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# Cationic Starch Iodophores

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**ABSTRACT:** Cross-linked cationic starches *N*-(2-hydroxyl)propyl-3-trimethyl ammonium starch chloride (CQS chloride), *N*-(2-hydroxyl)propyl-3-trimethyl ammonium starch iodide (CQS iodide), and *N*-(2-hydroxyl)propyl-3-trimethyl ammonium starch iodide–iodine (CQS triiodide) with the degree of substitution (DS) according to cationic groups from 0.04 to 0.62, as well as cross-linked starch–iodine complexes were synthesized and tested as potential antibacterial agents. Cationic starch iodine derivatives were obtained during ion exchange reaction between CQS chloride and iodide or iodide–iodine anions in aqueous solutions. $CQS_{DS\leq 0.3}$ chloride can form several types of iodine complexes, such as the blue amylose–iodine inclusion complex and ionic $CQS^{+}I^{-}\cdot(I_2)_m$ complex ($m \geq 1$). The antibacterial activity of modified starches–iodine samples against different pathogenic bacterial cultures and contaminated water microorganisms was evaluated. CQS chloride and CQS iodide were found to be bacteriostatic. A strong antibacterial activity was characteristic of CQS triiodides in which molecular iodine is present in both ionic and inclusion complexes. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 128: 4346–4354, 2013

**KEYWORDS:** polysaccharides; adsorption; crosslinking; Biopolymers & renewable polymers

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## INTRODUCTION

Formaldehyde, phenol, chlorine, iodine, and quaternary ammonium compounds are well known as effective antimicrobial agents.[1,2] However, low-molecular weight antimicrobial compounds exhibit numerous disadvantages, such as toxicity to the environment and humans as well as short-term antimicrobial ability.[3,4] In order to overcome these problems, antimicrobial polymers – polymeric biocides can be prepared by attaching antimicrobial agents to macromolecules. Polymeric biocides offer many advantages over conventional antimicrobial compounds as they are non-volatile, chemically stable, do not permeate through skin and show a reduced residual toxicity.

Antimicrobial polymers can be bioactive themselves or be able to release bioactive compounds. Quaternary ammonium or phosphonium groups containing polymers (water-soluble or insoluble) are typical examples of the first group of materials.[1,5-7] Furthermore, these materials, depending on their chemical constitution, can act as bacteriostatics or bactericides.[8] Their biological activity relies on direct contact between the macromolecules and bacteria. Studies on the bactericidal efficiency of water-soluble quaternary polyammonium salts revealed a higher activity of polymeric compounds as compared with that of monomers.[9,10]

Many literature data refer to the studies of biocides obtained from biodegradable polysaccharides, i.e., chitosan and starch. Numerous attempts have been made to use chitosan and its derivatives in food, medical, cosmetic, and textile industries.[11-15] Antimicrobial-modified starch was synthesized by covalently bonding guanidine oligomer with starch via coupling reaction.[16] One wt % of cationic starch was incorporated into paper; as a result, the growth inhibition of *Escherichia coli* and *Staphylococcus aureus* reached almost 100%.

Polymeric bactericides (those able to release bioactive materials) can be obtained by immobilizing antibacterial compounds or molecules (including molecular iodine) in polymers. The synthesis of such polymeric iodophores is advantaged by the ability of iodine and its ionic complexes, i.e., tri- or pentaiodide ($I_3^-$ or $I_5^-$), to form associates and different types of complexes (charge transfer, ionic, or inclusion) with macromolecules.

Polyvinylpyrrolidone and other synthetic polymers containing quaternary ammonium or amino groups are often used as basic

materials for the synthesis of polymeric biocides able to realease bioactive compounds. The most commonly employed iodophor is the charge-transfer complex of polyvinylpyrrolidone and iodine, also known as povidone iodine. Povidone iodine is used for pre-operative skin disinfections, wound disinfections, as an antiseptic, also in the production of antibacterial coatings, nanoparticles, and nanofibers.[17] Meanwhile, water-insoluble, stable complexes composed from strong-base exchange resins in salt form, or water-soluble polydialyldimethylammonium chloride and triiodides or pentaiodides are used for water disinfection and inhibit the growth and replication of pathogenic bacteria, spores, and viruses.[1,18,19]

Biodegradable natural polymers and their derivatives, especially cationic polysaccharides, are promising materials for the production of polymeric iodophores. Ionic complexes between positively charged chitosan macromolecules and anionic triiodide in a hydrogel form are suitable for direct application to intact skin.[20] Such complexes slowly release active iodine for disinfection of wounds. Chitosan can play a very important role in an antibacterial synergetic composition, e.g., cationic chitosan, silver nanoparticles, and iodine.[21] Chitosan, silver nanoparticles (Ag NPs) and iodine all possess antimicrobial properties individually. Meanwhile, chitosan–Ag NPs composite showed a higher antibacterial activity at a concentration lower than that of its components, i.e., chitosan and Ag NPs.

Starch iodophores can be used for technical and medical applications as the means of disinfection, components in cosmetics. Iodine can be bound to starch by forming an inclusion complex with amylose,[22] or it can be immobilized into modified starch derivatives. Cross-linked dextrin (cadexomer) can be saturated with iodine by incorporating up to 20 wt % of molecular iodine without any chemical or intermolecular interaction with the polymer.[23,24] However, a cadexomer–iodine complex containing only 0.9 wt % of iodine is used for practical applications.[25]

In stable iodine solutions, i.e., in the presence of iodide ions and at pH $< 7$, the formation of triiodide ($I_3^-$) and/or pentaiodide ($I_5^-$) ions may take place. The anionic species of iodine can interact with cationic groups of various polymers and form polymeric iodophores as ionic complexes.[26,27] Starch is a low-cost natural renewable polymer that can be cross-linked with epichlorohydrin and cationized with 2,3-epoxypropyltrimethylammonium chloride (EPTMAC) with a high reaction efficiency.[28] The obtained cationic cross-linked starches (QCS) with the degree of substitution (DS) from about 0.2 to 0.6 can adsorb anionic species of iodine from aqueous solutions. In our recent work, the equilibrium adsorption of iodine on QCS with a different DS from an aqueous iodine – potassium iodide solution has been investigated.[29] The obtained results confirmed that during adsorption on QCS, iodine could be introduced both into an inclusion complex (blue amylase – iodine complex) and into an ionic complex due to interaction with cationic groups. The amount of iodine introduced into the inclusion complex depended on the DS of QCS and could comprise 5 to 25 wt % of the total amount of iodine adsorbed on QCS. It could be suggested that the different nature of modified starch–iodine complexes could influence their antibacterial efficiency. The aim of the present work was to prepare cationic starch derivatives of different composition for complex formation with iodine and to evaluate their antimicrobial properties.

## EXPERIMENTAL

### Materials

The native potato starch (Antanavas Starch Plant, Lithuania) ($[\eta] = 0.393$ dl/kg) was dried at a temperature of 104°C before use. EPTMAC (Aldrich, Saint Louis, USA) (70%, Fluka) and epichlorohydrin (Aldrich, Saint Louis, USA) (99%, Aldrich) were used as received. $I_2$-KI and KI fixanals (Fluka, Germany) were purchased from Fluka. L-Tyrosine (Sigma - Aldrich, Germany) (98%) was obtained from Sigma-Aldrich. Sodium thiosulphate ($Na_2S_2O_3$) and sodium hydroxide (NaOH) were of analytical grade.

The bacterial strains used for antibacterial activity tests included *Listeria monocytogenes* ATCC 19117, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus epidermidis* ATCC 12228. Natural surface water of the Griciupis stream was used in water disinfection experiments. Oxoid membrane lauryl sulphate agar CM 919B (Oxoid) and plate count agar (BioChemika) were used for the cultivation of bacteria.

### Preparation of Cross-Linked Starch (CS)

The native potato starch was dispersed in water in order to obtain a 50% (wt/wt) slurry. The molecular mass of the anhydroglucoside unit (AGU) was assumed as a mole of starch. Starch macromolecules were cross-linked with 0.1 mol of epichlorohydrin per AGU in the presence of NaOH. The reaction at 45°C was completed after 24 h. Cross-linked starch (CS) was water washed and dried at 50°C.

### Preparation of Cationic Starch (QS) Chloride and Cross-Linked Cationic Starch (CQS) Chloride

Starch or prepared cross-linked starch was cationized with EPTMAC in the presence of sodium hydroxide as a catalyst (the molar ratio AGU : EPTMAC : NaOH : $H_2O$ was 1 : 0.2–0.6 : 0.04 : 16) at 45°C for 24 h.[28] After reaction, QS chloride was washed five times with isopropanol. Meanwhile, CQS chloride was washed five times with water and two times with a water–isopropanol mixture. The obtained materials were purified by Soxhlet extraction with methanol for 16 h and dried at 50°C.

The number of cationic groups in QS chloride or CQS chloride was expressed as the DS, which was calculated from the nitrogen content estimated by the Kjeldahl method:[30]

$$DS = \frac{162 \times N}{1400 - 151.5 \times N}$$

where $N$ is nitrogen content estimated by the Kjeldahl method (%), 162 is the molecular weight of the AGU, and 151.5 is the molecular weight of EPTMAC. The prepared cationic starches with a various DS were designated as $QS_{DS}$ and $CQS_{DS}$.

### Preparation of Cationic Starch Iodide and Cross-Linked Cationic Starch Iodide

Dry QS chloride or CQS chloride was poured over KI solution and mixed with a magnetic stirrer for 2 h. The molar ratio of

QS chloride or CQS chloride (according to cationic groups) and KI in a solution was 1 to 1.5. After adsorption, the suspension was filtered through a glass filter, washed with distilled water until no traces of $Cl^-$ or $I^-$ in filtrate were detected, dried, and the nitrogen content was estimated by the Kjeldahl method. Iodide content in the prepared QS iodide or CQS iodide was calculated by evaluating the nitrogen content in the samples before and after iodide adsorption.

### Equilibrium Iodide Adsorption Studies

KI solutions were prepared from KI fixanal by dilution with distilled water to get the required concentration. The pH of KI solutions was 5.4–5.6 in all experiments. 0.1 g of dry CQS chloride was placed into an Erlenmeyer flask, and 100 ml of KI solution of a desired concentration was added. The flask was stoppered and shaken for 30 min at a temperature of 35°C and a fixed shaking intensity in a thermostated water bath with the temperature control of ±1°C. Then the mixture was filtered through a glass filter, and the residual iodide concentration in solution was estimated.

The Langmuir equilibrium isotherm equation was used for the description of iodide adsorption.[31] The thermodynamic parameters such as the change in free energy ($\Delta G^o$), enthalpy ($\Delta H^o$), and entropy ($\Delta S^o$) were determined according to the commonly used equations.

### Preparation of CS–Iodine and CQS–Iodine Complexes

Iodine–potassium iodide ($I_2$-KI) solutions were prepared from $I_2$–KI fixanal (molar ratio of $I_2$ to KI was 1 to 3) by dilution with distilled water to get the required concentration. One gram of dry CS or CQS chloride (or such amount of CQS iodide) was placed into Erlenmeyer flask and 100 ml of $I_2$–KI solution of desired concentration was added. The flask was stoppered and shaken for 30 min at ambient temperature. Then the mixture was filtered through a glass filter. After the reaction, CS-iodine or CQS-iodine complexes were washed with water and dried at 50°C.

### Determination of Iodine and Iodide Content

The concentration of iodine in the samples was determined by iodometric titration with sodium thiosulphate solution, whereas iodide concentration was determined spectrophotometrically with a Unicam UV3 UV-Vis spectrometer by measuring the absorption intensity of samples at the 226 nm wavelength.

### Particle Size Measurements

The particle size distribution of starch and modified starch microgranules was determined using a Coulter LS200 particle size analyzer equipped with a Coulter fluid module (Beckman Coulter). A set of measurements was performed for each sample, and the geometric mean value of a particle diameter was calculated.

### Determination of Water Retention

A precisely weighed amount of CQS or CS (0.25 g of dry basis) was suspended in 20 ml of distilled water and stirred for 30 min at 20°C. Then the samples were centrifuged at 3000 rpm for 10 min. The supernatant was carefully removed and a sample was weighed. The retention of water (RS, g/g) was calculated according to the equation:[32]

$$RS = \frac{(W_2 - W_1)}{W_1}$$

where $W_2$ is the weight of a starch sample after supernatant removal, and $W_1$ is the weight of dry starch. Experiments were performed in triplicate, and the deviations did not exceed ±5%.

### Reactivity of Iodine Towards L-Tyrosine

The reactivity of iodine towards L-tyrosine was estimated as follows:[33] the CQS–iodine microgranules containing 0.0127 g of iodine were dispersed in 20 ml of 0.02 *M* sodium acetate solution, and 5 ml of 10 m*M* L-tyrosine solution was added. The initial concentration of both iodine and L-tyrosine in the reaction mixture was 2 m*M*. The mixture was stirred with a magnetic stirrer at room temperature. The time of mixing was taken as the beginning of the reaction. After a desired time, an excess of 0.01 *M* sodium thiosulphate solution was added and after 5 min titrated with 0.01 *M* iodine solution. Each reaction was run for at least three times, and the mean values of iodine consumption were calculated.

### Antibacterial Activity Tests

The antimicrobial activity of modified iodinated starch derivatives was evaluated by the diffusion method according to the procedure of LST EN ISO 20645:2005. Bacterial cells were incubated at 37°C for 18 h in broth, diluted, and mixed with the corresponding nutrient agar. Meanwhile, the suspension of *B. subtilis* spores was cultured at 37°C for 5 days, heat-treated at 80°C for 10 min, diluted, and mixed with nutrient agar. Ten millilitres of each mixture was spread on the plate and left to solidify at room temperature. Wells 8 mm in diameter were made in agar plates seeded with different organisms, and each well was filled with 5 mg of the test polymer and 50 μl of sterilized distilled water. All the plates were incubated at 37°C for 24 h (for 48 h in some cases), and then the diameter of the inhibition zones was measured.

### Antibacterial Activity Against Water Microorganisms

The bacterial contamination of the surface water upon treatment with the modified and iodinated starches was assessed by the shaking flask method, taking into account both the number of heterotrophic bacteria (NHB) and of coliformic bacteria (NCB) in the samples. NHB and NCB were determined following the procedures of LST ISO 6222:2000 and LST EN ISO 9308-2:2001 standards, respectively. The surface water samples were treated with different dosages of a polymer for 24 h and filtered through a sterile paper filter. Surface water samples without polymer addition were regarded as the reference samples. For NHB evaluation, 1 ml of a sample was mixed with 10 ml of the corresponding sterilized melted medium and left to solidify at room temperature. In the case of NCB, 0.25 ml of a sample was seeded on lauryl sulphate agar plate. The number of colonies was counted after the inoculated plates had been incubated at 37°C for 24 h. All experiments were performed in triplicate and the number of viable cells was determined as colony-forming units per 1 ml of a sample (CFU/ml) as follows:

$$CFU = \frac{a \cdot 10^n}{V}$$

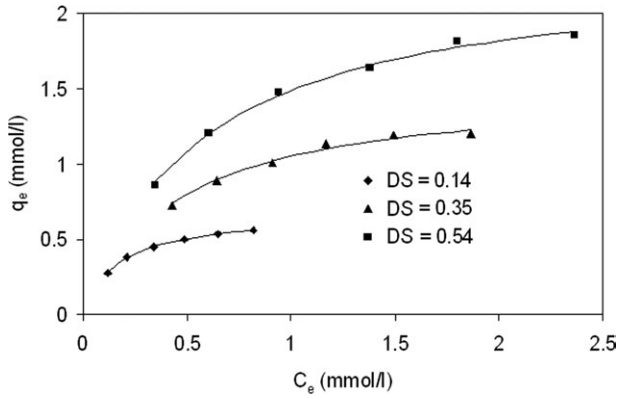


**Figure 1.** Adsorption isotherms of potassium iodide onto CQS chloride with different DS at 35°C temperature. Symbols represent experimental data and lines represent fitted curves of the Langmuir model.

where $a$ is the average number of CFU in the plate, $10^n$ is the dilution coefficient, and $V$ is the sample volume (ml).

## RESULTS AND DISCUSSION

### Characterization of Cationic Starches and Their Iodine Derivatives

**Cross-Linked N-(2-Hydroxyl)Propyl-3-Trimethyl Ammonium Starch Iodide (CQS iodide)** was synthesized during spontaneous ion exchange reaction between cross-linked *N*-(2-hydroxyl)-propyl-3-trimethyl ammonium starch chloride (CQS chloride) of different DS and potassium iodide in water at a temperature of 35°C. The obtained polycations still contained some amount of chloride counterions.

The equilibrium of iodide adsorption from KI solution on CQS chloride is best described by the Langmuir model ($R^2 > 0.99$). Iodide can interact with quaternary ammonium groups due to ion exchange between chloride and iodide. As one can see in Figure 1, the amount of adsorbed iodide increased with increasing the DS of CQS chloride. The calculated Langmuir maximum adsorption capacity data have shown that by increasing the DS of CQS chloride, the adsorption of iodide is increased from 0.87 to 0.97 mol per 1 mol of cationic groups. Iodide adsorption on CQS chloride proceeded spontaneously as indicated by the calculated values of the Gibbs free energy ($\Delta G^o$), which range from −4.06 to −5.67 kJ/mol. The negative values of $\Delta H^o$ and $\Delta S^o$ suggest that the adsorption process is exothermic and the order of the system undergoes major changes.

**Cationic Starch–Iodine (QS–Iodine) and Cationic Cross-Linked Starch–Iodine (CQS–Iodine) Complexes.** It is well known that starch amylose forms a blue starch–iodine inclusion complex in iodine–potassium iodide aqueous solutions. Meanwhile, the colour of QS iodine or QS chloride microgranules treated with aqueous potassium triiodide solution depends on the DS of modified starches (Figure 2). With increasing the DS, the colour changes from blue to yellow-brown.

The type of the obtained QS–iodine complexes can be determined by assessing the absorption spectra of aqueous QS dispersions with added potassium triiodide (Figure 3). As shown in Figure 3(a), the blue colour light ranging from about 380 to 480 nm is not absorbed by native starch or QS with a low DS and iodine derivatives. At the same iodine concentration and by increasing the DS of QS, the blue colour of the dispersion

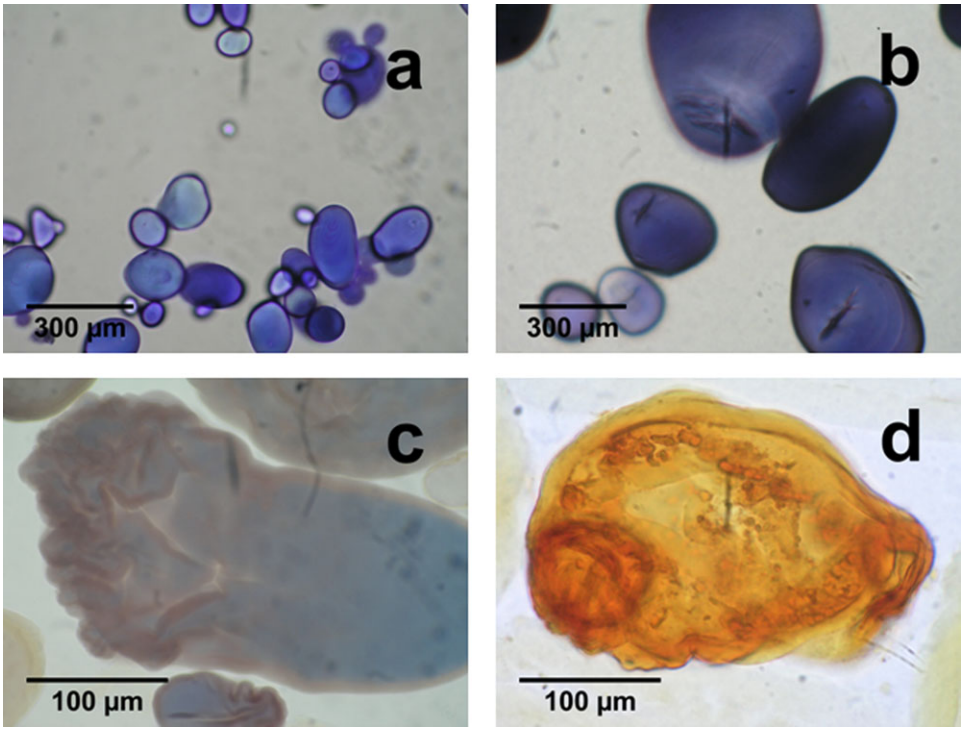


**Figure 2.** Photographs of microgranules of starch–iodine and QS–iodine complexes: a, starch–iodine; b, QS DS = 0.075-iodine; c, QS DS = 0.32-iodine; and d, QS DS=0.61-iodine. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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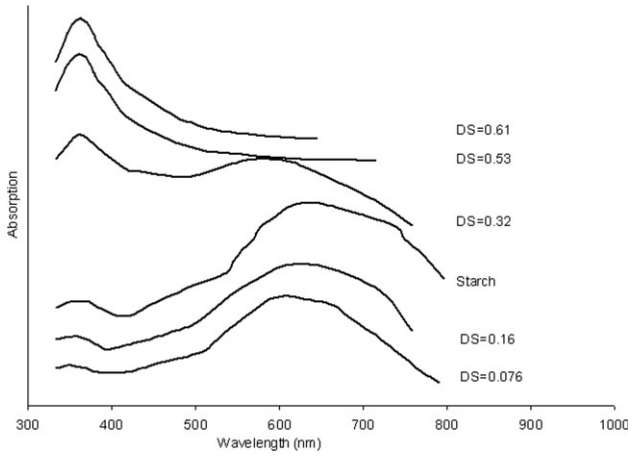


**Figure 3.** Absorbance spectra of aqueous native starch–iodine and QS–iodine dispersions depending on the DS of QS.

gradually diminishes (Figure 4). Some amount of the blue starch–iodine complex and higher content of yellow-brown material are present in the QS microgranules with DS = 0.32 after treatment with potassium triiodide solution [Figure 2(c)]. In the latter material, iodine is incorporated into the polyionic complex through interaction with the *N*-(2-hydroxyl)propyl-3-trimethyl ammonium groups. When DS > 0.32, the QS–iodine dispersions absorb light in the blue-green region of the spectrum (Figure 3), and the colour of dispersion becomes yellow-brown, which is typical of the ionic complex,[19] e.g., the $QS^{+}{}_{DS=0.61}I^{-}{\cdot}(I_2)_m$ dispersion [Figure 2(d)]. The above results indicate that modified starches with cationic groups and iodine can form several types of complexes—a blue amylose–iodine inclusion complex,[22,34] and ionic $QS^{+}I^{-}{\cdot}(I_2)_m$ or $CQS^{+}I^{-}{\cdot}(I_2)_m$ (Figure 5) complexes. The content of these complexes depends on the DS of cationic starch.

Assessment of iodine adsorption from aqueous potassium triiodide solution on cationic starches with different DS has shown that QS and CQS polycations with the DS ranging from 0.2 to 0.54 can attach around 2.3–2.4 mol of iodine per 1 mol of

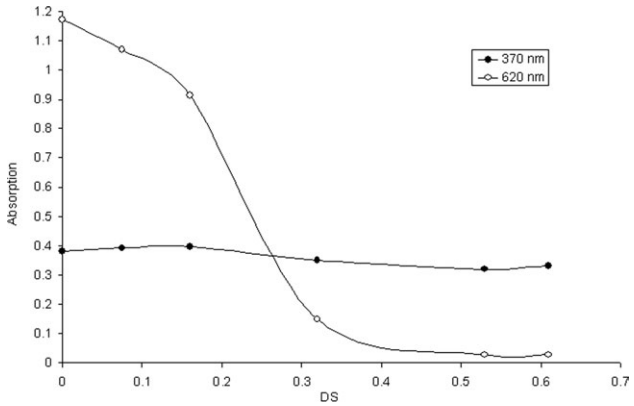


**Figure 4.** The absorbance intensity of aqueous native starch–iodine and QS–iodine dispersions at 370 nm (black circles) and 620 nm (white circles) wavelengths depending on the DS of QS.

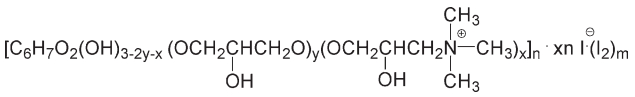


**Figure 5.** Ionic complex of cationic cross-linked starch and iodine derivatives.

cationic groups. Thus, molecular iodine in CQS can be incorporated into ionic complexes of various stoichiometry as follows:

$$CQS^{+}Cl^{-} \text{ or } CQS^{+}I^{-} + I_3^{-} \longrightarrow CQS^{+}I^{-} \cdot I_2 + Cl^{-} \text{ or } I^{-},$$
$$CQS^{+}I^{-} \cdot I_2 + I_3^{-} \longrightarrow CQS^{+}I^{-} \cdot I_2 \cdot I_2 + I^{-}.$$

By washing cationic starch-pentaiodide with water, a molecule of iodine is released, and the complex can transform into cationic starch–triiodide. The cross-linked cationic starch–triiodide complex $(CQS^{+}I^{-}{\cdot}I_2)_m$ could be easily obtained by dispersing dry CQS chloride microgranules in potassium triiodide solution in which two iodine equivalents are supplied per one cationic group of modified starch.

The ion exchange reaction is responsible for conformational changes of the polycation, followed by changes in the size of modified starch microparticles (Figure 6) and affinity to water (Figure 7). The microgranules of native potato starch with the average diameter of 36.2 $\mu$m (curve 1 in Figure 6) are several times smaller as compared with water-swollen microparticles of crosslinked cationic starch chloride or cationic starch iodide (curves 4 and 5 in Figure 6). The size of cross-linked cationic starch microgranules was also found to depend on the counterion of the cationic group. By changing chloride as a counterion with iodide or triiodide, the diameter of the microgranules of cationic starch derivatives is reduced (Figure 6) due to changes in the conformation of CQS macromolecules. Alongside with the compactization, iodine increased the hydrophobicity of cationic starch microgranules because of the reduced hydration of functional groups in iodinated cationic starch. The water retention of native or crosslinked starches is much lower as compared with that of cationic starches (Figure 7). Meanwhile, the affinity of CQS chloride to water is higher than that of CQS iodide or CQS triiodide.

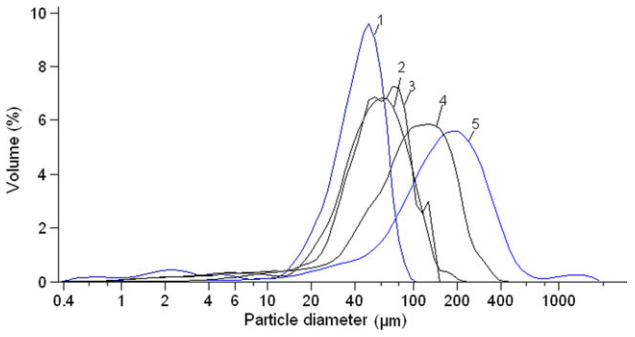


**Figure 6.** Size distribution by volume of native starch (1) and modified starch microgranules: 2, CQS DS = 0.32 iodide; 3, CQS DS = 0.33 triiodide; 4, CQS DS = 0.33 chloride; and 5, QS DS = 0.33 iodide. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

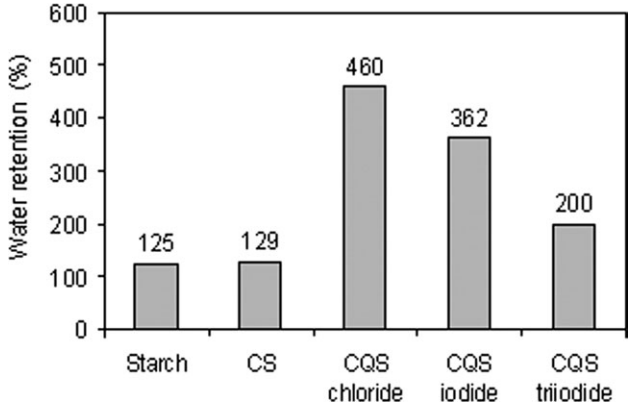


**Figure 7.** Water retention in the microgranules of native starch, CS, CQS DS = 0.29 chloride and its iodine derivatives.

### Antibacterial Assessment of Cationic Starches and Their Iodine Derivatives

The antibacterial activity of QS and CQS triiodides and the CS–iodine inclusion complex was evaluated. It is worth mentioning that CQS triiodide can contain three potential antimicrobial species, namely a quaternary ammonium group and molecular iodine incorporated in both the ionic and/or inclusion complexes. The antimicrobial capacity of modified starches against pathogenic bacterial cultures was assessed by the diffusion method (Table I). A strong antibacterial activity was characteristic of ionic CQS–iodine complexes (CQS triiodides) produced from CQS with DS from 0.21 to 0.62 (Table I, samples Nos. 5–7). The $CQS_{DS=0.62}$ triiodide was effective on all test species despite their different sensitivity and morphological properties. The inhibition zone diameter ranged within 12–42 mm and revealed the least effect on gram-positive *L. monocytogenes* bacteria (the inhibition ring 12 mm). A higher sensitivity was typical of *E. coli* and *S. aureus* species because they showed large and distinct inhibition zones 35–42 mm in diameter. No difference for both *B. subtilis* vegetative cells and endospores was found, the inhibition ring being 26 mm in diameter. The $CQS_{DS=0.3}$ triiodide was also very effective against all test bacterial species. The sensitivity of different bacteria was very similar; however, the difference in the sensitivity of *B. subtilis* vegetative cells and endospores was obvious: the inhibition zone diameter for endospores was by about 15% smaller than that for the cells. Similar tendencies were observed while assessing the antibacterial efficiency of $CQS_{DS=0.21}$ triiodide. In this case, the highest immunity was characteristic of *P. aeruginosa* bacteria with the inhibition zone diameter of only 19 mm. Meanwhile, the difference in sensitivity for *B. subtilis* vegetative cells and endospores was more prominent: the inhibition zone diameter for cells was by about 25% larger than for spores. It is worth noting that the antibacterial efficiency of $CQS_{DS=0.21}$ triiodide and $CQS_{DS=0.3}$ triiodide preparations, in which molecular iodine is included in both ionic and inclusion complexes, against some microorganisms is higher when that of only ionic complex ($CQS_{DS=0.62}$ triiodide).

Samples Nos. 1 and 2 (Table I) are CS–iodine inclusion complexes, and samples Nos. 3 and 4 are complexes of iodine with CQS with a low DS. All these complexes are blue in colour

**Table I.** Antimicrobial Activity of Modified Starches and Their Iodine Derivatives against Different Microorganism Species (Diffusion Method)

| No | Sample | $I_2$ content in the sample, wt % | *Inhibition ring diameter, mm* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *E. faecalis* | *B. subtilis* vegetative cells | *B. subtilis* endospores | *P. aeruginosa* | *S. typhimurium* | *S. aureus* | *E. coli* | *L. monocytogenes* |
| 1 | CS–iodine | 2 | 0 | 17.3 ± 0.5 | 12.0 ± 0.0 | 0 | 0 | 40.0 ± 0.0 | 11.0 ± 0.0 | 40.0 ± 0.0 |
| 2 | CS–iodine | 10 | 0 | 18.0 ± 2.9 | 13.0 ± 0.8 | 0 | 0 | 40.0 ± 0.0 | 11.0 ± 0.0 | 40.0 ± 0.0 |
| 3 | $CQS_{DS=0.04}$ triiodide | 2 | 0 | 23.7 ± 1.7 | 17.0 ± 0.0 | 0 | 0 | 40.0 ± 0.0 | 13.3 ± 1.4 | 14.0 ± 0.0 |
| 4 | $CQS_{DS=0.04}$ triiodide | 10 | 0 | 35.3 ± 3.4 | 23.0 ± 0.8 | 9.0 ± 0.0 | 17.0 ± 0.0 | 40.0 ± 0.0 | 23.7 ± 4.1 | 14.0 ± 0.0 |
| 5 | $CQS_{DS=0.21}$ triiodide | 22 | 34.0 ± 0.0 | 48.5 ± 2.5 | 36.3 ± 2.9 | 19.0 ± 0.8 | 25.0 ± 4.1 | 40.0 ± 0.0 | 25.0 ± 0.0 | 28.0 ± 0.8 |
| 6 | $CQS_{DS=0.3}$ triiodide | 20 | 35.0 ± 0.0 | 35.0 ± 0.0 | 30.0 ± 0.0 | 33.7 ± 2.5 | 32.0 ± 0.0 | 40.0 ± 0.0 | 42.7 ± 2.1 | 29.0 ± 0.8 |
| 7 | $CQS_{DS=0.62}$ triiodide | 22 | 30.0 ± 0.5 | 26.0 ± 1.6 | 26.0 ± 1.6 | 18.3 ± 0.5 | 27.3 ± 2.1 | 35.0 ± 0.0 | 42.0 ± 0.0 | 12.0 ± 0.0 |

**Table II.** Antibacterial Activity of QS (DS = 0.33) and CQS (DS = 0.33) and Their Iodine Derivatives against NCB and NHB Bacteria in Water

| | NCB/NHB ratio expressed as CFU/ml × $10^{-3}$ The concentration of starch derivative in water, g/l | | | | | | |
|---|---|---|---|---|---|---|---|
| Sample | 0 | 0.1 | 0.3 | 0.5 | 1.0 | 3.0 | 5.0 |
| QS chloride | 5.7/25.5 | – | – | 0.5/3.8 | 0.4/2.5 | 0.5/2.3 | 0.3/2.2 |
| CQS chloride | 5.6/27.1 | – | – | 0.5/4.0 | 0.4/3.5 | 0.3/2.5 | 0.3/2.0 |
| CQS iodide | 5.3/28.5 | – | – | 3.3/7.8 | 2.1/7.5 | 1.8/8.4 | 1.7/9.5 |
| CS–iodine | 7.0/31.3 | – | – | 3.8/3.8 | 3.0/3.0 | 2.6/2.6 | 1.0/1.0 |
| QS triiodide | 6.1/36.3 | 0/0.05 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |
| CQS triiodide | 6.8/33.5 | 0.01/0.05 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 |

(Figure 2). The content of immobilized iodine in such complexes is 5 to 10 times lower.[29] A positive inhibition was produced by these samples (Nos. 1–4) for some of the test bacterial species; however, the difference in sensitivity was more obvious. While assessing species with different morphological properties, a higher sensitivity of *B. subtilis* vegetative cells than of endospores was found again. A higher sensitivity towards low iodine content samples was typical of gram-positive bacteria species, meanwhile *P. aeruginosa* and *S. typhimurium* bacteria remained viable in the environment of the blue starch amylose–iodine inclusion complex. Thus, the effect of modified starch–iodine complexes is much more pronounced against gram-positive bacteria species in which cell wall permeability is much higher because of the thin peptidoglycan layer.

The antimicrobial activity of cross-linked cationic starch and iodinated modified starch derivatives against bacteria species in natural surface water samples was evaluated also by the shaking flask method (Table II). The number of NHB and the number of NCB were determined upon treating water samples with starch derivatives. Cationic starches with quaternary ammonium groups and chloride or iodide as counterions were found to be bacteriostatics rather than bactericides. The literature sources provide different opinions about the effect of counterions on the antimicrobial efficiency of polymeric bactericides.[33,35] As regards our results, the bacteriostatic effect of cationic starches was increasing in the following order: CQS iodide < CQS chloride ≤ QS chloride. Consequently, the antibacterial activity was reduced by screening quaternary ammonium groups with iodide counterions.

As could be expected, a strong bactericidal activity against water microorganisms was characteristic of cationic starch–triiodide complexes (Table II). Bacteria were instantly killed upon treating the samples with both CQS triiodide and QS triiodide. Even after several minutes of the contact (results not presented), no viable heterotrophic and coliformic microorganisms were present in water. The bactericidal efficiency of these cationic starch derivatives reached 100% when the concentration of a bactericide was above 0.1 g/l.

The antibacterial efficiency of CQS iodine derivatives should be related to iodine release from the complexes. Pictures of some samples after test carried out by the diffusion method are presented in Figure 8. The growth of microorganisms was largely suppressed in the case of CQS–iodine. The brown colour was still characteristic of the CQS triiodide complex after 24 h of incubation [Figure 8(a)]. In a bacteria-containing medium, CQS triiodide gradually loses its distinctive brown colour until all iodine is consumed [Figure 8(b)]. It is evident that molecular iodine is released from starch iodophore and reacts with the proteins of the bacterial cells. The bacterical efficiency of iodine is assumed to involve several different reactions.[21] First, iodine can oxidize the sulfhydryl group (SH) of the amino acid

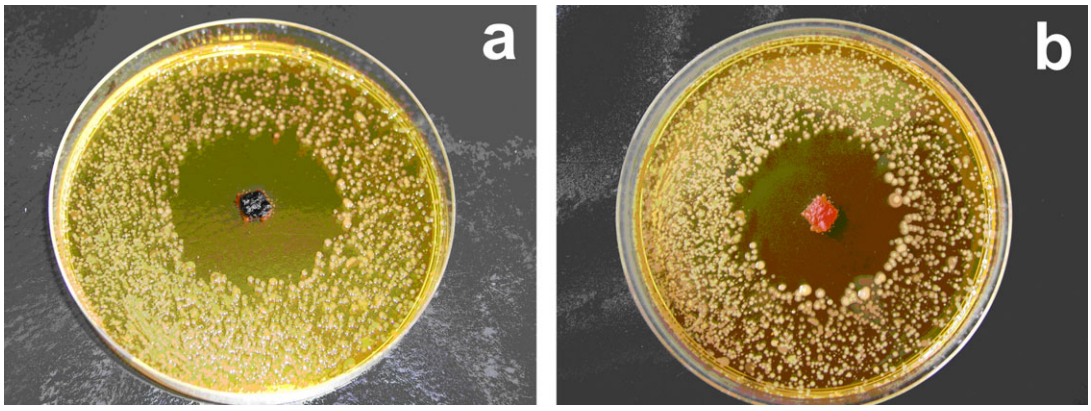


**Figure 8.** Inhibition zone of CQS DS = 0.3 triiodide sample against NCB after: a, 24 h; and b, 48 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

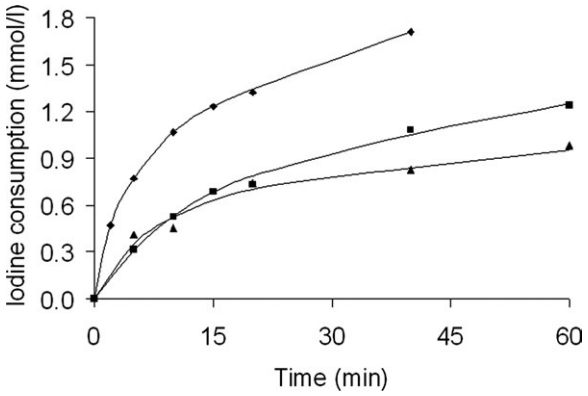


**Figure 9.** Iodine consumption in reaction with L-tyrosine in potassium triiodide solution (diamond) and starch iodophore samples: square, CQS DS = 0.33 triiodide; and triangle, CS-iodine. The initial concentration of iodine and L-tyrosine in solution was 2 mmol/l.

cysteine, thereby interfering with the formation of disulfide (—S—S—) bridges, which are important in protein folding. Second, *N*-iododerivatives block a hydrogen bond in certain amino acids or nucleotides, e.g., in lysine, histidine, arginine, adenine, cytosine, and guanine. As a result, a lethal disorder due to the impaired functioning of proteins and other biomolecules may occur. Third, iodine can react with the phenolic group of tyrosine, forming mono- or diiododerivatives. Last, iodine can react with the carbon-carbon double bond of unsaturated fatty acids, leading to changes in membrane functions.

The bactericidal efficiency of cationic starches and iodine derivatives is related to the amount of iodine released into bacterial environment. The reactivity of iodine released from the CS–iodine inclusion complex or CQS triiodide and potassium triiodide solution towards L-tyrosine as a component of proteins was examined. Figure 9 shows the dynamics of iodine consumption in reaction with L-tyrosine for different antibacterial preparations. When the CQS triiodide complex was used as an iodination reagent, iodine consumption was more rapid as compared with that of CS-iodine; however, iodine consumption was highest in the case of potassium triiodide solution.

The consumption of iodine released from an iodine preparation in the reaction with L-tyrosine correlated very well with antimicrobial tests performed with the same preparations. The antibacterial activity of samples containing the same iodine concentration is presented in Table III. As could be expected, the strongest antibacterial effect was identified in the case of potassium triiodide and was caused by the highest content of free iodine molecules in solution. Meanwhile, the bactericidal efficiency of starch iodophores was determined by the nature of the modified starch-iodine complex. The CQS triiodide was most effective among starch iodophores.

Thus, the antimicrobial efficiency of starch iodophores is determined by the stability of the preparations in the microbial environment and the amount of free released iodine. The results of the present study give a strong presumption for the production of starch iodophores with desirable technological properties

**Table III.** Antimicrobial Activity of Iodine-Containing Samples against Different Bacteria Species (Diffusion Method)

| Sample[a] | Inhibition ring diameter, mm | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *P. aeruginosa* | *S. epidermidis* | *L. monocytogenes* | *S. typhimurium* | *E. coli* | *B. subtilis* | *E. faecalis* | *M. luteus* | *S. aureus* |
| CS–iodine | 16.0 ± 1.6 | 32.3 ± 1.2 | 32.7 ± 1.7 | 34.7 ± 3.7 | 21.7 ± 0.5 | 34.7 ± 0.5 | 37.3 ± 1.7 | 40.7 ± 2.5 | 40.7 ± 2.5 |
| $CQS_{DS=0.3}$ triiodide | 28.7 ± 1.2 | 39.0 ± 2.2 | 70.0 ± 4.3 | 61.7 ± 3.5 | 74.7 ± 4.1 | 46.7 ± 3.5 | 76.7 ± 2.5 | 48.0 ± 1.6 | 77.7 ± 1.2 |
| Potassium triiodide solution | 39.3 ± 1.2 | 54.3 ± 3.7 | 72.0 ± 3.3 | 80.0 ± 4.3 | 75.3 ± 4.1 | 64.7 ± 3.7 | 78.7 ± 1.9 | 51.3 ± 1.9 | 75.3 ± 2.5 |

[a]Iodine content in the samples was 0.65 mg.

from cationic starches with a different content of *N*-(2-hydroxyl)propyl-3-trimethyl ammonium groups.

## CONCLUSIONS

In the present study, novel antimicrobial starches were successfully prepared from cross-linked *N*-(2-hydroxyl)propyl-3-trimethyl ammonium starch (CQS) chloride by ion exchange with iodide or triiodide in solution at ambient temperature. The microgranules of CQS iodide and triiodide were more hydrophobic and less water-swellable than those of CQS chloride. Based on the experimental findings, the following conclusions were made:

1. CQS chloride with DS $\leq$ 0.3 forms two kinds of iodine complexes: a blue amylose–iodine inclusion complex and an ionic $CQS^{+}I^{-}\cdot(I_2)_m$ complex ($m \geq 1$). The content of attached iodine in the complexes depends on the DS of CQS. The cationic starch–triiodide complex is more stable than the cationic starch–pentaiodide complex, however, it could release iodine into the aqueous solution of the iodine acceptor.
2. Starch derivatives containing quaternary ammonium groups are bacteriostatics and according to their increasing antibacterial activity may be arranged in the following order: CQS iodide < CQS chloride < QS iodide.
3. CQS triiodide showed a higher antibacterial activity against pathogenic bacteria than did only cross-linked starch–iodine complexes. The highest antibacterial activity was characteristic of CQS triiodides in which molecular iodine is included in both ionic and inclusion complexes. The antibacterial effect of cationic starch iodophores was much higher against gram-positive bacteria species.
4. The results of antimicrobial tests correlated with the results of L-tyrosine reaction with iodine released from modified starch complexes and showed the antimicrobial efficiency of starch iodophores to depend on the stability of preparations in the microbial environment.

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