The Use of Constant Potential Amperometry to Determine the Oxidation Rate of [R-(Z)]-α-(methoxyimino)-1-azabicyclo[2.2.2]-octane-3-acetonitrile by Dimethyldioxirane

*Julie N. Ennis**
Astra Charnwood
Bakewell Road
Loughborough, Leics
LE11 ORH

P.C. Buxton
SmithKline Beecham
Pharmaceuticals
Third Avenue
Harlow, Essex CM19 5AW

**Corresponding author*

*Constant potential amperometry was used to study the reaction rate of the oxidation of [R-(Z)]-α-(methoxyimino)-1-azabicyclo[2.2.2]-octane-3-acetonitrile by dimethyldioxirane. The reaction was found to follow pseudo first order reaction kinetics in the presence of an excess of [R-(Z)]-α-(methoxyimino)-1-azabicyclo[2.2.2]-octane-3-acetonitrile and gave a pseudo first order rate constant of 3.95 s^{-1}. The rate constant is of a similar order to the 5.86 s^{-1} obtained on reaction of 4-nitro-*N*,*N*-dimethylaniline, also a tertiary amine, with dimethyldioxirane in water, recorded using a spectrophotometric technique.*

Constant potential amperometry was designed to be used primarily in the study of electrochemical sensors, particularly with regards to investigating the efficiency of electrode coating and membrane formation. Eddy *et al.* monitored the coating of a platinum electrode with modifiers, such as phenols, by recording current versus time profiles for the loss of response to the modifying species due to film formation (1). In this paper we report another use of the technique: to study reaction kinetics by recording current versus time profiles for the loss of dimethyldioxirane (DMD) at a mercury drop electrode, on addition of [R-(Z)]-α-(methoxymino)-1-azabicyclo[2.2.2]-octane-3-acetonitrile ***(F1)***.

F1 is a compound currently under development at SmithKline Beecham Pharmaceuticals which has been found to degrade on storage in tablets to its *N*-oxide. DMD ***(F2)*** oxidizes ***F1*** to its *N*-oxide rapidly and in quantitative yield ***(F3)*** (2). Constant potential amperometry was used to study the rate of this oxidation process. The technique has also been extended to study reaction rates and mechanisms of other non-cyclic peroxides (3).

The kinetics of DMD oxidations have already been determined for a number of substrates by periodic sampling of the reaction mixture and measurement of the reactants or products by chromatographic or spectroscopic methods (4,5). Ultraviolet spectroscopic measurement of DMD is complicated by its extremely weak chromophore and the associated presence of acetone from which the reagent is derived. Furthermore, many of the substrates examined absorb UV light in the DMD region, leading to further potential problems in interpreting the data. We wished to carry out reactions with tertiary amines in a substantially aqueous medium and we have shown that under these conditions the oxidations involving DMD can be rapid. Periodic sampling and subsequent analysis would not, therefore, be a practical proposition. It seemed to us that an electrochemical method based on the reduction of DMD would provide good specificity and

sensitivity such that we could monitor the oxidant directly without interference from the substrate or solvent.

Experimental Procedures

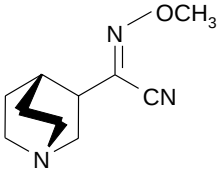
Polarographic and kinetic experiments were performed using a Bioanalytical Systems CV-50W voltammetric analyzer with a controlled-growth mercury electrode detector.

The polarographic peak potential of DMD (0.14 V vs. AgCl/Ag) was recorded in 0.1 M potassium nitrate:acetonitrile solution 50:50 v/v under the following conditions:

Mode:	differential pulse polarography
Initial potential:	+ 0.25 V
Final potential:	- 0.5 V
Purge time:	100 s (Helium)
Pulse amplitude:	50 mV
Pulse width:	50 ms
Drop time:	1000 ms
Sensitivity:	1 μA
Drop size:	8

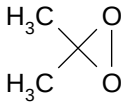
F1

[R-(Z)]-α-(methoxyimino)-1-azabicyclo[2.2.2]-octane-3-acetonitrile.



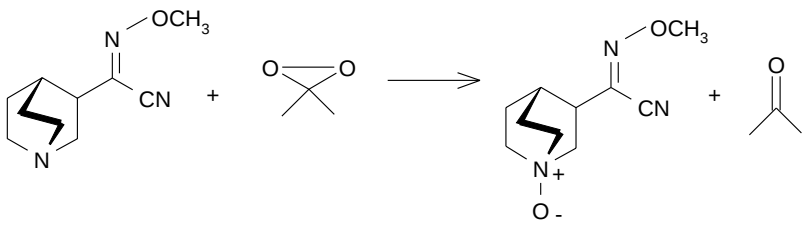
F2

Dimethyldioxirane.



F3

*DMD oxidizes **F1** to its N-oxide rapidly and in quantitative yield. Constant potential amperometry was used to study the rate of this oxidation process.*



Dimethyldioxirane was prepared according to a literature procedure (6).

Kinetic experiments were performed using the timebase function with the following parameters:

Initial potential:	80 mV
Sampling time:	1 s
Run time:	200 s
Sensitivity:	1 μA
Drop size:	8
Stirrer speed:	600 rpm

There was no need to purge the system since the operating potential of 80 mV was not sufficiently negative to reduce any oxygen in the system.

The samples were prepared for the kinetic experiments as follows: dimethyldioxirane solution (1 ml) was transferred by pipette into a volumetric flask (100 ml) and the volume made up with 0.1 M potassium nitrate:acetonitrile 50:50 v/v. The stock solution (20 ml) was then transferred by pipette into a volumetric flask (100 ml) and the volume again made up with 0.1 M potassium nitrate:acetonitrile, 50:50 v/v. The working solution (10 ml) was then transferred using a measuring cylinder into the polarographic cell. The solution was stirred and the run started. Once a steady baseline was established, a solution of ***F1*** in acetonitrile at a concentration of at least five times the concentration of the dimethyldioxirane (50 - 250 μl) was added to the cell using a microsyringe.

The pseudo first order rate constant (k_{obs}) for the reaction between ***F1*** and DMD was obtained from the slope of ln current against time.

Results

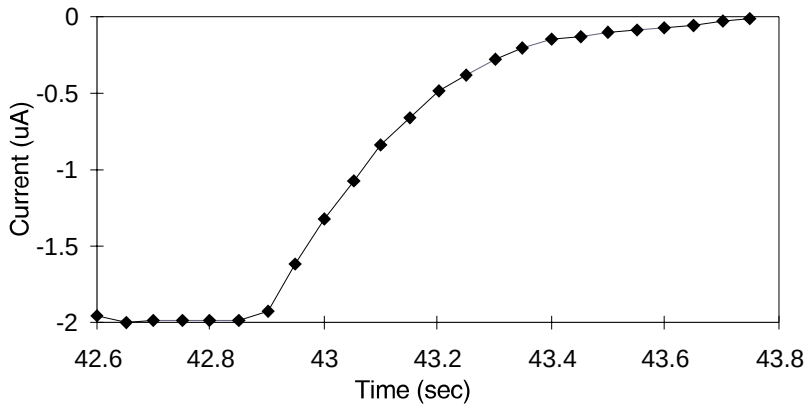
The electrochemical properties of dimethyldioxirane and other peroxides are such that satisfactory reduction waves or peaks can only be observed with a mercury working electrode and an unbuffered supporting electrolyte (7). Continuous monitoring of the oxidant requires the mercury drop to be stationary and the current needs to be sampled automatically. This is essentially a controlled potential amperometric technique which is based on hydrodynamic voltammetry, but rather than keeping the solution still and rotating the electrode, the electrode is held still and the solution stirred. In this way mass transport of the oxidizing agent is occurring by both convection, in the bulk solution, and diffusion, at the electrode surface.

In hydrodynamic voltammetry the concentration gradient only extends across the diffusion layer, so the oxidant is brought to the electrode (and the reduced species is removed) at a fast rate. As a result, there is a limiting current which, on addition of the substrate to the stirred solution of the oxidant, decreases as the reaction between the oxidant and substrate proceeds ***(F4)***. Quantitation of DMD is facilitated by the direct relationship that would be expected from an amperometric technique between current and concentration. This was confirmed by measurement of the amperometric current for varying concentrations of DMD. A summary of the linearity data is presented in ***T1***.

When ***F1*** was added to the electrochemical cell containing DMD there was a rapid loss of current ***(F4)*** and the reaction was essentially complete within 400 milliseconds. Transformation of the data showed the expected adherence to pseudo first order kinetics ***(F5)*** with the mean rate constant determined to be 3.95 s^{-1} ***(T2)***. This rate constant is of a similar order to the rate constant of 5.86 s^{-1} obtained on reaction of 4-nitro-*N*,*N*-dimethylaniline, also a tertiary amine, with dimethyldioxirane in water, recorded using a spectrophotometric technique (8).

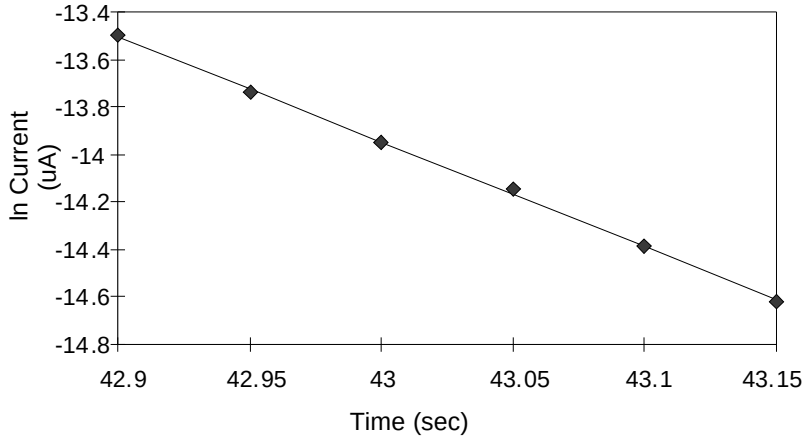
F4

*Loss of polarographic current with time on addition of **F1** to DMD solution.*



F5

Example pseudo first order plot for the loss of current with addition of SB202026 to DMD.



T1

Hydrodynamic linearity over concentration range 11-59 μg/ml.

The 95% confidence intervals intersected the origin.

Concentration (μg/ml)	Current (nA)
59.20	1010
23.68	388
17.76	248
11.84	154
Correlation coefficient	**0.999**
Slope	**18.1**
Intercept	**59.2**

T2

	Rate constant (s^{-1})	Correlation coefficient
1	4.42	0.997
2	3.48	0.996
3	4.02	0.996
4	3.86	0.997
Mean	**3.95**	

Conclusions

It has been shown that constant potential amperometry is a technique which can be used to study reaction rates and mechanisms. The use of the technique in this novel way provides significant advantages over other techniques currently available for studying DMD oxidations. In this particular instance the rate of oxidation of ***F1*** by DMD provided additional data on the rate of oxidative degradation of a drug molecule.

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References

1. *S. Eddy, K. Warriner, I. Christie, D. Ashworth, C. Purkiss, P. Vadgama, Biosensors & Bioelectronics 10 (1995) 831.*
2. *P. C. Buxton, J. N. Ennis, Analytical Communications 33 (1996) 261.*
3. *P. C. Buxton, J. N. Ennis, in print.*
4. *R.W. Murray, D.L. Shiang, J. Chem. Soc., Perkin Trans. 2 (1990) 349.*
5. *A.L. Baumstark, P.C. Vasquez, J. Org. Chem. 53 (1988) 3437.*
6. *R.W. Murray, R. Jeyaraman, J. Org. Chem. 50 (1985) 2847.*
7. *P.A. Giguère, D. Lamontagne, Can. J. Chem. 29 (1951) 54.*
8. *P. C. Buxton, J. N. Ennis, B.A. Marples, V. L. Waddington, T. R. Boehlow, J. Chem. Soc., Perkin Trans. (1998) 265.*