POLAROGRAPHY IN DRUG AND PHARMACEUTICAL ANALYSIS

Mark Cilia

Introduction

Electroanalysis is the separation and determination of chemicals by electrolysis. Voltammetry is a term used to describe all electroanalytical techniques, which involve the application of an external potential on the system and utilize the current-potential relationship arising at a polarizable microelectrode to calculate the concentration of the electroactive species. When the working electrode is a mercury electrode, the technique is called polarography.

The purpose of this work is to illustrate the applicability of these techniques, especially polarography in pharmacy. This is because the past decase has revealed a remarkable and renewed interest in the application of electroanalytical techniques to the analysis of drugs and pharmaceuticals.

Electroanalytical methods, in particular voltammetric techniques are well established in pharmaceutical and biomedical analysis. The requirements of analytical techniques in clinical chemistry, toxicology and drug control laboratories increase as more potent compounds are developed which give lower therapeutic concentrations. Moreover, information concerning the absorption, distribution, biotransformation and elimination of pharmacologically active compounds is useful for detecting human diseases and for determining the bioavailability and pharmacokinetic profile of a new drug and its metabolites. Therefore, highly selective and sensitive analytical methods are required. A sophisticated development in electronics and an increased understanding of the techniques themselves have resulted in voltammetric techniques that allow quantitative determination at the 10^{-6} - 10^{-9}M concentration range (Vire et al, 1988).

In this project, a brief explanation of the theory and instrumentation behind these techniques, together with an overview on pharmaceuticals treated by voltammetric techniques and other related applications is presented. As an illustration, various stability studies were carried out on ascorbic acid (Vit. C), using the polarographic method of determination.

Methodology

The method used for ascorbic acid polarographic determination was that described in Metrohm's Application Bulletin No. 98. For quantitative

analysis, either a calibration curve or the standard addition method was used, as specified for each study.

Study 1

In this study, two experiments were carried out:

1. To investigate the chemical stability of ascorbic acid in 100mg B.P. tablets upon exposure to dry heat, at temperatures of 4°, 20°, 40°, 60° and 80°C over a period of 105 days.

2. To study the stability of ascorbic acid tablets when subjected to different relative humidities (RHs) of 22.9%, 35%, 52%, 75% and 95% over a period of 50 days.

For both experiments, batches containing 20 tablets of ascorbic acid 100mg each, representing one of the 5 RHs or temperatures respectively, were analysed at specified points in time using the polarographic method mentioned above, in order to determine the residual % weight of ascorbic acid remaining. The shelf-life for ascorbic acid tablets was then determined using the Arrhenius equation.

Study 2

The polarographic method was used to study the aerobic oxidation of ascorbic acid. The pH-log profile of the rate of disappearance of ascorbic acid from aqueous solution under aerobic conditions, was determined at 4°C in the pH range of 4.00 - 5.00, which falls within the pH range (3.52 - 6.22) of most liquid formulations containing ascorbic acid (Blaug et al., 1972). The pKa, was determined by measuring the pH at 4°C of solutions containing 0.035 mole L^{-1} each of ascorbic acid and sodium monohydrogen ascorbate. The average of three readings gave a value of 4.72.

Study 3

In this part of the project, the accuracy and precision for the polarographic method and the British Pharmacopoeia (B.P.) 1988 assays for ascorbic acid, were determined and compared using statistical tests. The B.P. 1988 gives the ammonium cerium (iv) sulphate titration for ascorbic acid in tablet formulations, and the iodometric titration for ascorbic acid in injections (B.P. 1988 Addendum 1989), which together

with the previously used polarographic method, were carried out to assay different potency strengths of ascorbic acid.

Precision is often numerically expressed as the standard deviation or relative standard deviation (RSD), given by s/x, or the percentage RSD, known as the coefficient of variation (CV) given by 100s?x; s being an overall standard deviation and x the mean. Accuracy is determined by how close the result is to the actual concentration and its ± interval. This interval being defined by ± $ts/n^{1/2}$, where t is a value found from statistical tables.

The statistical 't' test was then used to compare the results of the different methods, and determine whether a statistical difference exists between their means - the 95% confidence interval being used. (Analysis by Tyson, 1988).

Results

Study 1

Ascorbic acid in tablet form showed good chemical stability under accelerated studies of:

1. Varying humidity over a period of 50 days;
2. Temperatures of 4°, 20°, 40° and 60°C in the 105 days period of the experiment, while for the 80°C the percentage of active ingredient went below 95% only after the 6th week.

The shelf-life, determined using the Arrhenius equation was found to be 4.4 years (95% potency retention).

Study 2

Yellowish discolouration occurred in all pH's for acetate buffer after the third day of the experiment, while those for phosphate buffer remained all colourless except pH5.00 and 4.80, for the 15 day period of the study.

Table 1: Specific rate constants (K) and half-life periods ($t^{1}/2$) for oxidation of ascorbic acid at a range of pH 4.00 - 5.00 for acetate and phosphate buffers.

pH	Buffer	$Kx10^{-2}$ $days^{-1}$	$\log K$	$t^{1}/2$ days
4.00	Acetate	40.69	-0.391	1.70
	Phosphate	13.29	-0.876	5.21
4.20	Acetate	40.98	-0.387	1.69
	Phosphate	11.41	-0.943	6.08
4.40	Acetate	50.45	-0.297	1.37
	Phosphate	12.96	-0.887	5.35
4.60	Acetate	26.20	-0.582	2.65
	Phosphate	55.92	-0.252	1.24
4.80	Acetate	91.98	-0.036	0.75
	Phosphate	18.02	-0.744	3.85
5.00	Acetate	55.06	-0.259	1.26
	Phosphate	21.34	-0.671	3.25

Discussion

The shelf-life for ascorbic acid tablets; determined using the Arrhenius equation, was found to be 4.4 years as assayed by the polarographic method, the latter being rapid, easy to use and precise. Several possibilities were outlined in an attempt to explain the chemical stability of ascorbic acid tablets to different moistures. These are followed by some practical recommendations and areas where the pharmacist can utilise his knowledge in stability-related areas.

In the study of the effect of H ion concentration on rate of ascorbic acid oxidation, the slopes of the lines were calculated by regression analysis. All of the rate constants were calculated from the first-order rate equation, using the method of least squares. The aerobic oxidation of ascorbic acid solutions of 4°C was shown to have lowest rate constant in phosphate buffer pH 4.20 with a $t^{1}/2$ of 6 days. The pH-log rate profile at 4°C showed a maximum near the pKa, of ascorbic acid. According to Blaug et al., (1972), a reaction can be proposed whose rate law predicts a

Study 3

Table 2: Showing precision expressed as coefficient of variance (CV) and accuracy, for iodometric titrations versus polarography

		94.50%	95.00%	95.50%	105.00%	105.50%
CV	Titn.	1.01	1.02	1.02	1.00	1.02
	Pol.	0.86	0.36	0.42	0.66	0.70
Accuracy	Titn.	94.36±1.00%	94.88±0.01%	95.50±1.02%	104.75±1.11%	105.54±1.13%
	Pol.	93.97±0.85%	95.71±0.36%	95.51±0.42%	104.97±0.73%	105.44±0.77%

Table 3: Showing precision expressed as CV and accuracy, for ammonium cerium (iv) sulphate titrations versus polarography

	94.50%	95.00%	95.50%	107.50%	108.00%
CV Titn.	0.35	0.31	0.26	0.21	0.26
CV Pol.	0.48	1.04	0.36	0.57	0.31
Accuracy Titn.	94.40±0.35%	94.81±0.32%	95.40±0.26%	107.26±0.24%	107.79±0.29%
Accuracy Pol.	94.37±0.47%	94.95±0.99%	95.47±0.36%	107.41±0.64%	107.65±0.35%

decrease in rate of ascorbic acid oxidation with increasing ascorbic acid oxidation.

In this study the polarographic method was ideal since very small volumes (1.0ml) of buffer were needed for each assay.

When compared to the iodometric titrations, polarography tends to have a better precision and accuracy as shown by a narrower ±interval; while the ammonium cerium (iv) sulphate titrations tend to have better precision but relatively the same accuracy. However these differences are small and were found to be not significant when using the statistical 't' test.

Hence the polarographic advantages - mainly accuracy, precision and sensitivity - are best pronounced in trace analysis and low drug concentrations rather when compared for high molarities; while the advantages of rapidity, automation capability, selectivity and specificity (most often no separation of constituents is needed), and ease of operation become important in routine analysis. Varous impressive features which represent the state-of-the art technology in polarographic analysis were not able to be used in the conducted experiments, such as automated standard addition for greater accuracy and smaller standard deviation.

Indeed polarography offers other advantages which were not able to be shown in the work carried out. These include for instance, simultaneous determination of drugs and their metabolites or degradative substances, and non interference from other drugs, minerals, or constituents in multidrug e.g. multivitamin preparations. This could perhaps provide ground for future studies.

References

Blaug S.M., Hajratwala B. Kinetics of Aerobic Oxidation of Ascorbic Acid. J. oif Pharmaceutical Sciences 1972; 61: 556=562.

Tyson J. Analaysis. RSC publications 1988: 125.

Vire J.C. et al. Adsorptive stripping voltammetry applied to drug analysis: a powerful tool. J. of Pharmaceutical and Biomedical Analysis 1989; 7: 1323-1335.