Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 19–25
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of thio-based additives for biopharmaceuticals by pulsed electrochemical detection following HPLC

Swati J. Modi[a], William R. LaCourse[a,*], Richard E. Shansky[b]

[a] *Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore 21250, MD, USA*
[b] *Genzyme Corporation, 45 New York Avenue, P.O. Box 9322, Framingham 01701-9322, MA, USA*

Received 23 January 2004; accepted 3 August 2004
Available online 28 October 2004

Abstract

Pulsed electrochemical detection (PED) following liquid chromatographic separation has been applied to the direct (i.e., without derivatization) determination of two major sulfur-containing compounds used as pharmaceutical additives, isopropyl-thio-β-D-galactopyranoside (IPTG) and monothioglycerol (MTG). Limits of detection of IPTG and MTG were found to be 1 ppb (0.2 pmol, 50 μL) and 0.2 ppb (0.1 pmol, 50 μL), respectively, using optimized potential-time waveforms applied to a Au electrode. In addition to high sensitivity as compared to optical detection, the simultaneous detection of free thiols and disulfides can be used to study the kinetics of these conversions, as is shown for MTG. A practical application of HPLC–PED is demonstrated in determining MTG in a pharmaceutical formulation. The high selectivity of PED for thiocompounds reduces sample preparation and produces simpler chromatograms in a variety of matrices.

Keywords: IPTG; Monothioglycerol; Electrochemistry; Pulsed electrochemical detection; Thiol; Sulfur

1. Introduction

Pharmaceutical additives are secondary ingredients that function to enable or enhance the delivery of the primary drug or medicine in a variety of dosage forms, and thereby improve its efficacy, control of bioavailability, uniformity, and/or flow characteristics [1,2]. Additives may also be used for their antimicrobial or antioxidant properties, to extend the shelf life of a product, or to increase the stability of active ingredients [1].

Isopropyl-β-D-thiogalactopyranoside (IPTG) is used to trigger gene expression that is under control of the lac promoter for the overexpression of proteins [3]. IPTG is a chemical analog of galactose which cannot be cleaved by the enzyme β-galactosidase. Thus, it can serve as an inducer for activity of the *Escherichia coli* lac operon by binding and inactivating the lac repressor. Normally, IPTG is used to provide a means for color detection of recombinant plasmids. During experiments or manufacturing processes, multiple additions of IPTG are often necessary for longer induction times as the compound decays under culture conditions. Monothioglycerol (MTG) is a sulfur-containing compound used in buffers and for cellular studies [4–8]. Unlike IPTG, it contains a free thiol group and so it readily converts to its disulfide in solution. Although these compounds are not considered "active" ingredients, they are a part of the manufacturing process and often are a part of the final product. Hence, monitoring their presence and concentration is of critical importance.

Several high-performance liquid chromatography (HPLC) methods are available for the determination of thioethers, thiols, disulfides, and other sulfur-containing compounds [9–11]. Since many of these compounds of interest have poor chromophores, the most commonly used method of detection is based on the formation of fluorescent derivatives using reagents such as *o*-phthalaldehyde or *N*-substituted maleimides [12–14]. These procedures are often labor inten-

* Corresponding author. Tel.: +1 410 455 2105; fax: +1 410 455 2608.
E-mail address: lacourse@umbc.edu (W.R. LaCourse).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.08.042

sive, time-consuming, and the derivatization reaction may not go to completion, or may offer poor selectivity [14,15]. Electrochemical detection of sulfur compounds with an Au–Hg amalgam electrode operated at constant potential (DC amperometry) has also been a popular method; however, a single Au–Hg electrode is not useful in detecting both thiols and disulfides [15,16]. Additionally, these types of electrodes are susceptible to fouling.

In contrast, pulsed electrochemical detection, in combination with HPLC, has been used as a highly sensitive and selective means of detecting thiols, disulfides, and other sulfur-containing compounds [17–20]. Thio-compounds are detected via an oxide-catalyzed mechanism, in which surface oxide formation occurs simultaneously with the detection process. The current from the surface oxide formation leads to large background signals, reduced analyte signal, and unstable baselines. These problems are greatly mitigated with the integrated pulsed amperometric detection (IPAD) waveform [9,14,19], which applies a potential-time sequence to the electrode that incorporates a linear cyclic scan between two potentials in the detection step followed by pulsed potential cleaning to maintain uniform and reproducible electrode activity. In addition, PED at a gold electrode has proven to be selective for sulfur-containing compounds under mildly acidic conditions [10,19,20]. The use of PED following HPLC has been applied to methionine [20,21], glutathione oxidized/reduced [10,22], cysteine [21,22], homocysteine [21,23], antibiotics including penicillins and cephalosporins [11,24–26], and other reduced and oxidized sulfur-containing compounds.

In this paper, PED following HPLC is extended to determine IPTG and MTG in biopharmaceutical formulations. Electrochemical characterization via cyclic voltammetry is the basis of optimization of the IPAD waveform, and mechanistic information is used to select the proper choice of the chromatographic solvent system. In addition to analytical figures of merits for standard compounds, the high selectivity and sensitivity of PED is demonstrated with its application to an MTG-containing formulation buffer and pharmaceutical samples. The analytical utility of PED is further demonstrated by its ability to monitor disulfide formation in MTG solutions.

2. Experimental

2.1. Reagents

All solutions were prepared from reagent grade chemicals (Fisher Scientific, Springfield, NJ). All solvents were HPLC grade. Mobile phases were filtered with 0.45-μM Nylon-66 filters (Fisher Scientific) and a solvent filtration apparatus (Rainin). Water was purified using a reverse osmosis system coupled with a multi-tank/ultraviolet/ultrafiltration station (US Filter/IONPURE, Lowell, MA).

Isopropyl-β-D-thiogalactopyranoside (IPTG) was obtained from Research Products International Corporation (Mt. Prospect, IL) and the monothioglycerol (MTG) standard from Sigma (St. Louis, MO). MTG formulations were provided by Genzyme Corporation (Framingham, MA). All sulfur-containing compounds were used as received without further purification. Standards were stored in the refrigerator at 1–5 °C, while MTG formulations, pharmaceutical samples, and the IPTG aged solution were stored in the freezer at −20 °C.

2.2. Instrumentation

Voltammetric data were obtained at a 3 mm Au rotated disk electrode (RDE) using a Model AFMSRX analytical rotator (Pine Instrument, Grove City, PA) and a Epsilon potentiostat (Bioanalytical Systems, Inc., West Lafayette, IN). Data acquisition and potentiostat control were accomplished with a 800/100 MHz Dell Dimensions 2100 computer and BAS Epsilon software. For all experiments, a Pt auxiliary electrode was used. All voltammetric electrode potentials are reported versus an Ag/AgCl reference electrode (Model 13-620-53, Fisher Scientific). The glass electrochemical cell held a volume of ca. 125 mL and had two side arms separated from the cell body by fine glass frits.

For separation and detection, a DX-500 liquid chromatography system (Dionex Corporation, Sunnyvale, CA) with a Dionex Model ED40 electrochemical detector was used. The electrochemical detector was equipped with a gold working electrode, a combination pH and Ag/AgCl reference electrode, and a titanium auxiliary electrode. Solutions were injected with either an AS40 autosampler (Dionex) and/or an injection valve (Model 9126, Rheodyne, Inc., Cotati, CA) fitted with a 50-μL injection loop. Data collection and system control was accomplished using Peaknet software (Dionex, version 5.21) on a 200/33 MHz Compaq Presario computer.

Separation of MTG and IPTG was achieved using a Phenomenex Luna C18, 5-μm particle size, 4.6 mm × 250 mm analytical column (Phenomenex, Torrance, CA) and a Phenomenex security (C_8) guard column. The columns and electrochemical cell were temperature controlled at 30 °C with an LC-30 chromatography oven (Dionex). Unless otherwise specified, the mobile phase solvents were 'solvent A' = sodium acetate (NaOAc) buffer (pH 4.54, 0.01 M) and 'solvent B' = 100% acetonitrile (ACN), (Fisher Scientific, Pittsburgh, PA) delivered at a flow rate of 1.00 mL/min. All solvents were filtered, degassed, and kept under pressure (N_2, ca. 10 psi).

2.3. Sample preparation

All samples were prepared using deionized water, and no special precautions were taken for preparation of IPTG solutions. For MTG solutions, the water was first degassed thoroughly to minimize formation of disulfide. MTG stan-

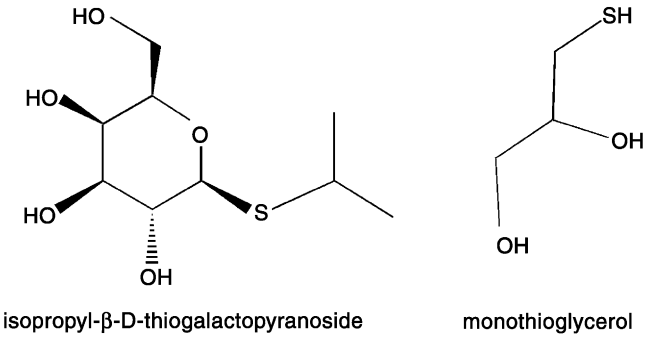

Fig. 1. Structure of thioether: IPTG (left) and reduced thiol MTG (right).

dards were injected within one hour of preparation. Formulation and pharmaceutical samples were weighed and diluted 1:10000 (v/v) in degassed, deionized water. Samples were injected immediately after preparation.

3. Results and discussion

The thioether group of IPTG (Fig. 1A), the thiol of MTG (1B), and the disulfide of the oxidized form of MTG are considered to be the site of electrocatalytic detection under the conditions presented here. Although the electrochemical responses of these compounds are similar, the mechanisms by which each detects are different [9,17,18].

3.1. Electrochemical response and waveform optimization

Cyclic voltammetry has proved useful in selection of approximate PAD waveform potentials. The current–potential (*i–E*) response is shown in Fig. 2A for a Au RDE in NaOAc buffer (pH 4.54, 0.01 M)/CH_3CN (90/10, (v/v)) with dissolved oxygen (- - -), and degassed in the absence (. . .) and presence (—) of 100 ppm IPTG. Under these mildly acidic conditions, the residual response shows an anodic peak at ca. +1350 mV (wave a) during the forward scan due to the formation of surface oxide. On the reverse scan, a cathodic peak at ca. +300 mV (wave b) corresponds to dissolution of the surface oxide formed on the forward scan. Solvent breakdown occurs at ca. +1600 mV (c) and −600 mV (d), resulting in O_2 generation and H_2 evolution, respectively. Dissolved O_2 reduction (- - -) occurs at both the forward and reverse scans and commences at ca. +200 mV (wave e). Addition of IPTG results in an anodic peak which begins at ca. +1000 mV (wave f), coinciding with oxide formation, and reaching a maximum at ca. +1400 mV. Fig. 2B shows the *i–E* plots for MTG (—) in NaOAc buffer (pH 4.54, 0.01M)/CH_3CN (98/2, (v/v)) in the absence of dissolved O_2. The residual for the supporting electrolyte (. . .) is also shown. An anodic wave (f) is observed on the positive scan beginning at ca. +500 mV, corresponding to the oxidation of the thiol group. The formation of the surface oxide commences to a limited degree in the potential

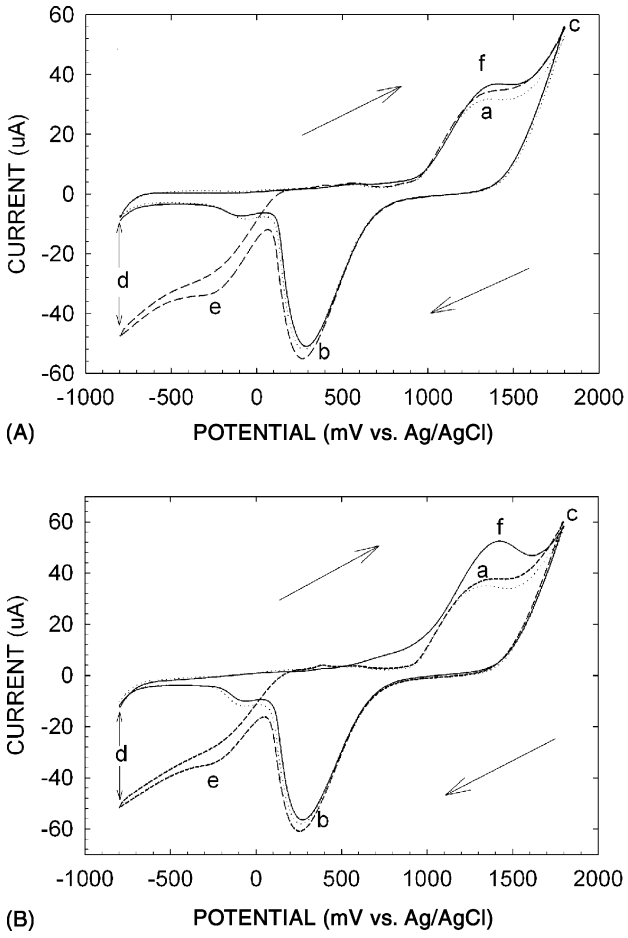

Fig. 2. Voltammetric response for (A) IPTG, 100 ppm in NaOAc buffer (0.01 M, pH 4.54)/CH_3CN (90/10 (v/v)) (B) MTG, 1 ppm (98/2 (v/v)) at 3 mm Au RDE. Rotation speed: 900 rpm; scan rate: 200 mV s^{-1}; (—) analyte response (. . .); residual degassed and (—-) with dissolved O_2.

region of +500 to +1200 mV. For both IPTG and MTG, the current increases with increasing concentration.

Since electrocatalytic detection mechanisms are dictated by the surface state of the electrode, all sulfur-containing compounds are expected to detect at similar potentials. The proposed mechanism is that R–S–R or R–SH is first adsorbed to the electrode and, subsequently, oxidized. Oxidation is concomitant with surface oxide formation, which facilitates the transfer of oxygen to the analyte [9,10]. The term for this phenomenon is "oxide-catalyzed" detection. The IPAD waveform has been shown to be effective in electronically rejecting the "background" signal from oxide formation, which is orders of magnitude larger than the total analyte signal. Table 1 shows the optimized waveforms for the detection of IPTG and MTG. The detection step is comprised of a series of triangular potential scans to maximize the signal from the transient oxide intermediates of the oxide-catalyzed mechanism. The waveform starts at a potential prior to oxide formation, scan to a maximum potential that includes oxide formation and analyte, and ends at a potential that is more negative than that which is required for cathodic dissolution

Table 1
Optimized waveforms for IPTG and MTG

Time (s)	MTG potential[a] (mV)	IPTG potential[a] (mV)	MTG/IPTG integration
0.00	0	−200	
0.05	0	−200	Begin
0.15	1400	1550	
0.25	0	−200	
0.35	1400	1550	
0.45	0	−200	
0.55	1400	1550	
0.65	0	−200	
0.75	0	−200	End
0.80	0	−200	
0.81	−2000	−2000	
0.82	−2000	−2000	
0.83	1600	1600	
0.93	1600	1600	
0.94	−200	−200	
1.55	−200	−200	

[a] Vs. a pH reference.

of the formed oxide. The starting and ending potentials are also more positive than the reduction of dissolved O_2. Hence, the contribution to the overall signal from the reduction of dissolved O_2 is minimized. On-line degassing as part of solvent delivery system can relax constraints on the starting/ending potential [9]. Note that the electrochemical cell utilized a pH reference electrode, while CV was carried out versus a Ag/AgCl reference electrode. The difference between pH and Ag/AgCl was determined to be +170mV by measurement of the mobile phase on a pH/voltmeter, which is reflected in the optimized waveforms presented in Table 1.

The differences in the initial/final and maximum scan potentials for IPTG and MTG is due to differences in the detection mechanisms of a thioether versus a thiol. Fig. 3 shows plots of modulated hydrodynamic voltammograms (MHDVs) for IPTG and MTG in their respective solvent

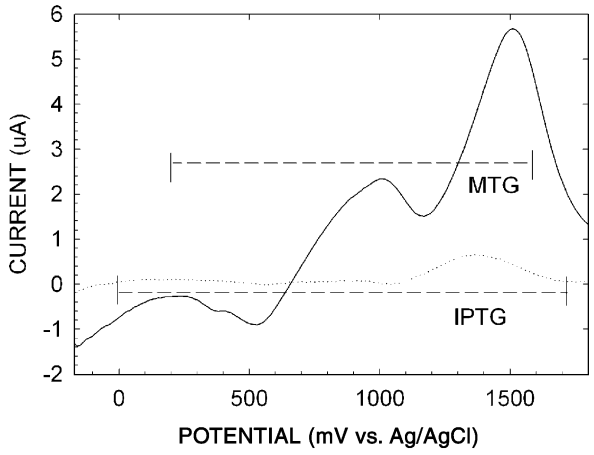

Fig. 3. Modulated hydrodynamic cyclic voltammetry response of (A) 1 ppm MTG (—) and (B) 100 ppm IPTG (. . .) at 3 mm Au RDE in NaOAc buffer (0.01 M, pH 4.54)/CH_3CN (98/2 (v/v) for MTG and 90/10 (v/v) for IPTG). Rotation speeds: high, 2500 rpm; low, 900 rpm; subtracted. (——-) Indicates the potential range for integration of MTG and IPTG signals.

systems. These plots delineate the potential range of the highest mass-transport dependent component of detection by changing the rotation speed of the RDE. As indicated by the MHDVs, the scan potential parameters of the optimized IPAD waveforms corresponds to the potential range of greatest mass-transport. The upper limit of signal collection for IPTG extends further than for MTG to allow for maximum signal collection for the thioether; As a consequence, the greater amount of surface oxide formation must be removed by starting and ending at a more negative potential, which allows for more time for oxide reduction to occur. The detection parameters were also empirically optimized using HPLC–PED to give an optimal signal-to-noise, stable response without post-peak dipping, and the widest linear dynamic range.

The detection step in both waveforms is followed by a large negative potential pulse (i.e., −2000 mV for 10 ms) to induce both cathodic cleaning of the electrode [27] and reduce any partially-solubilized Au [28]. This potential pulse is necessary to extend the life of the electrode for months of continuous use. Next, a short positive potential pulse (i.e., +1600 mV for 100 ms) is used to induce anodic cleaning of the electrode and this is followed by 600 ms at −200 mV to allow for pre-adsorption of the analyte. The Johnson group has shown that amines can be pre-concentrated 10-fold with the use of an adsorption step in the potential–time waveform [29]. In agreement with these findings, LaCourse and Owens demonstrated that the optimal response for sulfur-containing compounds is obtained by maximizing the time of the adsorption step without negatively affecting the chromatographic integrity of the peaks [10].

3.2. Reversed-phase chromatography with PED

Retention of IPTG and MTG was achieved using a reversed-phase column with 10 and 2% ACN, respectively, with the remainder being a NaOAc buffer (10 mM, pH 4.54). A C18 column was chosen to maximize the retention of these highly polar compounds. Fig. 4A shows a chromatogram of IPTG standard, eluting at 5.1 min ($k' = 1.40$), with an oxygen peak observed at 9.7 min ($k' = 3.59$). Dissolved O_2 is found in both the standard and the blank injection, and is relative to the amount of dissolved oxygen in the sample versus the amount in the mobile phase, which is filtered, degassed, and kept under the pressure of N_2. Therefore, the size of this peak is highly variable. Fig. 4B shows a chromatogram of MTG standard with a retention time of 5.7 min ($k' = 1.55$). The disulfide, or oxidation product, eluted at ca. 13.5 min ($k' = 4.82$). The identity of this peak was determined on the basis that it elutes later than the MTG, its growth is correlated with the disappearance of the MTG peak, and PED at pH 4.54 is specific to the detection of sulfur-containing compounds. The peak observed at ca. 11.5 min is due to dissolved O_2. As expected for a reversed-phase separation, an increase in the concentration of the organic modifier decreases the retention time. Since the oxidation of thiols occurs more rapidly at high

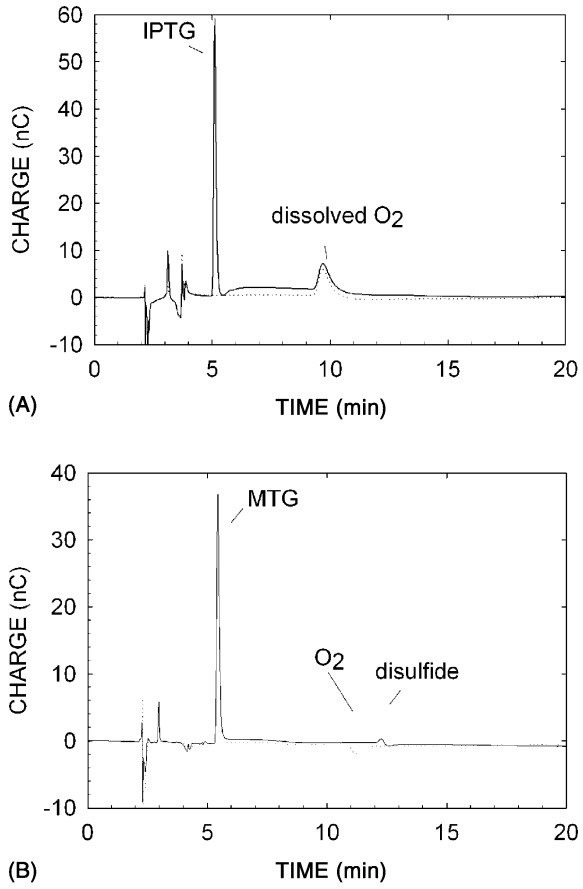

Fig. 4. Chromatogram of HPLC separation of (A) IPTG and (B) MTG. Conditions: Phenomenex 5u Luna C_{18} column 250 mm × 4.6 mm, mobile phase NaOAc buffer (0.1M, pH 4.54)/CH_3CN 90/10 (v/v) for IPTG, 98/2 (v/v) for MTG); flow rate 1.0 mL min^{-1}; 50 μL injection volume, temperature 30 °C; optimized IPAD waveforms listed in Table 1. Concentrations: IPTG 1.0 ppm and MTG 0.1 ppm. (—) Analyte response and (. . .) blank.

pH [15], acetate buffer pH (4.54) was chosen for the mobile phase as it allowed for minimal disulfide formation, and is well below the pK_a value for MTG, which is 9.46 [30].

The analytical figures of merit for IPTG and MTG are listed in Table 2. All compounds are linear over two to three decades with correlation coefficients of 0.9986 (IPTG) and 0.9965 (MTG). Under their respective conditions, limits of detection (LOD) for IPTG and MTG are 1 ppb (0.2 pmol, 50 μL) and 0.2 ppb (0.1 pmol, 50 μL), respectively. The lower LOD for MTG is reflected in the greater signal with less oxide-formation as seen from the background of the cyclic voltammogram of MTG as compared to IPTG.

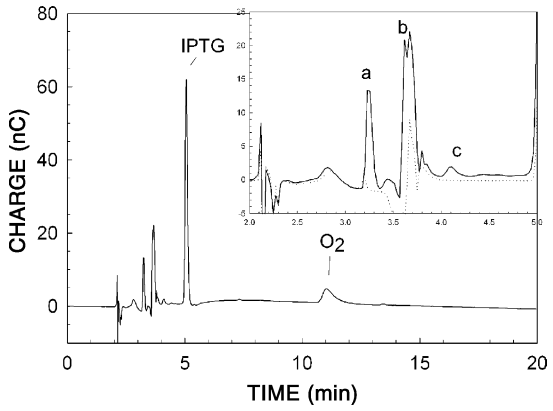

Fig. 5. Chromatogram of aged solution (5.9 years) of 1 ppm IPTG (—). Inset shows degradation product peaks a, b, and c in aged solution as compared to fresh IPTG solution (. . .).

Table 3
Summary of formulation buffer I assay results

Trial	Concentration in sample directly determined (ppm, mM)	Concentration in sample, standard addition, three points (ppm, mM)
1	747, 6.9	733, 6.8
2	753, 7.0	760, 7.0
3	793, 7.3	767, 7.1
Average	764, 7.1	753, 7.0
S.D.	0.04	0.02
% R.S.D.	3.21	2.16

3.3. Application

The following applications are used to highlight the utility of HPLC–PED and not to be analytically rigorous—all samples were used ‘as is’.

Fig. 5 shows the chromatogram of an aged (6.7 years) sample of IPTG stock (—) and the inset shows an enlargement of the same chromatogram versus a ‘fresh’ 1.00 ppm IPTG standard (——). The amount in the aged sample was determined to be 97.8 ± 1.5% (n = 3) of the fresh sample. Note the appearance of new peaks (a, b, c) in the aged sample. The *S*-glycosidic linkage of IPTG is susceptible to chemical oxidation and cleavage reactions. No decomposition measurements in cellular media were carried out because samples were not available; however, it is known that the compound degrades under culture conditions and that more stable substitutes are currently being explored [3].

Formulation buffers containing citrate, phosphate, polysorbate 80, dextrose, and 0.1% monothioglycerol were

Table 2
Quantitative parameters of thio-compounds at an Au electrode by PED

Compound	Linear range $nC = a$ (ppb) + b				Repeatability R.S.D. (ppb, n)
	LOD (ppb, pmole)	a	b	r^2	
IPTG	1, 0.2	0.0597	0.3480	0.9986	2.3 (600, 6)
MTG	0.2, 0.1	0.3355	1.9449	0.9965	2.8 (100, 6)

LOD was calculated at 3 *S/N* from concentration within 5 LOD.

Table 4
Summary of formulation buffer II and drug product sample assay results

Sample	Concentration MTG in sample[a] (ppm, mM)	Percent MTG in sample	Percent MTG Expected	Repeatability RSD (*n*)
Formulation buffer	1405, 12.8	0.11	0.1	0.5 (3)
Drug product air overlay	367, 3.4	0.03	0.1	1.5 (3)
Drug product N_2 overlay	851, 7.8	0.07	0.1	0.2 (3)
Drug product high aggregate control	802, 7.5	0.06	0.1	2.9 (3)

[a] Calculation as determined from calibration curve.

also assayed using HPLC–PED. The method of standard addition was used to assay the first formulation buffer, which contained a significant disulfide peak (chromatogram not shown). Results are summarized in Table 3. Results via the standard addition approach to quantitation showed no significant difference between the two values at the 95% confidence level, which indicates no matrix effect.

The second set of samples that were supplied included a fresh formulation buffer that was diluted 20 μL to 200 mL with degassed, deionized water and showed very little disulfide product. Fig. 6A shows the chromatograms of (—) formulation buffer, (———) 100 ppb MTG standard, and (. . .) and a blank injection. Results for the formulation buffer agree with the reported amount of MTG in the sample to the same number of significant figures as supplied (0.1% MTG by volume). Fig. 6 (B and C) are injections of a sample of a protein drug product in formulation buffer with air overlay and N_2 overlay, respectively. It is clearly evident from the chromatograms that the sample stored under air has significant disulfide formation, while the N_2 layer allows the thiol some protection from oxidation. The two samples differed significantly in percent reduced MTG remaining (0.03 for air overlay versus 0.07 for N_2 overlay). A fourth sample containing MTG, high aggregate control, was also run and was also shown to contain a significant amount of disulfide. Table 4 summarizes the results of assaying the second formulation buffer and samples.

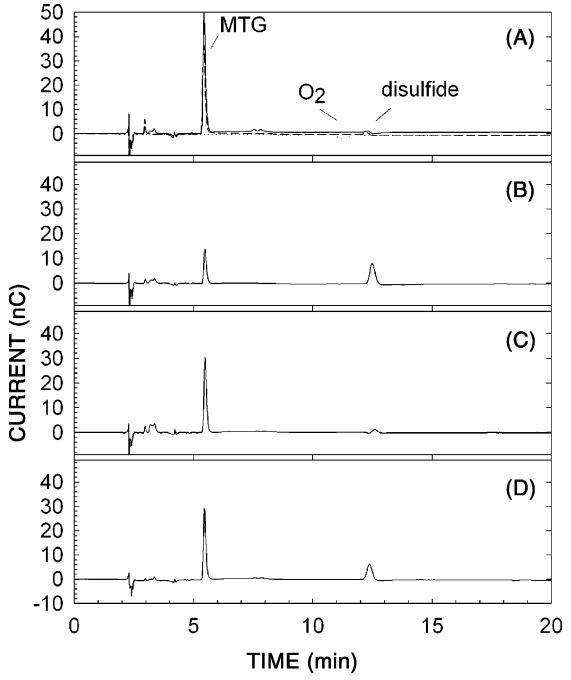

Fig. 6. Chromatograms of (A) formulation buffer containing 0.1%MTG (—) vs. MTG standard, 100 ppb, (—) and water blank (. . .). (B) Protein drug product 2.5 mg/mL in formulation buffer, air overlay and (C) nitrogen overlay. D-Protein drug product 1 mg/mL high aggregate control in formulation buffer. All samples were diluted 20 μL–200 mL, using degassed deionized water. All other conditions as listed in Fig. 4B.

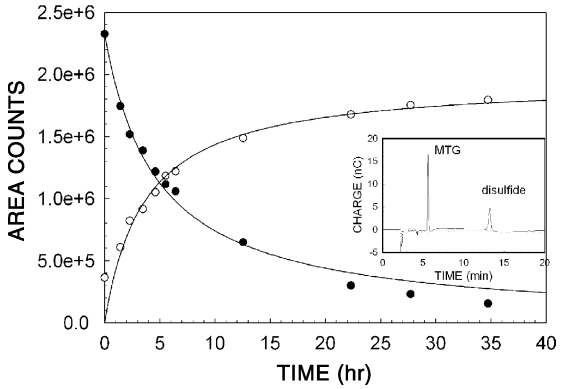

Fig. 7. Monitoring the loss of MTG (● —) and formation of disulfide (◯ —) over a period of 35 h. Inset shows chromatogram of MTG/disulfide at time 3.5 h. Conditions as listed in Fig. 4B.

In order to better understand the kinetics of disulfide formation, MTG standard was dissolved in water and kept at ambient temperature, and no effort was made to stabilize the thiol. Fig. 7 shows a plot of the (●) disappearance of MTG and (◯) appearance of the disulfide of a standard solution. The insert shows a chromatogram of the conversion of MTG with a starting concentration of 100 ppb to disulfide at time point 3.5 h. Effects of sample diluent, presence of metal ions, pH, and amount of dissolved oxygen can cause variability in rate of oxidation. The reaction followed first order kinetics, as determined by a log (area) versus time (h) plot ($R^2 = 0.985$). As both reduced and oxidized thiols are detected in the same run, this method can be used to study the rate of disulfide formation or to monitor the quality control of the formulation.

4. Conclusions

IPTG, MTG, and MTG's disulfide product are readily detected using PED. Excellent analytical figures of merit and

low detection limits are obtained by the use of an IPAD waveform for the determination of sulfur compounds. The high selectivity of this approach assures that the matrix components of the assay do not interfere with their determination as shown by the MTG formulation buffer and protein drug product samples. The assay is also useful in monitoring disulfide formation kinetics and assuring quality control for biopharmaceuticals.

References

[1] M. Ash, I. Ash, Handbook of Pharmaceutical Additives, Ashgate Publishing Company, December 1996.
[2] L.A. Damani, M. Mitchard, in: L.A. Damani (Ed.), Sulphur-Containing Drugs and Related Organic Compounds, vol. 1, part A, Ellis Horwood Ltd., Chichester, England, 1989, pp. 93–111.
[3] K. Ko, J. Kruse, N.L. Pohl, Org. Lett. 5 (2003) 1781–1783.
[4] L.V. Allen Jr., Int. J. Pharm. Compd. 3 (1999) 52–55.
[5] K. Jensen, G.T. Javor, Antimicrob. Agents Chemother. 19 (1981) 556–561.
[6] G.T. Javor, J. Bacteriol. 171 (1989) 5607–5613.
[7] G.T. Javor, E.F. Febre, J. Bacteriol. 174 (1992) 1072–1075.
[8] W.K. Seltzer, G. Dhariwal, H.A. McKelvey, E.R.B. McCabe, Life Sci. 39 (1986) 1417–1424.
[9] W.R. LaCourse, Pulsed Electrochemical Detection in High Performance Liquid Chromatography, Wiley, New York, 1997.
[10] W.R. LaCourse, G.S. Owens, Anal. Chim. Acta 307 (1995) 301–319.
[11] C.O. Dasenbrock, W.R. LaCourse, Anal. Chem. 70 (1998) 2415–2420.
[12] C.C. Yan, R.J. Huxtable, J. Chromatogr. B 672 (1995) 217–224.
[13] K. Mopper, D. Delmas, Anal. Chem. 56 (1984) 2557–2560.
[14] P.C. White, N.S. Lawrence, J. Davis, R.G. Compton, Electroanalysis 14 (2002) 89–98.
[15] T.J. O'Shea, S.M. Lunte, Anal. Chem. 65 (1983) 247–250.
[16] J.A. Stenken, D.L. Puckett, S.M. Lunte, C.E. Lunte, J. Pharm. Biomed. Anal. 8 (1990) 85–89.
[17] D.C. Johnson, W.R. LaCourse, Anal. Chem. 62 (1990) 589A–596A.
[18] D.C. Johnson, W.R. LaCourse, Electroanalysis 4 (1992) 367–380.
[19] G.S. Owens, W.R. LaCourse, Curr. Separat. 14 (1996) 82–88.
[20] P.J. Vandeberg, D.C. Johnson, Anal. Chem. 65 (1993) 2713–2718.
[21] P. Jandik, J. Cheng, J. Evrovski, N. Avdalovic, J. Chromatogr. B 759 (2001) 145–151.
[22] Y. Hiraku, M. Murata, S. Kawanishi, Biochim. Biophys. Acta 1570 (2002) 47–52.
[23] K. Rasmussen, J. Møller, Ann. Clin. Biochem. 37 (2002) 627–648.
[24] C.O. Dasenbrock, W.R. LaCourse, J. Pharm. Biomed. Anal. 7 (1998) 239–252.
[25] C.O. Dasenbrock, W.R. LaCourse, Adv. Chromatogr. 38 (1998) 189–232.
[26] V.P. Hanko, W.R. LaCourse, C.O. Dasenbrock, J.S. Rohrer, Drug Dev. Res. 53 (2001) 268–280.
[27] P.J. Vandeberg, J.L. Kowagoe, D.C. Johnson, Anal. Chim. Acta 260 (1992) 1–11.
[28] R.D. Rocklin, A.P. Clarke, M. Weitzhandler 70 (1998) 1496–1501.
[29] D.A. Dobberpuhl, D.C. Johnson, Anal. Chem. 67 (1995) 1254–1258.
[30] C. Toniolo, A. Fontana, in: S. Patai (Ed.), The Chemistry of the Thiol Group Part 1, Wiley, New York, 1974, pp. 355–415.