reduction in electrostatic repulsion between chain segments greatly facilitates radical recombination, leading to predominance of crosslinking over degradation.

In neutral solutions, where the linear charge density is high due to deprotonated carboxylate groups, high concentration of CMC is required to impose the chains to be physically close one to each other in order to allow efficient recombination and gel formation (otherwise irradiation causes degradation). In contrast, at low pH gel formation is observed for CMC concentrations as low as 0.5% and the gelation doses are lower than 2 kGy. In these conditions, up to 70% of CMC is converted into the macroscopic gel.

Equilibrium degree of swelling for the obtained gels decreases with increasing dose. For a typical sterilization dose of 25 kGy, values in the range of 50–200 g/g can be obtained at the conditions used in this study.

We conclude that this new approach to crosslinking of anionic polysaccharides in solutions of low concentration and low pH is an attractive alternative for the known synthetic methods based on highly concentrated solutions, i.e., paste-like state polysaccharide-water systems. Straightforward preparation, easy-to-handle physical form (liquid of relatively low viscosity), no gas bubbles, less amount of polymer used to obtain firm gel, relatively low irradiation dose required – there are advantages of currently developed method.

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