POLAROGRAPHIC AND VOLTAMMETRIC DETERMINATION OF CHLOROBENZENE, BENZYL CHLORIDE AND MELPHALAN*

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Dedicated to Professor Jaroslav Zýka on the occasion of his 70th birthday.

Conditions were found for the determination of chlorobenzene and benzyl chloride in dimethylformamide and of Melphalan in ethanol–water medium (4 : 1) using tast polarography at a classical dropping mercury electrode in the concentration range $1 . 10^{-3}-2 . 10^{-5}$ mol l^{-1}. Differential pulse polarography permits the range for the determination of benzyl chloride to be extended to $1 . 10^{-5}-2 . 10^{-6}$ mol l^{-1}. The use of fast scan differential pulse voltammetry and linear scan voltammetry at a hanging mercury drop electrode did not lead to an increase in the sensitivity of the determination because of the very negative half-wave potential values and high irreversibility of the processes involved.

Benzyl chloride and Melphalan are effective alkylation agents whose reactivity for cellular nucleophiles leads to high genotoxicity[1,2]. In addition, Melphalan (4-bis-(2-chloroethyl)amino-L-phenylalanine) is utilized clinically in the treatment of various types of tumours[3]. Chlorobenzene is an acutely toxic substance[4] and prolonged chronic exposure can lead to damage to the liver and kidneys. It can be seen from a review of the methods used to determine chlorinated hydrocarbons[5] and Melphalan[6] that chromatography or spectrometry is used most often. Far less attention has been paid to polarography or voltammetry for the determination of these substances. The difficulty of polarographically reducing the C—Cl bond[7] makes it necessary to work in nonaqueous medium using a base electrolyte decomposing at a sufficiently negative potential. DC polarography of chlorobenzene in a medium of dimethylformamide and 0·05 mol l^{-1} tetraethylammonium bromide ($E_{1/2}$ = = −2·6 V vs SCE) can be utilized analytically[8,9] in the range $1 . 10^{-3}-2 . 10^{-4}$ mol. . l^{-1}. This is also roughly true for benzyl chloride in dioxane–water medium (3 : 1) with 0·05 mol l^{-1} tetraethylammonium bromide ($E_{1/2}$ = −1·94 V vs SCE)[10].

However, no mention is made in the literature of the determination of chlorobenzene or Melphalan using modern techniques such as tast polarography and differential pulse polarography (DPP) at a classical dropping mercury electrode

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(DME) or fast scan differential pulse voltammetry (FS DPV) and linear scan voltammetry (LSV) at a hanging mercury drop electrode (HMDE). Consequently, this work was devoted to a study of the usefulness of these techniques for improving the sensitivity of the determination of these substances The base electrolyte for the determination of chlorobenzene was tetraethylammonium iodide, whose cation facilitates the reduction[11] compared to the tetrabutylammonium cation, in whose presence the wave of chlorobenzene coincides with the decomposition of the base electrolyte[8]. On the other hand, for benzyl chloride, where the tetraethylammonium cation has no favourable effect, tetrabutylammonium iodide was used as the base electrolyte. This substance decomposes at more negative potentials[12] and mercury continues to form regular drops at very negative potentials in its presence.

EXPERIMENTAL

Reagents

The stock solutions of benzyl chloride ($c = 0{\cdot}1$ mol l^{-1}) and chlorobenzene ($c = 0{\cdot}1$ mol l^{-1}) were prepared by dissolving an exactly weighed amount of the p.a. substance (Lachema, Brno) in dimethylformamide. The stock solution of Melphalan ($c = 0{\cdot}04$ mol l^{-1}) was prepared by dissolving an exactly weighed amount of the substance provided by the International Agency for Research on Cancer, Lyon, France, in methanol. The purity of the chlorobenzene and benzyl chloride were checked using gas chromatography, while that of Melphalan was tested using thin-layer and high-performance liquid chromatography[13]. The dimethylformamide employed (Lachema, Brno) was purified as follows: the solvent was stored for one week over solid sodium hydroxide, which was changed once every two days. The solvent was then doubly distilled, first under decreased pressure and then under a vacuum. The distillate was collected in a vessel containing annealed molecular sieve, over which it was stored. The remaining chemicals were of p.a. purity (Lachema, Brno).

Apparatus

Work was carried out using a PA 3 polarographic analyzer with XY 4105 recorder (both from Laboratorní přístroje, Prague). DC, tast and DP polarography were carried out using a dropping mercury electrode with the following parameters: at a mercury reservoir height of $h = 25$ cm, the flow rate $m = 0{\cdot}42$ mg s^{-1} and drop time $\tau = 13{\cdot}99$ s (measured at an applied potential of 0 V vs SCE in medium of $0{\cdot}05$ mol l^{-1} tetrabutylammonium iodide in dimethylformamide). A capillary with a low flow rate and long drop time was chosen intentionally to avoid difficulties encountered at very negative potentials when working with a capillary with higher flow rate. A polarization rate of 5 mV s^{-1} was used and, where not stated otherwise, a drop time of 1 s was adjusted in tast and DP polarography, with a mercury reservoir height of 25 cm and DPP modulation amplitude of -100 mV. FS DPV and LSV were carried out using an SMDE 1 static mercury drop electrode (Laboratorní přístroje, Prague), connected as a HMDE. Where not stated otherwise, a polarization rate of 20 mV s^{-1} was used, with a maximum drop size attained by opening the valve for 160 ms and FS DPV modulation amplitude of -100 mV. All experiments were carried out in the three-electrode arrangement with a saturated calomel reference electrode and platinum foil auxiliary electrode. The SCE was connected with the test solution using a salt

bridge filled with the base electrolyte in the appropriate solvent. Oxygen was eliminated from the test solution by bubbling for ten minutes with nitrogen, which was purified by passing through a solution of chromium(II) ions in dilute (1 : 1) hydrochloric acid over zinc amalgam. Before entering the polarographic vessel, the nitrogen was passed through a prebubbler containing the test solvent.

Procedure

Calibration curves were measured in triplicate and evaluated statistically by the least squares linear regression method. The determination limit was calculated as ten times the standard deviation of ten determinations of the analyte with a concentration corresponding to the lowest point on the given calibration curve[14]. Where not stated otherwise, measurements were carried out at laboratory temperature.

RESULTS AND DISCUSSION

DC *Polarography*

In a medium of 0·05 mol l^{-1} tetrabutylammonium iodide in dimethylformamide, benzyl chloride yields one well-developed, strongly irreversible wave that is diffusion controlled and has a half-wave potential of −2·25 V vs SCE. The slope of the logarithmic analysis equals 135 mV. The wave height depends linearly on the depolarizer concentration in the range $1 . 10^{-3} - 1 . 10^{-4}$ mol l^{-1}.

In medium of 0·05 mol l^{-1} tetraethylammonium iodide in dimethylformamide, chlorobenzene also yields a single irreversible wave with a half-wave potential of −2·58 V vs SCE. The slope of the logarithmic analysis equals 90 mV. The wave height is proportional to the concentration of chlorobenzene in the range $1 . 10^{-3}$ to $1 . 10^{-4}$ mol l^{-1}.

On the basis of preliminary experiments[13], 0·05 mol l^{-1} tetrabutylammonium iodide in an ethanol–water mixture (4 : 1) was selected as the base electrolyte for the determination of Melphalan. Under these conditions, Melphalan yields one strongly irreversible wave with a half-wave potential of −1·88 V vs SCE; to suppress the maximum, it is necessary to add 0·1 ml of 0·5% gelatine solution to 10 ml of the polarographed solution. The slope of the logarithmic analysis of this wave equals 155 mV and cyclic voltammetry at the HMDE confirmed that this is a completely irreversible process. The height of this wave is proportional to the Melphalan concentration in the range $1 . 10^{-3} - 1 . 10^{-4}$ mol l^{-1} and is independent of the mercury reservoir height. Consequently, the polarographic behaviour of unsubstituted α-phenylalanine was studied under the same conditions and it was found that this substance yields a practically identical, irreversible wave, whose height is also independent of the mercury reservoir height. It can be assumed that the observed Melphalan wave is connected with reduction in the phenylalanine part of the molecule,

whereas the expected reduction of the C—Cl bonds is overlapped by the decomposition of the base electrolyte. The observed wave of Melphalan and phenylalanine probably corresponds to the reduction of hydrogen ions provided by the carboxyl group, and the wave height is controlled by the dissociation kinetics, similar to boric acid[15]. This suggestion is supported by the fact that the wave disappears in a medium of 0·05 mol l^{-1} tetramethylammonium hydroxide in 80% ethanol.

TABLE I

Parameters of calibration curves for the determination of benzyl chloride (*I*), chlorobenzene (*II*) and Melphalan (*III*) by various polarographic and voltammetric techniques

Technique	Substance	Concentration mol l^{-1}	Slope mA mol^{-1} l	Intercept nA	Correl. coef.	L_Q[a] mol l^{-1}
Tast	*I*	$(1-10) . 10^{-4}$	2·32	12	0·9995	—
		$(1-10) . 10^{-5}$	2·00	2	0·9999	$1·1 . 10^{-5}$
	II	$(1-10) . 10^{-4}$	4·93	16	0·9999	—
		$(1-10) . 10^{-5}$	5·03	3	0·9998	$1·0 . 10^{-5}$
	III	$(1-10) . 10^{-4}$	1·02	15	0·9991	—
		$(1-10) . 10^{-5}$	0·95	2	0·9963	$2·1 . 10^{-5}$
DPP	*I*	$(1-10) . 10^{-4}$	1·84	20	0·9997	—
		$(1-10) . 10^{-5}$	1·48	7	0·9983	—
		$(2-10) . 10^{-6}$	1·22	−1	0·9919	$2·9 . 10^{-6}$
	II	$(1-10) . 10^{-4}$	3·92	8	0·9998	—
		$(1-10) . 10^{-5}$	4·09	−3	0 9999	$0·4 . 10^{-5}$
	III	$(1-10) . 10^{-4}$	0·35	−8	0·9979	—
		$(1-10) . 10^{-5}$	0·33	1	0·9994	$0·8 . 10^{-5}$
FSDPV	*I*	$(1-10) . 10^{-4}$	3·04	27	0·9999	—
		$(1-10) . 10^{-5}$	3·06	−7	0·9998	$0·4 . 10^{-5}$
	II	$(1-10) . 10^{-4}$	0·81	13	0·9997	—
		$(1-10) . 10^{-5}$	0·76	6	0·9975	$1·5 . 10^{-5}$
	III	$(1-10) . 10^{-4}$	1·27	26	0·9994	$0·7 . 10^{-4}$
LSV	*I*	$(1-10) . 10^{-4}$	2·17	16	0·9998	—
		$(1-10) . 10^{-5}$	2·06	8	0·9988	$1·2 . 10^{-5}$
	II	$(1-10) . 10^{-4}$	3·97	−15	0·9998	—
		$(1-10) . 10^{-5}$	4·93	6	0·9992	$1·0 . 10^{-5}$
	III	$(1-10) . 10^{-4}$	1·15	−20	0·9952	$1·6 . 10^{-4}$

[a] Determination limit.

Tast Polarography at the Dropping Mercury Electrode

This technique permits the determination of concentrations of test substances an order lower than that attainable using classical DC polarography. The dependence of the wave height of the test substance is linear in the range $1 . 10^{-3} - 2 . 10^{-5}$ mol . $. l^{-1}$; the parameters of these curves are given in Table I. Lower concentrations cannot be determined using tast polarography because of the very negative half-wave

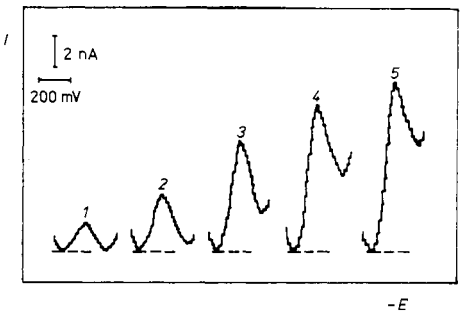

FIG. 1

Differential pulse polarograms of benzyl chloride (*I*) in 0·05 mol l^{-1} tetrabutylammonium iodide in dimethylformamide. *c*(*I*) (μmol l^{-1}): *1* 2; *2* 4; *3* 6; *4* 8; *5* 10. Initial potential −2·1 V. The dashed line corresponds to the baseline from which the peak heights were measured

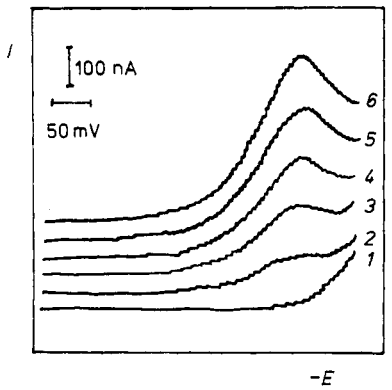

FIG. 2

Differential pulse polarograms of chlorobenzene (*II*) in 0·05 mol l^{-1} tetraethylammonium iodide in dimethylformamide. *c*(*II*) (μmol l^{-1}): *1* 0; *2* 20; *3* 40; *4* 60; *5* 80; *6* 100. Initial potential −2·25 V. The peak height was measured from the base electrolyte curve

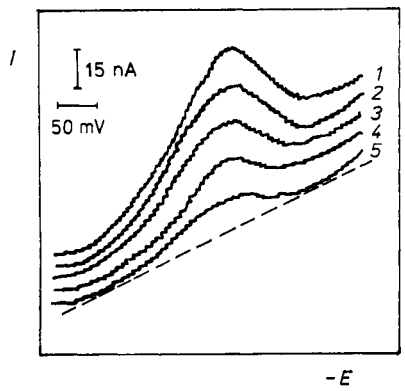

FIG. 3

Differential pulse polarograms of Melphalan (*III*) in 0·05 mol l^{-1} tetrabutylammonium iodide in mixed ethanol–water (4 : 1) solution. *c*(*III*) (μmol l^{-1}): *1* 100; *2* 80; *3* 60; *4* 40; *5* 20. Initial potential −1·7 V. The dashed line corresponds to the baseline from which the peak heights were measured

potential of the test substances and the high residual current in this region. The residual current could not be decreased even by further distillation of the solvent or electrochemical purification of the base electrolyte by electrolysis on a large-area mercury electrode at the potential of the limiting currents of the test substances.

Differential Pulse Polarography at a Dropping Mercury Electrode

This technique permitted a decrease in the determination limit of chlorobenzene and Melphalan to about one half of that attainable using tast polarography. A linear concentration dependence was still obtained for benzyl chloride in the range $(2-10) . 10^{-6}$ mol l^{-1} (see Table I), connected with its more positive half-wave potential. The peaks obtained in DPP are more easy to evaluate than the waves obtained in tast polarography. The manner of evaluating these peaks is depicted in Figs 1–3, where it can also be seen that the peaks are very wide and relatively low as a consequence of the high irreversibility of the electrode processes. Consequently, DPP does not yield a much lower determination limit compared with tast polarography, as is true of more reversible systems.

Fast Scan Differential Pulse Voltammetry and Linear Scan Voltammetry at a Hanging Mercury Drop Electrode

These techniques were employed for the determination of chlorobenzene using a smaller hanging mercury drop electrode obtained by opening the valve for 40 ms. (When a larger drop was used, it was frequently torn off at potentials more negative than −2·5 V.) An attempt was made to eliminate the coalescence of the peak of chlorobenzene with the decomposition of the base electrolyte by decreasing the pulse height to −12·5 mV. The parameters of the calibration curves obtained are given in Table I, where it can be seen that the very negative potentials of the peaks and the high irreversibility of the processes involved prevent these techniques from being used to decrease the determination limit compared with tast and DP polarography; in fact, for Melphalan, determination becomes impossible in the concentration range $(1-10) . 10^{-5}$ mol l^{-1}. Thus the only advantage remains the decrease in the time required to record the voltammetric curve and the lower mercury consumption.

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