Voltammetric optimisation of TEMPO-mediated oxidations at cellulose fabric

Yun Jin, Karen J. Edler, Frank Marken, Janet L. Scott

Received (in XXX, XXX) Xth XXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXX 20XX
5 DOI: 10.1039/b000000x

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.

15 Introduction

Selective, partial oxidation of the primary hydroxy 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); 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.5 Such oxidation is most productively carried out at elevated pH5 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-oxidation8 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.
voltammetric methods have been effectively employed to predict mediator/enzyme compatibility.\textsuperscript{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

10 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 \textit{ca}. 270 \textmu m 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\textsuperscript{+}) with cellulose, i.e. chemical rather than electrochemical reduction of TEMPO\textsuperscript{+}. 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\textsuperscript{13} and methyl α-D-glucopyranoside.\textsuperscript{3}

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\textsuperscript{+} 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\textsuperscript{+} 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\textsuperscript{+} and/or TEMPOH through the electrolyte.

(TEMPOH is assumed to undergo comproportionation with TEMPO\textsuperscript{+} regenerating TEMPO, as described in detail by Israeli \textit{et al.}\textsuperscript{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 mM 2a (iii) and cotton in CBS with 1 mM 2a (iv) at pH 10; potential scan rate 1 mVs\textsuperscript{-1}; (b) cotton with 1 mM (i) and 2 mM 2a (ii) in CBS pH 10, potential scan rate 10 mVs\textsuperscript{-1}; (c) cotton with 1 mM 2a in CBS pH 10 at potential scan rates of (i) 1 mVs\textsuperscript{-1}, (ii) 10 mVs\textsuperscript{-1}, (iii) 20 mVs\textsuperscript{-1}, and (iv) 100 mVs\textsuperscript{-1}.

An increase in scan rate from 1 to 10 mVs\textsuperscript{-1} resulted in a significant increase in measured current, but a further increase to 20 mVs\textsuperscript{-1} had less effect, Figure 2c. More importantly, further increases in scanning rate resulted in appearance of a reduction peak, until, at 100 mVs\textsuperscript{-1}, the shape of the reversible TEMPO only voltammogram is recovered (Figure 2c), suggesting that the chemical reaction between TEMPO\textsuperscript{+} and cellulose no longer

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 ς.
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 $\delta$ (Figure 1b) is too small to allow interaction with the cellulose (with $\delta = \sqrt{\left(\frac{D F}{v F}\right)/R}T$) [add reference] the higher limit for scan rate can be estimated as $v_{upper} < 1 \text{Vs}^{-1}$, and (ii) very slow scan rates where the diffusion layer thickness extends beyond the fabric into solution (with $\delta = 270 \mu m$ 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 $k_{cellulose}$ 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 $v_{trans} \approx 50 \text{mVs}^{-1}$, which suggests that $k_{cellulose}$ under these conditions is $k_{cellulose} = \sqrt{\left(\frac{D v_{trans} F}{R F}\right)} (RT) = 4 \times 10^{-3} \text{ms}^{-1}$. 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.

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.

![Figure 4: Cyclic voltammograms](image)

**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, TEMPO, $2a$, exhibited slightly higher reactivity towards cellulose at pH 10 than its 4-acetamido analogue, $2b$. The sequence in reversible potentials is $2a \leq 2c \leq 2b$ 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 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 \( I_{\text{lim}} \) is the limiting current of the diffusion controlled anodic peak in the presence of the N-oxyl mediator alone. \( I_{\text{lim}} \) 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. \( I_{\text{lim}} \) 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 \( \alpha \)-hydrogen atom have been discussed at length,\(^6,16,17,18,15\) 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.

**Experimental**

**Materials and Reagents**

Cotton samples (Phoenix Calico, US) were woven non-fluorescent, 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 85 (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 mM concentration unless otherwise specified.

**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. 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.
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™, Windsor Scientific).

Following methodology developed previously, 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.

**Notes and references**


