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Graphical Abstract

Voltammetric optimisation of TEMPO-mediated oxidations at cellulose fabric

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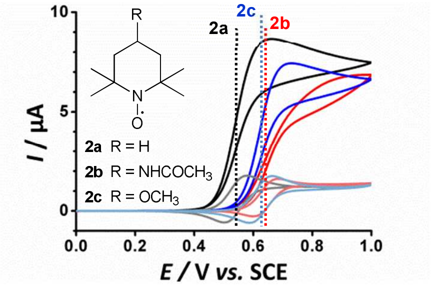
A simple method for rapid voltammetric screening of *N*-oxyl based mediators and reaction conditions for electrochemical oxidation of cellulose is described. Using immobilised cellulose (cotton cloth) the screening requires small quantities of mediator (so is suitable for screening new synthetic or natural mediators) and yet yields information about relative efficiency of mediator, optimum pH, and preferred buffer identity.



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FULL PAPER

Voltammetric optimisation of TEMPO-mediated oxidations at cellulose fabric

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N-oxyl mediated oxidations are becoming widely applied to the selective oxidation of a range of substrates bearing primary alcohol groups, including renewable biopolymers such as cellulose, but screening of mediators and oxidation conditions can be a laborious process, potentially limiting discovery of new mediators and/or optimised conditions for reaction. We describe a simple method for rapid voltammetric screening of mediators and reaction conditions that requires only small quantities of mediator (so is suitable for screening new synthetic or natural mediators) and yet yields information about relative efficiency of mediator, optimum pH, and preferred buffer identity. Such methodology may be used to speed mediator screening and selection of optimal and greenest reaction conditions prior to preparation or isolation of large quantities of mediators.

Introduction

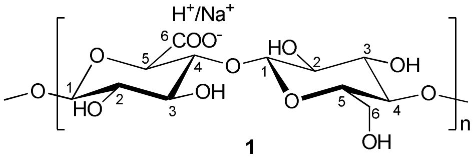
Selective, partial oxidation of the primary hydroxyl groups of cellulose, **1**, may be applied in the preparation of a range of materials,[1] which have the following features in common:

(i) a solid structure reflecting the form of the cellulose in the starting material at the micro- or nano-level (gross form may change significantly, particularly when further processing is applied)[2]; and

(ii) surface charge, in the form of carboxylate groups, which may be present in the acid form COOH, or as a salt, such as the sodium carboxylate (COONa).

Such oxidation is frequently effected by application of the method of de Nooy,[3] or variations thereof, which utilise NaOCl as the primary oxidant, 2,2,6,6-tetramethylpiperidin-1-yl-oxyl (TEMPO, **2a**) as the oxidation catalyst or mediator and NaBr as a co-catalyst. Many TEMPO derivatives have been tested and 4-acetamido-TEMPO, **2b**, is often used in cost sensitive applications.[4] Such oxidation is most productively carried out at elevated pH[5] and constant addition of NaOH solution is usually applied to maintain a constant pH of around 10-11.[6] Such conditions have been reported to lead to a decrease in the degree of polymerisation of the polysaccharide and concerns about the use of chlorine containing oxidants and large quantities of salts have led to investigation of alternative conditions.

Of particular interest, from a Green Chemistry perspective, are electro-[7] or bio-oxidation[8] procedures, which avoid the use of chlorine based primary oxidants and, in many cases also decrease the depolymerisation of cellulose often noted.[9] In the former direct oxidation of a mediator, such as TEMPO, obviates the need for chemical oxidants including hypochlorite, while in the latter an enzyme, such as a laccase, oxidizes the small molecule mediator. In all such protocols it is the oxidized small molecule, water soluble mediator that effects oxidation of the primary C6 alcohol of cellulose and so attention turns to mediator selection.



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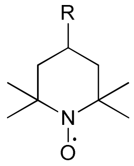

	R
2a	H
2b	$NHCOCH_3$
2c	OCH_3
2d	COOH
2e	OH

Apart from **2a** and its derivatives (**2b-2e** represent only a small number of the TEMPO derivatives that have been reported, although many are inherently unstable under oxidative conditions), synthetically derived mediators such as 2-azaadamantane *N*-oxyl and analogues have been prepared and shown to be highly active in some cases.[10] The preparation of bespoke mediators can be synthetically challenging and screening of mediators, both for activity and optimum conditions, particularly pH, is particularly laborious when using an insoluble substrate such as cellulose (largely due to the need for titrimetric analytical methods to discern the degree of oxidation). To overcome this mediators may be tested for activity against simple soluble substrates (benzyl alcohol being the most common), but this is not a good predictor of the capacity to oxidise cellulose, or voltammetric methods may be used.[11] The latter are appealing, yet the reported methods still require significant quantities of mediator, may lack sensitivity and have only been applied to soluble substrates, although voltammetric methods have been effectively employed to predict mediator/enzyme compatibility.[12]

Herein we report a simple, yet sensitive, voltammetric method for rapid screening of mediators employing cotton fabric, even where these are only available in very small quantities (e.g. during early stage research). In addition to comparing mediators, conditions of pH and buffer type (seldom considered) can be quickly scanned and, in addition, mediator (in)stability probed.

Results and Discussion

Fabric Voltammetry I: Reaction of TEMPO with Cellulose

Initial studies were carried out at pH 10 in aqueous carbonate buffer (CBS) to explore the reactivity of TEMPO with cellulose (cotton fabric with *ca*. 270 µm thickness, Figure 1) immobilised at the electrode surface as described in the Experimental section. Comparison of cyclic voltammetry traces of CBS alone, cotton fabric in CBS, TEMPO in CBS and cotton fabric in CBS with TEMPO, Figure 2a, reveals that the oxidation of a substrate is readily distinguished from reversible oxidation/reduction of TEMPO alone. The increase in oxidation peak and diminution of the reduction peak in the presence of cotton and mediator was a result of reaction of the TEMPO oxoammonium cation (TEMPO^+) with cellulose, i.e. chemical rather than electrochemical reduction of TEMPO^+. A significant increase in measured current resulted from rapid diffusion of reduced TEMPO back to the surface of the working electrode in the confined region between cotton and electrode (see Figure 1B). Doubling the mediator concentration resulted in an approximate doubling of the anodic current (Figure 2b) suggesting that the rate of oxidation of cellulose is proportional to the TEMPO concentration, in accordance with previous results for both cellulose[13] and methyl α-D-glucopyranoside.[3]

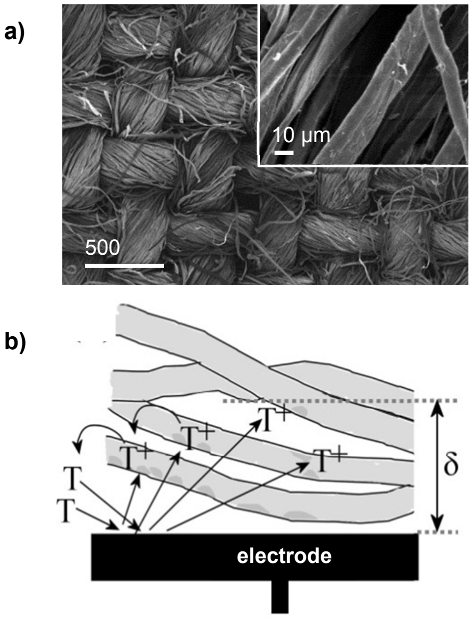

Fig. 1 a) SEM images of cotton fabric, showing weave and fibre dimensions (inset) and (b) schematic drawing of mediator generation and reaction in the cotton fabric with diffusion layer thickness δ.

The effect of altered scan rate on voltammetric response provided a means of probing the transport processes and rate of chemical reaction. The measured current is a function of rate of reduction of TEMPO^+ upon reaction with cellulose and rate of diffusion of TEMPO to and from the electrode surface (and the cellulose surface). Thus, a change in measured current could signify:

1. a change in the binding of TEMPO^+ to the cellulose surface (or release of the reduced hydroxylamine, TEMPOH, from the surface);
2. a change in rate of the chemical oxidation reaction; or
3. a change in the rate of transport of TEMPO^+ and/or TEMPOH through the electrolyte.

(TEMPOH is assumed to undergo comproportionation with TEMPO^+ regenerating TEMPO, as described in detail by Israeli *et al*.,[14] thus does not interfere with the measurements described below.) Together these processes (binding, chemical reaction, release) comprise the effectiveness of oxidation of cellulose by a given mediator. (The reasonable assumption is made that water soluble, small molecule mediators bearing similar charge exhibit similar rates of diffusion in water and therefore a simple change in transport rate can be ruled out.)

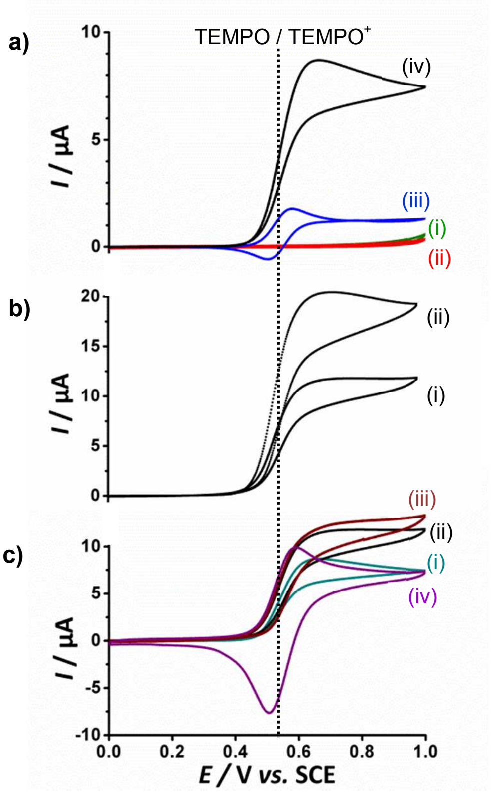

Fig. 2 Cyclic voltammograms of (a) CBS (i), cotton with CBS (ii), 1 m*M* **2a** (iii) and cotton in CBS with 1 m*M* **2a** (iv) at pH 10; potential scan rate 1 mVs^{-1}; (b) cotton with 1 m*M* (i) and 2 m*M* **2a** (ii) in CBS pH 10, potential scan rate 10 mVs^{-1}; (c) cotton with 1 m*M* **2a** in CBS pH 10 at potential scan rates of (i) 1 mVs^{-1}, (ii) 10 mVs^{-1}, (iii) 20 mVs^{-1}, and (iv) 100 mVs^{-1}.

An increase in scan rate from 1 to 10 mVs^{-1} resulted in a significant increase in measured current, but a further increase to 20 mVs^{-1} had less effect, Figure 2c. More importantly, further increases in scanning rate resulted in appearance of a reduction peak, until, at 100 mVs^{-1}, the shape of the reversible TEMPO only voltammogram is recovered (Figure 2c), suggesting that the chemical reaction between TEMPO$^+$ and cellulose no longer serves to remove TEMPO$^+$ in an irreversible process, on the timescale of a single cycle. In other words the reaction is now slow compared to scan rate, either because it is limited by the rate of diffusion of TEMPO$^+$, or by binding and chemical reaction with cellulose of TEMPO$^+$.

For the effect of the potential scan rate two limiting cases have to be considered with (i) very fast scan rates where the diffusion layer thickness δ (Figure 1b) is too small to allow interaction with the cellulose (with $\delta = \sqrt{(DRT)/(vF)}$ the higher limit for scan rate can be estimated[15] as $v_{\text{upper}} < 1\ \text{Vs}^{-1}$), and (ii) very slow scan rates where the diffusion layer thickness extends beyond the fabric into solution (with δ = 270 μm the lower limit for scan rate can be estimated as $v = 10\ \text{mVs}^{-1}$). Within this range of scan rates the competition of diffusion and reaction at the cellulose surface allows the apparent rate constant $k_{\text{cellulose}}$ to be estimated from the change from the transition scan rate v_{trans} for chemically irreversible to reversible voltammetric features. For data in Figure 2c this occurs at *ca*. $v_{\text{trans}} = 50\ \text{mVs}^{-1}$, which suggests that $k_{\text{cellulose}}$ under these conditions is $k_{cellulose} = \sqrt{(Dv_{trans}F)/(RT)} = 4 \times 10^{-5}\ \text{ms}^{-1}$. This has to be regarded as an estimate only as the reaction front will gradually move into the cellulose fibres, thereby reducing the apparent rate constant. It is interesting to note the *shape* of the reduction peak in the voltammogram depicted in Figure 2c(iv) is consistent with accumulation of TEMPO **2a**$^+$ probably due to strong interaction with the negatively charged cotton surface (the large reduction signal suggests a reservoir of **2a**$^+$).

Evidence for the bulk oxidation of the cotton fabric is obtained from ATR-FTIR; cotton discs removed from the electrode surface after repeated cycles of electrochemical oxidation exhibit clear signals due to carboxylate and carboxylic acid groups at *ca*. 1600 and 1700 cm^{-1}, Figure 3.

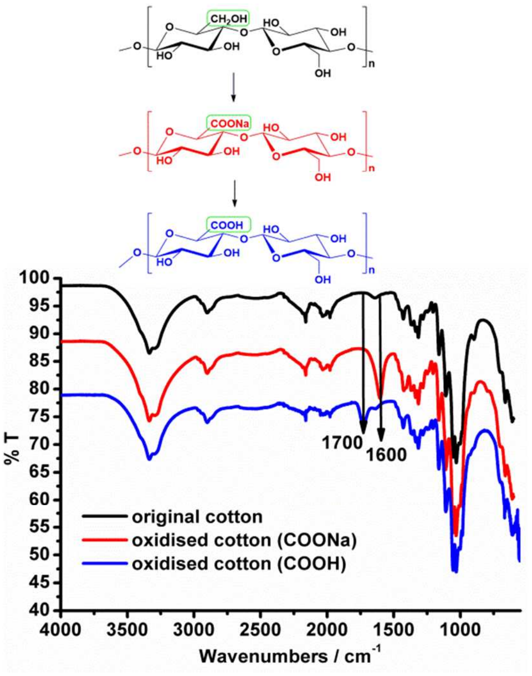

Fig. 3 ATR-FTIR data (for the cotton sample facing the electrode) for original cotton (black), oxidized cotton at pH 10 (red), and oxidized cotton at pH 4 (blue).

The effect of change of pH on the reaction was quite marked: at pH 4, while the cyclic voltammogram of TEMPO was unaltered, the voltammetric response in the presence of the cotton fabric was attenuated, Figure 4a. More importantly, there is now no evidence of the chemical reaction and it appears that the cotton acts only to either absorb TEMPO, or to obstruct diffusion of TEMPO from the bulk solution to the electrode. This presents the possibility of quickly and easily scanning across a range of pH values with the aim of maximising reactivity, or, indeed, to select an appropriate mediator for use at a specific pH.

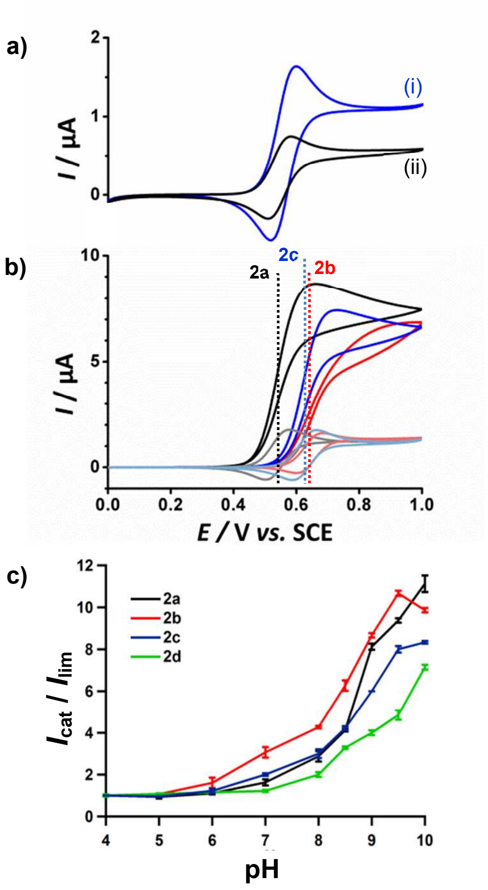

Fig. 4 Cyclic voltammograms of : (a) 1 m*M* TEMPO without (i) and with (ii) cotton in ABS pH 4, potential scan rate 1 mVs^{-1}; (b) mediators **2a**, **2b** and **2c** (1 m*M*) without and with cotton in CBS pH 10, scan rate 1 mVs^{-1}. (c) Influence of pH on the efficiency of *N*-oxoammonium oxidation of cotton by electro-oxidized **2a** (black), **2b** (red), **2c** (blue) and **2d** (green).

Fabric Voltammetry II: Comparison of TEMPO Derivatives

The effect of variation of substituents at the 4 position of TEMPO was considered, Figure 4b. As expected from previous studies,[16] TEMPO, **2a**, exhibited slightly higher reactivity towards cellulose at pH 10 than its 4-acetamido analogue, **2b**. The sequence in reversible potentials is **2a** < **2c** ≤ **2b** consistent with the electron withdrawing nature of the substituents. Perhaps surprisingly, the mildest oxidant, **2a**, also is the fastest (possibly due to penetration speed into or out of the cellulose fibres). This counter-intuitive result is clearly demonstrated in Figure 4.

Fabric Voltammetry III: Optimisation of pH and Buffer

To allow comparison of the efficiency of cellulose oxidation by

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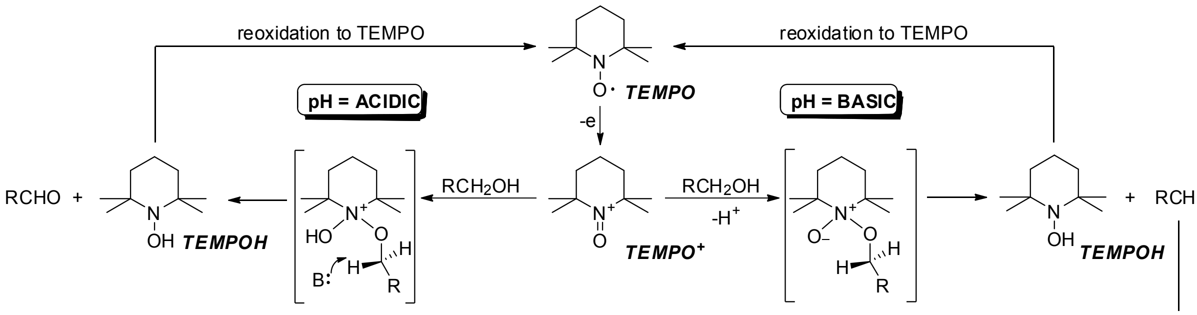

Fig. 5 Oxidation of primary alcohols by TEMPO$^+$ under different pH regimes.

TEMPO derivatives **2a-d**, the ratio I_{cat}/I_{lim} is defined, where I_{cat} is the anodic catalytic limiting current measured in the presence of the cotton and *N*-oxyl mediator and I_{lim} is the limiting current of the diffusion controlled anodic peak in the presence of the *N*-oxyl mediator alone. (I_{lim} is determined by the limiting current in the presence of cotton and **2a-d** at pH 4, where no reaction with cotton is noted on the time scale of the voltammetric scan. This is required as the presence of the cotton, in close contact with the working electrode, resulted in attenuation of the measured current by blocking diffusion of *N*-oxyl mediator from the bulk solution. I_{lim} is likely to be dominated by natural convection outside of the cotton fabric in the solution phase.)

In agreement with previous reports, all TEMPO derivatives tested exhibited a higher rate of reaction with cotton at elevated pH (due to a higher driving force) and all become significantly less effective as pH drops, Figure 4c. No reaction with the cotton substrate is detected, on the timescale of the experiment, at pH 4 and **2b** retains activity at lower pH than **2a, 2c** or **2d**, again, in accordance with previous reports.[5] The mechanism of TEMPO mediated oxidation of alcohols and rate limiting steps: formation of a TEMPO-alcohol complex and subsequent elimination via a cyclic transition state or intermolecular abstraction of the α-hydrogen atom have been discussed at length[17,18,19] (Fig. 5) while pH effects on the various *N*-oxyl derived species[20] and comproportionation reactions are known.[14] Here this is further complicated by more indirect mobility/concentration effects, e.g. the potential for changes in interaction of the positively charged oxoammonium, TEMPO$^+$, and its counterions with the increasingly oxidized cellulose surface.

Under the conditions employed here oxidation and reduction of mediators showed good reversibility over the entire pH window investigated, and throughout the experimental period (although one should note that this is short compared to other preparative studies[16]). Mediator **2d**, bearing a COOH group at position 4, will form a salt at high pH and, as oxidation of cellulose proceeds yielding COO$^-$ surface functionality, electrostatic interactions might limit diffusion of the oxoammonium cation of **2d** into the cotton substrate.

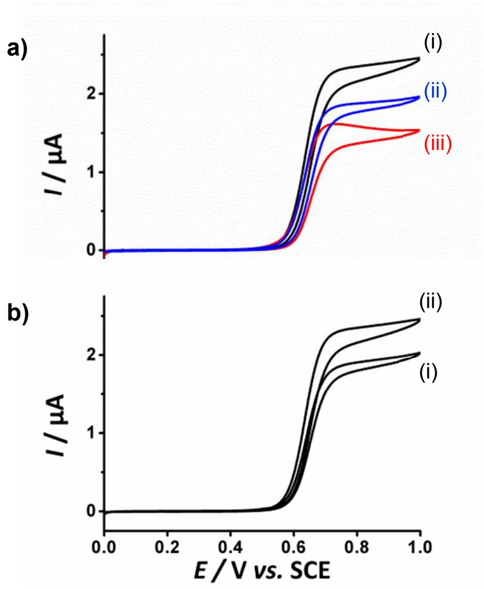

Fig. 6 Cyclic voltammograms of cotton with 1 m*M* **2b** in (a) 0.1 *M* phosphate buffer pH 7 (i), 0.1 *M* citrate buffer (iii) and in phosphate/citrate mixed buffer pH 7 (ii); potential scan rate 1 mVs^{-1}; (b) 0.2 *M* (i) and in 0.1 *M* phosphate buffer (ii), pH 7, potential scan rate 1 mVs^{-1}.

In these electrochemical reactions pH is controlled by an appropriate buffer, but the buffer may not be innocuous in the reaction (particularly at acid pH where a base is implicated in proton abstraction from the intermediate complex). Changing the identity of the buffer from phosphate to citrate, but maintaining pH at 7 (with **2b** as the mediator) results in a decrease in efficiency of oxidation of cotton, Figure 6a. Similarly, doubling the concentration of the buffer salts decreases the efficiency of **2b** mediated oxidation of cotton, Figure 6b. In part effects observed here could be caused by processes within the cellulose with additional effects from cellulose functional groups.

The counter-ion effect has been noted previously,[19] but not explored. Noting the deleterious effect of β-oxygen atoms on TEMPO mediated oxidation of alcohols[21] and the postulated formation of a complex that slows oxidation either by reducing positive charge on the nitrogen atom, or by forcing the hydrogen atom out of plane in the transition state,[22] we suggest that, in addition to changes in basicity of the anions, competitive complexation by the buffer anions may also lead to decreased reactivity of the oxoammonium cation. Thus, in optimising such reactions, buffer composition could be important and the voltammetric method described here allows this to be probed with relative ease. (It is noteworthy that citrate buffer is often the buffer of choice for laccase/TEMPO oxidations, yet these results suggest that high concentrations of citrate might serve to slow the rate of turnover of TEMPO derivatives.)

Conclusions

A simple voltammetric method for screening efficacy of mediators in electrochemical oxidation is reported. Applied here to TEMPO and derivatives, the method is sensitive, easy to deploy and yields information pertaining to relative rates of substrate oxidation, as well as stability of mediators under a range of conditions. As very small amounts of mediator are required for testing, this provides a means for screening new synthetic or natural mediators and selection of optimal conditions of pH and buffer. The TEMPO based oxidation process is widely considered to be green (see numerous reports in this and other journals) and thus our report of a simple to execute method for screening mediators (including new, potentially greener mediators) and reaction conditions will enable more rapid development of even more efficient, cleaner and ultimately scalable processes.

While oxidation of cellulose is of wide interest due to developing applications of partially C6 oxidized fibrils, the method described is applicable to a range of insoluble substrates and need not be limited to polysaccharides.

Experimental

Materials and Reagents

Cotton samples (Phoenix Calico, US) were woven non-fluoresecent, non-desized and non-mercerised. TEMPO **2a** (98 %), 4-acetamido-TEMPO **2b** (for EPR spectroscopy), 4-carboxy-TEMPO **2d** (97 %) and 4-hydroxy-TEMPO **2e** (97 %) were obtained from Sigma Aldrich, while 4-methoxy-TEMPO **2c** (>98 %) was from TCI. All other reagents were purchased from Aldrich and used without further purification.

In this study, carbonate buffer (CBS) pH 10; borate buffer (BBS) pH 9.5, 9 and 8.5; phosphate buffer (PBS) pH 5 and 7; acetate buffer (ABS) pH 6, 5 and 4; and citrate buffer pH 7 were used to maintain the pH of the reaction solution. All the buffers were of 0.1 *M* concentration unless otherwise specified. Mediators **2a-e** were of 1 m*M* concentration unless otherwise specified. Voltammetric experiments were carried out in buffered solutions prepared with ultrapure water (18.2 MΩ cm) from a PURELAB Classic system (EGLA, UK).

Voltammetric studies

For voltammetric studies an Autolab PGSTAT12 system (EcoChemie, NL) was employed with a KCl-saturated calomel (SCE) counter electrode (REF401, Radiometer, Copenhagen), and a Pt counter electrode. The working electrode was a 3 mm diameter boron-doped diamond electrode (Diafilm^{TM}, Windsor Scientific).

Following methodology developed previously,[23] a 5 mm disk of cotton fabric was placed in close contact with the working electrode and held in place with a fine nylon mesh and immobilised by an O-ring, Figure 6. Voltage range was from 0 to 1 V and scan rates as indicated for each experiment. For the effect of pH on rate of oxidation, each experiment was repeated three times on virgin cotton samples cut from the same bulk material.

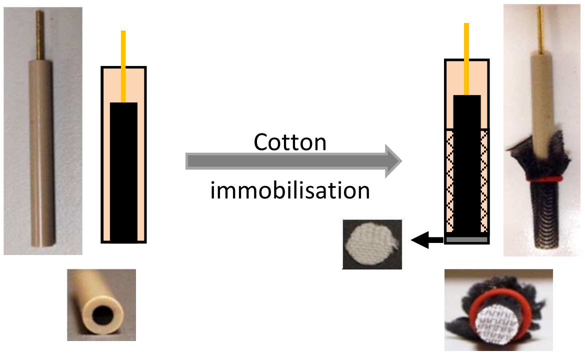

Fig. 6 A disk of cotton fabric is placed in intimate contact with the working electrode and secured by means of a fine nylon mesh.

Characterisation of Fabric

Fourier Transform Infrared Spectrometry (ATR-FT-IR) was employed at ambient temperature on a diamond single-crystal parallelepiped internal reflection element with PerkinElmer Frontier IR system to characterize freeze-dried, non-oxidized and oxidized cotton derived from multiple cycles at pH 10 (see Figure 3). The spectra were recorded with a width ranging from 4000 to 600 cm^{-1}, with an accumulation of 10 scans and a 1 cm^{-1} resolution.

Notes and references

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1 A plethora of examples exist, but some notable applications include: a) rheology modifiers – R. J. Crawford, Robert K. J. Edler, S. Lindhoud, J. L. Scott, G. Unali, *Green Chem*., 2012, **14**, 300-303; b) chromatographic ion exchange material - T. Saito and A. Isogai, *Carbohyd. Polym.*, 2005, **61**, 183-190; and c) films – H. Fukuzumi, Hayaka, T. Saito, Tsuguyuki, T. Wata, Y. Kumamoto, A. Isogai, *Biomacromolecules*, 2009, **10**, 162-165.

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