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Photobactericidal plastic films based on cellulose esterified by chloroacetate and a cationic porphyrin

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ABSTRACT

The synthesis and characterisation of pyridinium porphyrinic chloroacetyl cellulose ester chlorides, where photosensitizing agents are covalently bounded to the polymeric chain, is presented in this paper. First, cellulose was homogenously converted into chloroacetate cellulose ester in DMAc/LiCl solvent by using chloroacetyl chloride. The complete substitution of cellulose was achieved using 7 equiv of chloroacetyl chloride for a 2 h reaction at room temperature. The absence of base did not prove detrimental to reaction. The grafting of monopyridyltritolylporphyrin onto chloroacetate cellulose ester was then realised by alkylation of the photosensitizer in DMF. These new plastic films were found to be thermostable up to 55 °C; higher temperatures led to progressive deacetylation. First results of their photobactericidal activity against *Staphylococcus aureus* and *Escherichia coli* strains are very encouraging. Such materials could find applications in medical environments as an alternative to overcome the rampant bacterial multiresistance to classical antibiotics.

1. Introduction

Sever health hazards and diseases can be induced by the proliferation and adhesion of bacteria on the surfaces of numerous materials.[1] In consequence, alternative bactericidal and bacteriostatic materials preventing propagation and development of microorganisms on material surfaces must be developed.[2–6]

Photodynamic antimicrobial chemotherapy (PACT) is a recently developed technique based on the selective accumulation of a photosensitizer in targeted cells followed by their illumination with visible light. Photoactivation of the dye initiates chemical responses that proceed through two possible oxidative mechanisms. Type I photochemical mechanism leads to the building of free radicals[7] whereas type II mechanism results in the formation of singlet oxygen.[8,9] These species react with almost every cell component and can generate a number of harmful by-products such as reactive oxygen species (ROS)[10] that, in turn, induce cell damages and may ultimately lead to cell death. PACT appears as a useful alternative to defeat rampant bacterial multiresistance to classical antibiotics, particularly in hospitals.[11–14] A requisite of PACT is the binding of the photosensitizer to the bacterial cell wall prior to its penetration into cells.

However, this is not an obligation since Bezman[15] showed that illumination of *Escherichia coli* in presence of Rose Bengal-coated polystyrene beads resulted in bacterial death. Midden and Wang[16] subsequently demonstrated that a light-induced flow of singlet oxygen was responsible for this cell inactivation. With regard to these results, 'photobactericidal surface' emerged as a new concept. This concept relies on the incorporation of photosensitising molecule in a material and its further illumination that leads to a flow of singlet oxygen, thus providing photobactericidal properties to the material surface. Porphyrins and other tetrapyrroles were shown to be efficient PACT photosensitizers.[17,18] Cationic porphyrins proved more efficient than neutral and anionic ones, more especially against Gram-negative bacterial species.[19]

Porphyrins covalently bound to polymeric chains should lead to a material possessing an intrinsic and long-lasting antimicrobial activity. In a previous paper,[20] we have shown that photobactericidal plastic films could be obtained by esterification of cellulose with protoporphyrine IX and lauric acid. Because of their higher efficiency and in order to obtain material plastic with better photobactericidal activity, we planned to prepare cationic porphyrin derivatives. We present in this paper the synthesis and characterisation of pyridinium porphyrinic chloroacetyl cellulose chlorides starting from chloroacetyl cellulose esters and monopyridyltritolylporphyrin. Preliminary results showing photoinactivation of *E. coli* and *Staphylococcus aureus* strains by these new plastic films are also described.

2. Synthesis

Among many ways used to obtain cellulose esters, we chose the *N*,*N*-dimethylacetamide/lithium chloride (DMAc/LiCl) solvent

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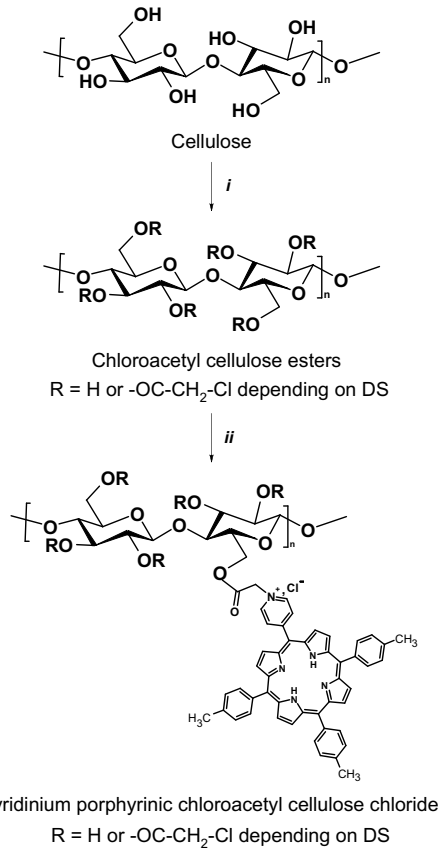


Scheme 1. Synthesis of porphyrinic plastic films. Reagents and conditions: (i) $ClCH_2COCl$, DMAc/LiCl, rt, 2 h; (ii) monopyridyltritolylporphyrin, $DMF_{anhydrous}$, 55 °C, 24 h.

system. Cellulose dissolved in DMAc/LiCl was reacted with chloroacetyl chloride according to a method previously described,[21] leading to halogenated plastic film (Scheme 1). Thus; cellulose reacted with 6 equiv of chloroacetyl chloride per AGU at room temperature. After 2 h of reaction under stirring, the modified polymer was isolated by precipitation with water. Purification was achieved using successive dissolution–precipitation cycles with THF and water, respectively.[22] Plastic films were then obtained by casting.

Monopyridyltritolylporphyrin was synthesised according to the method described by Little.[23] Condensation of the corresponding aromatic aldehydes with pyrrole in propionic acid gave after column chromatography purification the desired porphyrin in 6% yield (Scheme 2). This modest yield is usual for this kind of reaction.

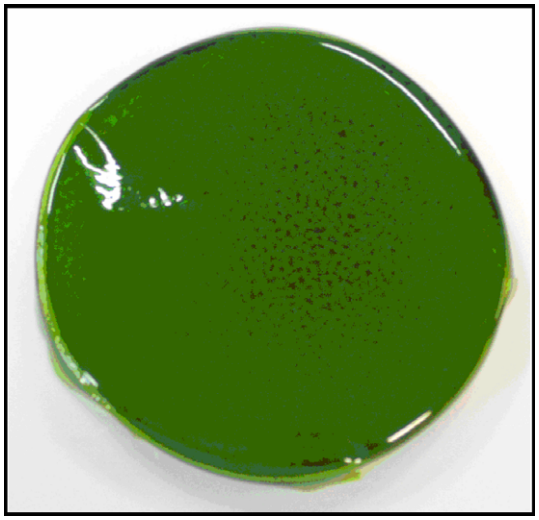


Figure 1. Picture of porphyrinic plastic film synthesised.

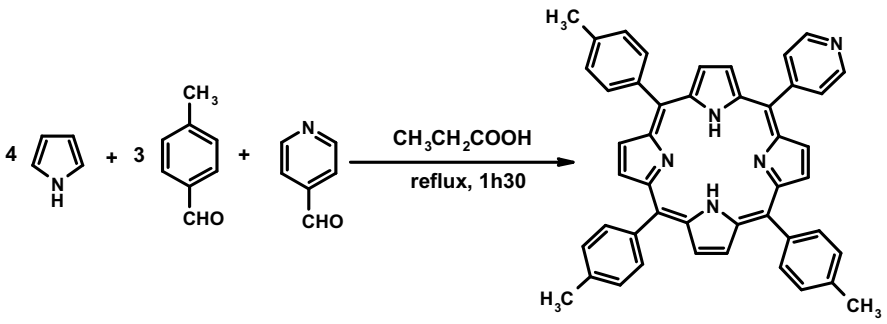
The next step of our work was the grafting of the photosensitizing agent on chloroacetyl cellulose esters. The plastic film previously synthesised (DS = 3) was dissolved in anhydrous DMF and reacted with monopyridyltritolylporphyrin, at room temperature for 24 h (Scheme 1). Porphyrinic plastics were isolated by precipitation with chloroform and purified with successive dissolution–precipitation cycles using THF and chloroform, respectively. The porphyrinic plastic films were then obtained by casting in a Petri dish. We present in Figure 1 a picture of such a plastic film.

3. Results and discussion

3.1. Synthesis of chloroacetyl cellulose

FTIR spectroscopy was used to monitor the reaction and compare chloroacetylated to unmodified cellulose. The appearance of the signal around 1750 cm^{-1} (C=O) corresponding to the ester function was observed and confirms the efficiency of acylation. In addition and as expected, increases of the signal around 2900 cm^{-1} (methylene of the chloroacetyl group) was found correlated with the decrease of the signal around 3400 cm^{-1} (hydroxyl groups).

^1H NMR spectra recorded in DMSO-d_6 displayed glucidic protons between 5.2 and 3 ppm and a singlet at 4.3 ppm attributed to the methylene protons of chloroacetyl groups. However, overlapping of these signals prevented their use for the determination of DS. In consequence, DS were calculated from elemental analysis data using carbon percentage.



Scheme 2. Synthesis of monopyridyltritolylporphyrin according the Little method.[23]

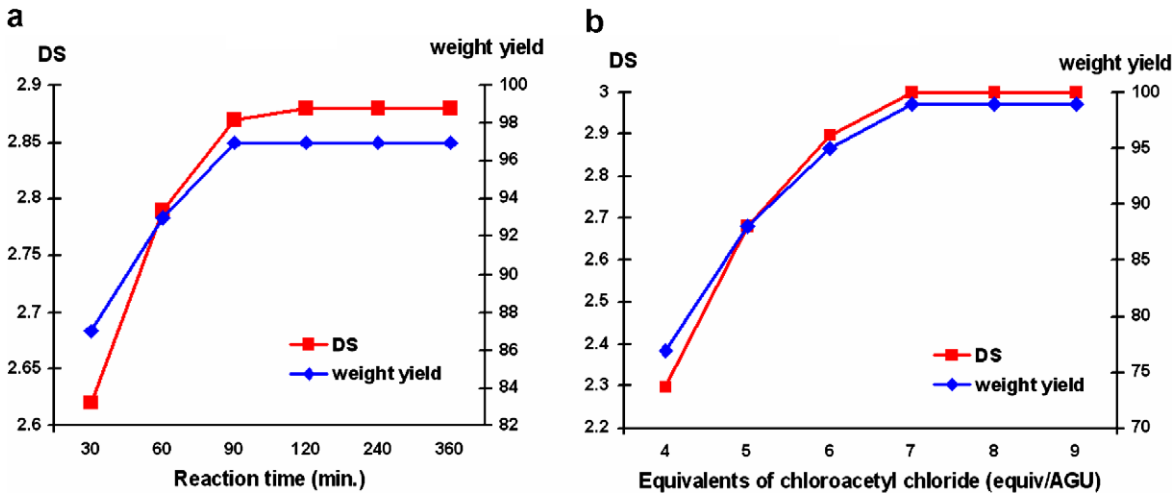


Figure 2. (a) Weight yield and DS versus reaction time and (b) weight yield and DS versus chloroacetyl chloride stoichiometry.

Study of the acylation reaction was first conducted in order to determine optimal parameters for the synthesis of a plastic film with a maximal DS in a high yield. Figure 2 summarises results in terms of DS and weight yield. A kinetic study was first achieved by reacting cellulose with 6 equiv of chloroacetyl chloride per AGU for reaction times between 30 min and 6 h at room temperature. Results showed first that chloroacetyl cellulose esters obtained displayed DS and weight yield higher than 2.6 and 87%, respectively, after 30 min reaction only (Fig. 2a). These two parameters increase with reaction times 30–90 min and reach a plateau around DS = 2.8 and yield = 97%. These high values and short reaction times can be explained by the strong reactivity of acetyl chloride due to withdrawing effect of the chloride atom in the α-position of the carbonyl group.

In addition, this strong reactivity allows the reaction to proceed at room temperature and in absence of proton-trapping bases such as pyridine or DMAP, with no detrimental effect of acidity on the polymer backbone. These conditions contrast with other acylation reactions that use less reactive agents like lauroyl chloride and which thus need heating. Combination of heat and protons accumulated by acylation reaction would lead to a progressive hydrolysis of the glycosidic bonds, hence the mandatory use of proton-trapping bases.[24] In consequence; a reaction time of 2 h has been hold for the following of the study.

Another series of experiments was conducted using amounts of chloroacetyl chloride between 2 and 9 equiv per AGU at room temperature for 2 h (Fig. 2b). DS and weight yield increase with chloroacetyl chloride amounts between 4 and 7 equiv. Full substitution of the polymer was obtained when 7 equiv were used resulting in a nearly maximal weight yield. It is to notice that only a slight excess of acylating agent (4 equiv/AGU) allows obtaining plastic films with a relatively high DS (2.3).

Other trials using 2 and 3 equiv of chloroacetyl chloride were conducted too. In these cases no product was isolated following the water precipitation step, due to the solubility of low DS esters in the DMAc/LiCl/water mixture. With care about reproducibility and homogeneity, we have always used samples taken from a film with DS close to 3 obtained from 5 g of cellulose reacted with 7 equiv/AGU of chloroacetyl chloride for 2 h at room temperature, for the next step of our work.

3.2. Synthesis of the photosensitizer

Monopyridyltritolylporphyrin was characterised by UV–visible, MALDI, and ^{1}H NMR spectroscopies. It was found to display classic electronic spectrum of *meso*-arylporphyrin. The Soret band near 420 nm and the four less intense Q bands present an *etio* outline. Its mass spectrum displays one main peak (protonated molecule MH^+). ^{1}H NMR spectrum recorded in DMSO-d_6 displays classical signals and figures at corresponding chemical shifts.

3.3. Synthesis of pyridinium porphyrinic chloroacetyl cellulose chlorides

FTIR spectra of porphyrinic and non-porphyrinic chloroacetyl cellulose esters are indistinguishable. UV–visible spectra of porphyrinic plastic recorded in THF present a similar allure to the spectrum of free porphyrin in solution in the same solvent (Fig. 3). It is to notice that all bands are slightly red shifted. The Soret band is found at 420 nm and the four less intense Q bands at 650, 595, 552 and 517 nm, respectively. This phenomenon has already been described and is classical for cationic porphyrin derivatives.[25] However, the spectra of porphyrinic plastic present an *etio* outline too. Moreover, the absence of shoulder of the Soret band traduce the absence of interaction between grafted macrocycles or π-stacking, because of the law value of porphyrin content, no major modification was observed on the UV–visible spectra. ^{1}H NMR spectra recorded in DMSO-d_6 display porphyrinic protons between 9.5 and 7.5 ppm, glucidic protons between 5.2 and 3 ppm and the methylene protons of chloroacetyl groups at 4.3 ppm. We can notice that the quaternisation of pyridylic nitrogen atoms induces the slight displacement of β-pyrrolic and pyridylic proton signals to down fields compared to free porphyrin (Table 1). The photosensitizer content of synthesised porphyrinic plastic films was determined by using the molar extinction coefficient of the Soret band. Optimal alkylation reaction parameters were firstly defined to synthesise porphyrinic plastic films of different photosensitizer contents with respect to porphyrin content. The porphyrin content was studied as a function of temperature, reaction time and photosensitizer stoichiometry. The experiments were performed by reacting 500 mg of chloroacetylated cellulose ester (DS = 3) with 14 mequiv of porphyrin per AGU. Results are presented in Figure 4.

The effect of temperature on porphyrin content was evaluated between 25 and 80 °C for 24 h. Results show that porphyrin content increased with heat activation from 0.06 to 0.19 for temperature up to 55 °C (Fig. 4a). These relatively low values are due to the steric hindrance of the porphyrinic macrocycle toward cellulosic backbone and to the low amount of porphyrin allowed to react (14 mequiv/AGU). For temperatures above 55 °C, the porphyrinic

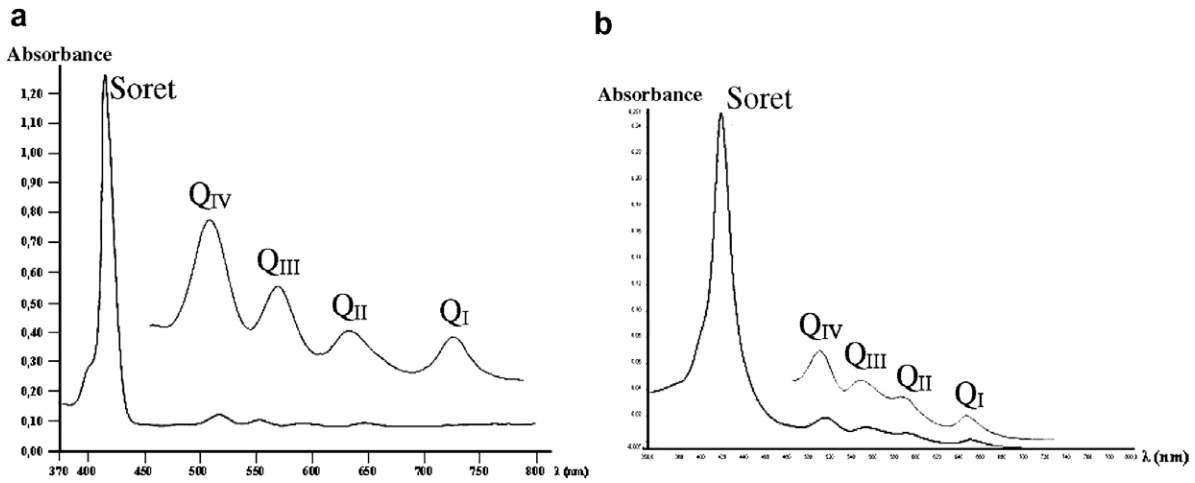


Figure 3. UV–visible spectra of free porphyrin (a) and porphyrinic plastic film (b) in THF.

Table 1
Effect of the quaternisation of pyridylic nitrogen on chemical shifts of pyrrolic and pyridylic protons

H	Monopyridyltritolylporphyrin	Porphyrinic plastic film	$\Delta\delta$ (porphyrinic plastic film – monopyridyltritolylporphyrin)
Pyrrol			
2,8	8.90 d (4.8)	8.96 d (4.4)	+0.06
3,7	8.76 d (4.8)	8.82 d (4.4)	+0.06
12	8.87 s	8.90 s	+0.03
13	8.87 s	8.90 s	+0.03
17	8.87 s	8.90 s	+0.03
18	8.87 s	8.90 s	+0.03
Pyridyl			
2,6	9.01 d (5.8)	9.51 d (5.6)	+0.50
3,5	8.16 d (5.8)	8.90 d (5.6)	+0.74

plastics obtained were found to be insoluble in any usual solvent used. Their insolubility is due to the partial or total deacetylation for temperatures higher to 55 °C. Indeed, because of the presence of the chloride atom on carbon α, these esters are much sensitive to thermal activation compared with the non-halogenated acetate cellulose esters. In conclusion, porphyrinic plastics were obtained with higher porphyrin content at 55 °C. Therefore, this temperature was used for the following of our experiments.

The effect of reaction time on porphyrin content was studied between 6 and 48 h of reaction at 55 °C. Results show that porphyrin content is not sensitive to reaction time between 6 and 18 h with a constant value of 0.14 (Fig. 4b). This content increases for reaction time longer than 18 h and reaches a plateau at 0.26 for longer reaction times up to 36 h. For reaction times of 48 h porphyrinic plastics isolated after precipitation step were found to be insoluble in any usual solvent or to form gels because of their partial or complete deacetylation. In consequence, 36 h of reaction seem to be optimal.

3.4. Synthesis of pyridinium porphyrinic chloroacetyl cellulose chlorides of various porphyrin contents

After optimisation of the alkylation reaction, we decided to correlate the stoichiometry of the photosensitizer with porphyrin content of the porphyrinic plastics. Relative amounts of porphyrin between 3 and 119 mequiv per AGU were allowed to react at 55 °C for 36 h with chloroacetyl cellulose esters. Results presented in Figure 4c show that porphyrin content, in the presence of porphyrin amounts up to 30 mequiv per AGU increases quasi-linearly up to 0.57. It was found to be constant when stoichiometry of porphyrin exceeded 30 mequiv. In comparison with protoporphyrinic laurate cellulose esters previously prepared,[20] these values were found twice weaker. The use of 119 mequiv of photosensitiser per AGU did not allow obtaining higher porphyrin content. These low values can be attributed to the absence of spacer which would have move the porphyrinic macrocycle away from the cellulosic backbone and at the same time overcome the steric hindrance drawback.

3.5. Photodynamic inactivation of *E. coli* and *S. aureus* immobilized on agar surface by pyridinium porphyrinic chloroacetyl cellulose chlorides

Porphyrinic plastic films with a range of photosensitizer content between 0.14 and 0.57 were first evaluated for singlet oxygen production ($^1O_2$) by ergosterol acetate photoperoxidation.[26–28] All tested porphyrinic plastic films were found to induce singlet oxygen production. These plastic films were then evaluated for their photobactericidal activity against *E. coli* and *S. aureus* stains. Usually, the photobactericidal activity of porphyrins is evaluated using antibiogram. This method requires the solubilisation of the tested molecules. However, we wanted to evaluate the photobactericidal activity of plastic films in their solid form (not in solution), that is why we have developed the following protocol with plastic film discs as already described in the literature.[15,16]

Plastic film discs of 2 cm diameter were deposited onto the nutrient agar seeded with the target strain. A series of five porphyrinic plastic films having photosensitizer contents between 0.14 and 0.57 were evaluated. Tested porphyrinic plastic films were found to display the nearly same photobactericidal activity against the two strains after illumination (Table 2).

Films with porphyrin content higher than 0.20 inactivated the two bacterial strains whereas films with lower porphyrin content were found more active against *S. aureus* than against *E. coli*. In comparison, protoporphyrinic lauryl cellulose esters need at least a porphyrin content value of 0.52. These results clearly show that cationic porphyrinic plastic films are more efficient against *E. coli* and *S. aureus* stains than protoporphyrinic ones.

The non-porphyrinic control allows a full growth of both bacteria; absolutely no inhibition did occur in the dark. To exclude a possible bacteriostatic effect, the treated plates were incubated for an additional 24 h after removing the discs. No change occurred

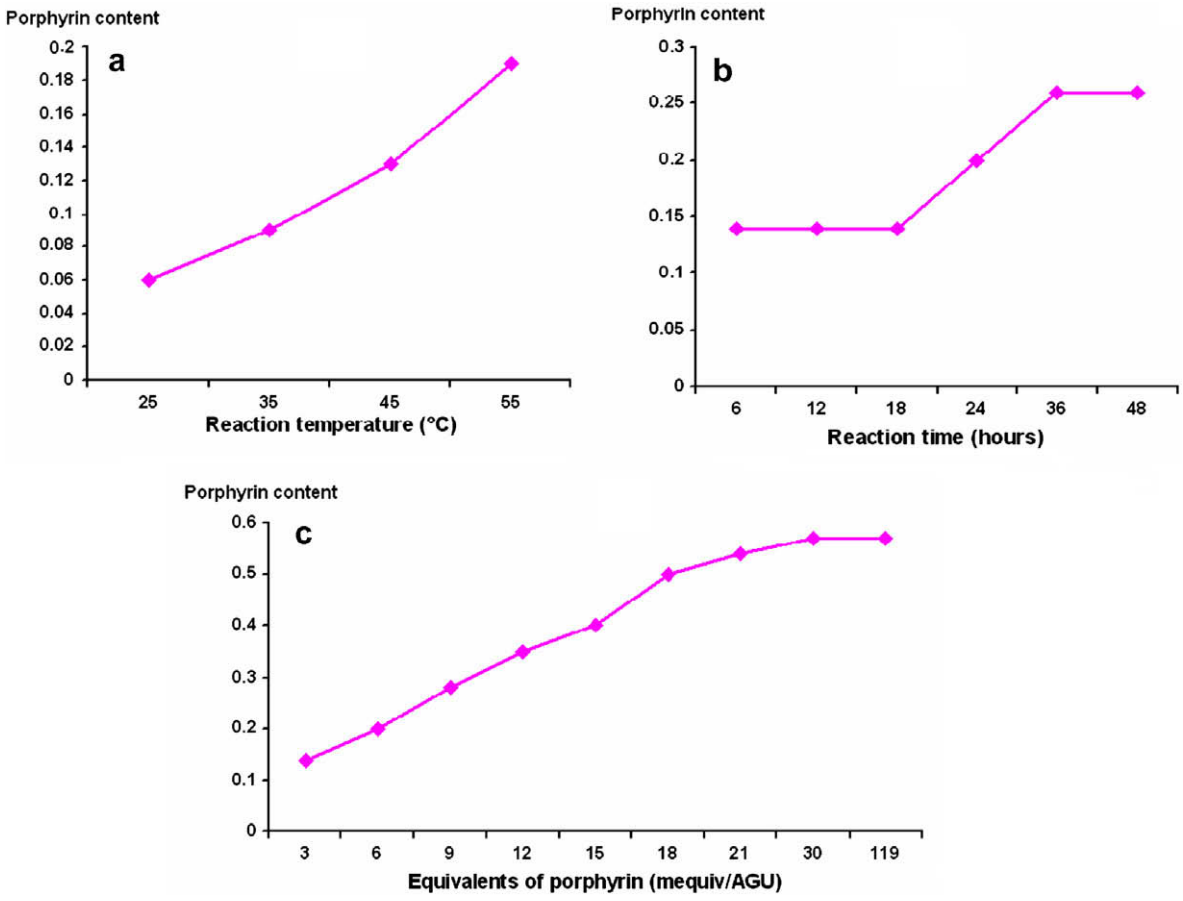


Figure 4. (a) Porphyrin content versus reaction temperature; (b) porphyrin content versus reaction time; (c) porphyrin content versus monopyridyltritolylporphyrin stoechiometry.

Table 2
Photobactericidal activity of pyridinium chloroacetyl cellulose chlorides as function of their porphyrin content

Porphyrin content (porphyrin/100 UAG)	0.14	0.20	0.35	0.50	0.57	0.00
S. aureus	±	–	–	–	–	+
E. coli	±	±	–	–	–	+

–, no colony observed under the discs.
±, presence of few colonies (<5) in disc areas.
+, numerous colonies observed in disc areas.

and no colony did appear in the sterile areas confirming the photobactericidal activity. The covalent bound was found to play its role; it did not allow the release and then diffusion of porphyrins. Indeed photobactericidal effect was limited to discs area; no halo of inhibition larger than plastic film disc size was observed. This is an advantage toward other classical bactericidal materials which often release bactericidal molecules in their environment. Interpretation of these results must take into account the insoluble and immobilized character of the photosensitizing agent. Porphyrinic plastic films induced cell death by oxygen-dependent reactions inducing cell membrane damage by peroxidation involving singlet oxygen.[14,29]

Indeed, type II photochemical reaction leading to the generation of singlet oxygen and diffusion of the latter has already been described first by Kautsky[30] then by Dahl[31] and Nitzan.[32] It was found that this oxidative agent is able to diffuse on the average 200 nm in water and 1 mm in air. Type I photochemical reaction has not been observed yet and seems not to be involved in this photobactericidal activity. Additional microbiological experiments will be realised for understanding the photobactericidal activity of these porphyrinic plastic films.

4. Conclusion

News plastic films bearing cationic photosensitizing agent covalently bound to the polymeric chain have been synthesised, starting from cellulose and porphyrin. The conversion of cellulose into its chloroacetyl ester was achieved using DMAc/LiCl solvent and chloroacetyl chloride. This very reactive acylating agent allowed the reaction to proceed at room temperature, and the obtaining of completely substituted cellulose in a quantitative yield after only 2 h of reaction. Moreover, the absence of base did not prove detrimental to the reaction since no hydrolysis was observed. Porphyrinic plastics were then obtained by alkylation of the photosensitizer. These materials were found to be thermostable up to 55 °C; increasing temperatures induced their deacetylation. The porphyrin content values are low because of the steric hindrance between the porphyrinic macrocycle and the cellulosic chain. However, their photobactericidal activity, tested against *S. aureus* and *E. coli* strains, are very encouraging. Type II photochemical reaction leading to the generation of singlet oxygen seems to be involved in the photoinactivation of these bacterial strains. Such materials could find applications in medical environments as an alternative to overcome the rampant bacterial multiresistance to classical antibiotics.[33]

5. Experimental

5.1. Materials and methods

All solvents and reagents were purchased from Aldrich or SDS. Pyrrole was distilled over CaH_2 under reduced pressure immediately before use. Analytical thin-layer chromatography (TLC) was performed on silica gel Merck $60F_{254}$. Column chromatography was carried out with silica gel (60 ACC, 20–40 μm, Merck). FTIR spectra were recorded with a Perkin-Elmer spectrum 1310 by direct transmission through the plastic films. UV–visible spectra were recorded on a Perkin-Elmer Lambda 25 double-beam spectrophotometer using 10 or 50 mm quartz cells. ^{1}H NMR spectroscopy was performed with a Brücker DPX-400 spectrometer. Chemical shifts are reported as δ ppm, downfield from internal TMS, and are listed according to the standard numbering of *meso*-arylporphyrins. Mass spectrometry (MALDI) was performed by the Paris VI University. MALDI mass spectra were recorded with a Voyager Elite time-of-flight mass spectrometer. Elemental analyses were carried out by the Service Central d'Analyse du CNRS of Vernaizon (France). The two bacterial strains used in PACT experiments were obtained from 'Institut Pasteur, Paris': *E. coli* (CIP 368548, class 2) and *S. aureus* (CIP 35053156, class 2).

5.2. Cellulose dissolution

Cellulose powder was dissolved into DMAc/LiCl according to a method described in a previous paper.[20] Cellulose (20 g/L) and LiCl (80 g/L) in DMAc were kept at 70 °C under stirring until homogeneity. A limpid solution was obtained after cooling down to room temperature and overnight stirring.

5.3. Synthesis

5.3.1. Chloroacetyl cellulose esters

In a typical experiment, one gram of cellulose (50 mL of the previous solution) was reacted with 6 equiv per anhydroglucose unit (AGU) of chloroacetyl chloride at room temperature for 2 h. The reaction product was then precipitated in water (500 mL) and purified by successive dissolution–precipitation cycles, using THF (50 mL) as solvent and water (300 mL) as precipitant. After drying in vacuum in the presence of phosphorous pentoxide, the modified polymer was dissolved in THF and filtered through a glass wool pad. The plastic films were obtained by casting in a glass Petri dish later on.

IR: 3504, 2959, 1747, 782 cm^{-1}; ^{1}H NMR (400.13 MHz, DMSO-d_6, 25 °C): 4.3 (s, 2H, CH_2 methylene), 5.2–3 (m, $H_{glucidic}$).

5.3.2. 5-(4-Pyridyl)-10,15,20-tritolylporphyrin

5-(4-Pyridyl)-10,15,20-tritolylporphyrin was prepared according to the Little[21] method by condensation of freshly distilled pyrrole (4 equiv), with *para*-pyridinecarboxaldehyde (1 equiv) and *para*-tolualdehyde (3 equiv) in propionic acid. After purification by column chromatography performed with a gradient of $CHCl_3$/petroleum ether from 80/20% to 100% mono-pyridyltritolyporphyrin was isolated in 6% yield.

R_f : 0.62 ($CHCl_3$/EtOH, 9:1); UV–vis (THF): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^{-3}): 417 (398.5); 513 (11.9); 548 (8.7); 591 (5.6); 647 (4.3). ^{1}H NMR (400.13 MHz, DMSO-d_6, 25 °C): δ −2.79 (br s, 2H, NH pyr), 2.70 (s, 9H, CH_3 tolyl), 7.55 (d, 6H, J = 7.8 Hz, $H_{3,5}$ Ar), 8.08 (d, 6H, J = 7.8 Hz, $H_{2,6}$ Ar), 8.16 (d, 2H, J = 5.8 Hz, $H_{3,5}$ Py), 8.76 (d, 2H, J = 4.8 Hz, $H_{3,7}$ β-pyr), 8.87 (s, 4H, $H_{12,13,17,18}$ β-pyr), 8.90 (d, 2H, 4.8 Hz, $H_{2,8}$ β-pyr), 9.01 (d, 2H, J = 5.8 Hz, $H_{2,6}$ Py). MS (MALDI) m/z 658.32 ([M+H]$^+$ monoisotopic calcd: 657.30).

5.3.3. Pyridinium porphyrinic chloroacetyl cellulose chlorides (porphyrinic plastic)

Chloroacetyl cellulose ester (DS = 3, 500 mg) was dissolved in DMF (50 mL) at room temperature. This solution was heated at the desired reaction temperature and *para*-5-pyridyl-10,15,20-tritolylporphyrin (14 mequiv/AGU) was then added while stirring. After 24 h of reaction, pyridinium porphyrinic chloroacetyl cellulose chloride (porphyrinic plastic) was recovered by precipitation with $CHCl_3$ (300 mL). Purification was achieved by successive dissolution–precipitation cycles, using THF (50 mL) as solvent and $CHCl_3$ (300 mL) as precipitant, until the filtrate became colourless. After drying in vacuum in the presence of phosphorous pentoxide, the polymer was dissolved in THF and filtered through glass wool. Porphyrinic plastic films were then obtained by casting in a glass Petri dish.

IR: 3504, 2959, 1747, 782 cm^{-1}; UV–vis (THF): λ_{max}, nm: 420; 517; 552; 595; 650. ^{1}H NMR (400.13 MHz, DMSO-d_6, 25 °C): δ −2.70 (br s, 2H, NH pyr), 2.65 (s, 9H, CH_3 tolyl), 4.30 (s, 2H, CH_2 methylene), 5.2–2.6 (m, $H_{glucidic}$), 7.50 (d, 6H, J = 7.7 Hz, $H_{3,5}$ Ar), 8.07 (d, 6H, J = 7.7 Hz, $H_{2,6}$ Ar), 8.82 (d, 2H, J = 4.4 Hz, $H_{3,7}$ β-pyr), 8.90 (d, 2H, J = 5.6 Hz, $H_{3,5}$ Py), 8.90 (s, 4H, $H_{12,13,17,18}$ β-pyr), 8.96 (d, 2H, 4.4 Hz, $H_{2,8}$ β-pyr), 9.51 (d, 2H, J = 5.6 Hz, $H_{2,6}$ Py).

5.4. Characterisation

5.4.1. Degree of substitution of chloroacetate cellulose esters

The degree of substitution (DS) indicating the number of chloroacetyl residues per anhydroglucose unit (maximum value 3) was determined using elemental analysis, from carbon percentage, according to the following formula, where %C is the percentage of carbon in the sample.

$$DS = \frac{72.06 - 162.14 \times \%C}{78.48 \times \%C - 24.02}$$

where

72.06 = carbon mass of an anhydroglucose unit,
162.14 = mass of a non-esterified anhydroglucose unit,
76.48 = mass of a chloroacetyl residue,
24.02 = carbon mass of a chloroacetyl residue.

5.4.2. Weight yield

The weight yield is defined as the ratio of $m_{plastic}$, the mass of the plastic film obtained to maximal theoretical mass, that it is possible to obtain (mass of completely substituted cellulose). One gram of cellulose theoretically yields 2.42 g of completely chloroacetylated product (DS = 3); the following formula is thus applicable when starting from 1 g of cellulose.

$$\text{Weight yield} = \frac{m_{plastic}}{2.42} \times 100$$

5.4.3. Porphyrin content of pyridinium porphyrinic chloroacetyl cellulose chlorides

The porphyrin content of porphyrinic plastic film indicates the number of grafted pyridyltritolyporphyrin residue per 100 anhydroglucose units. It was determined by UV–visible spectroscopy, using the molar extinction coefficient at the maximum of the Soret band, according to the following formula:

$$\text{Porphyrin content } (\%) = \frac{A \times V \times (162.14 + 76.48 \times DS)}{\varepsilon \times m} \times 100$$

where

DS = degree of substitution of chloroacetyl residues,
A = absorbance (maximum of the Soret band),

V = volume of solvent used for the dissolution of the plastic sample (L),
ε = molar extinction coefficient of the free porphyrin (maximum of the Soret band) (mol^{-1} L cm^{-1}),
m = mass of the analysed sample,
162.14 = mass of a non-esterified anhydroglucose unit,
76.48 = mass of a chloroacetyl residue.

5.5. Singlet oxygen production

Photosensitizing properties of *para*-5-pyridyl-10,15,20-tritolylporphyrin and porphyrinic plastics were carried out by trapping reactions of $^1O_2$ with ergosterol acetate.[22] Solutions of free porphyrin or porphyrinic plastics in THF were subjected to light illumination (150 W tungsten bulbs) in the presence of ergosterol acetate (10^{-5} M) for 2 h.[23,24] At the end of the reaction ergosterol acetate was completely converted into endoperoxide. Reference experiments with eosin, Rose Bengal and hematoporphyrin (Hp), well-kown singlet oxygen producers, gave ergosterol acetate endoperoxide in nearly quantitative yields.

5.6. Photoinactivation of *E. coli* an *S. aureus* cells immobilized on agar surface

These two strains were grown 24 h at 37 °C in test tubes containing 10 mL peptone water broth. 0.2 mL of 1/10 dilution of these broths were plated onto Müeller–Hinton agar. Discs (diameter 2 cm) were cut out of the porphyrinic plastic films and deposited onto the nutrient agar seeded with the target strain. Chloroacetyl cellulose discs (non-porphyrinic plastics with same DS) were taken as controls and used in the same conditions. Duplicate plates containing porphyrinic and non-porphyrinic discs were incubated in the dark and served as additional controls. Test plates were turned upside down, placed on a bench and incubated during 24 h at 37 °C under continuous illumination by four 150 W tungsten bulbs placed 60 cm above and 60 cm away from the flipped plates, totalling 1.7 mW cm^{-2} fluence rate at the level of the bottom of the plates. Each experiment was performed three times. Bacterial growth was examined visually. Results are given in term of positive (no colony) or negative response. After removing the discs, irradiated plates were incubated an additional 24 h in the dark before inspection: persistent absence of colonies in unveiled zone testified for a bactericidal photoactivity otherwise tested film would be provisionally qualified as a bacteriostatic device.

References and notes

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