Bioelectrochemistry became again object of interest since about 40 years, rapidly covering very different fields. Following examples are then mentioned giving some more details; Chemical metabolism; Membrane phenomena; Photosynthesis; Active transport; Electroanaesthesia; Transmission of information; Electropermeabilization, Ilectroporation and gene transfer. Medical diagnosis; Electrocardiography and electroencephalography.

ELECTROCHEMICAL BEHAVIOUR AND DETERMINATION OF CYTARABINE

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ABSTRACT

An electroanalytical study of the cytarabine reduction process at a dropping mercury electrode in aqueous supporting electrolyte solution using direct current polarographic technique has been carried out. The optimum parameters were found as 1000 dyne.cm^{-2} pressure on the mercury reservoir, 1s drop time, 4 mV.s^{-1} scan rate and 5.50-6.60 pH range. The reversibility of the reduction on the mercury electrode was ascertained as quasi-reversible and the polarographic current was mainly diffusion controlled. The results obtained by DC, SIAP and DP polarography allowed a method developed for the determination of cytarabine in the 1x10^{-4} - 5x10^{-4} mol.L^{-1} concentration range. Good results were obtained by applying the DC polarographic technique to the determination of cytarabine in a pharmaceutical preparation.

Keywords. Cytarabine determination, direct current polarography.

INTRODUCTION

Cytarabine (ara-C, 1-β-D arabinofuranosylcytosine) is a potent drug which is used in the treatment of acute myelegenous leukemia. After administration, cytarabine is converted to the active metabolite, cytosine 1-β-D arabinofuranoside-5'-triphosphate in the body. This metabolite supresses DNA synthesis both by inhibiting DNA polymerase and incorporating into the DNA molecule(1,2).

Cytarabine is stable both in solid state and in acidic media, although it is degradable by hydrolysis in neutral and basic aqueous solutions(3). Hydrolytic deamination of the molecule leads to the formation of uracil arabinoside (ara-U) which is a therapeutically

inactive compound. Since it seems to be a problem of stability of the molecule, it appears to be worthwhile to study the determination of both cytarabine and ara-U regarding to the quality control of pharmaceutical preparations, the elucidation of pharmacological effects and the determination of the pharmacokinetic parameters.

Some studies have been published on the determination of cytarabine and ara-U in body fluids and in pharmaceutical preparations. Previous quantitative methods for determination of unchanged cytarabine and its metabolite are based on spectrophotometry(4), radioimmunology(5,6), GC-Mass(7), HPLC(8-11) and derivative spectrophotometry(12). Electroanalytical methods do not appear to have been published.

The effect of pH, pressure on the mercury reservoir, drop time, and potential scan rate on the limiting current of cytarabine, as well as the electrocapillarity and reversibility of the processes were investigated in this study. After the optimization of polarographic and analytical parameters, the calibration studies using certain polarographic modes such as direct current (DC), superimposed increasing amplitude pulse (SIAP) and diferential pulse (DP) polarography were performed. In addition to these studies, the determination of cytarabine in a pharmaceutical preparation was achieved under the optimized conditions using only DC polarography among the modes.

The results were statistically evaluated and it was concluded that the method proposed in this study was fast, easy and precise.

EXPERIMENTAL

Apparatus.

A Polaropulse (Tacussel Model PRG-5) polarograph, polarographic and voltammetric cell stand (Tacussel Model EGMA) with three electrodes (a dropping mercury electrode as working, a saturated Ag/AgCl as reference, a platinum wire as auxillary electrode), X-Y recorder (BBC Goerz Metrawatt Model SE 790), pH meter (Consort Model P114) combined with glass electrode were used.

Chemicals and Reagents.

Standard cytarabine from Sigma (USA) and Alexan® Ampoules from Mack,Illert (Germany) were used. Other chemicals were of analytical grade (Merck) with no further purification. Double distilled mercury and distilled deionised water were used throughout the experiments. As supporting electrolyte an aqueous solution of 0.2 M KCl and 0.2 M phosphate buffer at pH 6 was used.

Polarographic Procedure.

An aliquot of 10 ml of the cytarabine solution prepared in supporting electrolyte was placed in the polarographic cell.The solution was deoxygenated for ten minutes with a stream of pure nitrogen and the nitrogen atmosphere was maintained all through the experiments.

Experimental Conditions:

Voltage scan	0 to -2000 mV	Drop time	1 s
Mercury reservuar pressure	1000 dyne.cm^{-2}	Pulse amplitude	50 mV
Potential scan rate	4 mV.s^{-1}		

RESULTS and DISCUSSION

The effect of pH.

In order to examine the effect of pH on the limiting current and on the half-wave potential, a series of polarograms were recorded using 2.03x10^{-4} M cytarabine in the 5.47-8.20 pH range. The variation of the limiting current and the half- wave potential versus pH were investigated and shown in Figure 1.

The limiting current values of cytarabine solution were almost equivalent in the 5.47-6.60 pH range. Well-defined polarograms were also exhibited in this range. The limiting currents decreased above pH 6.60, then the curves overlapped with the discharge of the supporting electrolyte.

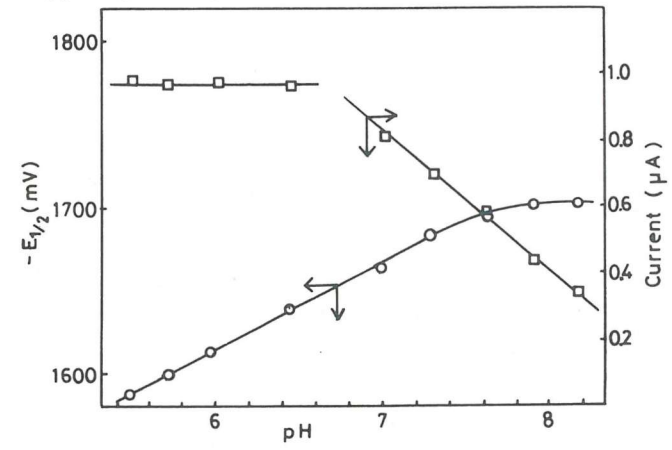

Figure 1. Variation of the limiting current and the half-wave potential against pH.

The half-wave potentials of the polarograms changed linearly in the 5.47-7.5 pH range; the linearity disappeared above this range.

The average electron numbers involved in the reduction of the molecule were calculated from the log i / i_d - i versus E (mV) relation and found to be 3.17 in the 5.47-6.60 pH range.

The results of this study is in agreement with the study published previously on cytosine(13).

Effect of the pressure on the mercury reservoir.

In order to investigate the effect of pressure on the mercury reservoir, a series of polarograms for 2.03×10^{-4} M cytarabine in 0.2 M KCl and phosphate buffer at pH 6 was recorded, varying the pressure from 500 to 2000 dyne.cm^{-2}. The linear plots of limiting current versus the square root of pressure are shown in Figure 2.

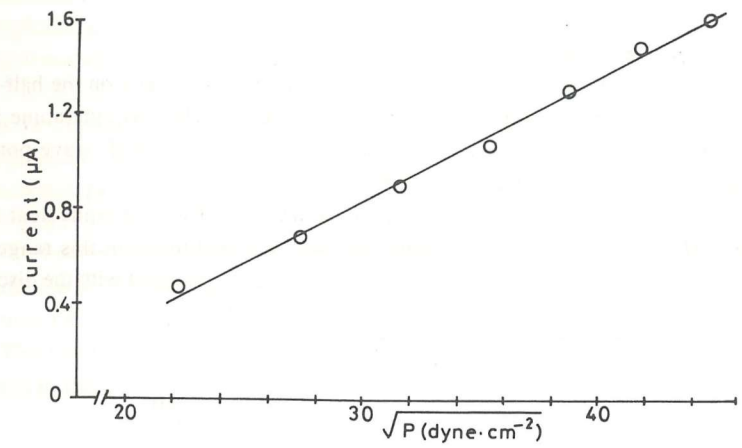

Figure 2. Variation of the limiting current as a function of square root of pressure on the mercury reservoir.

This relationship suggests that the polarographic current is diffusion controlled and to some extent by adsorption phenomena since the linear curve does not pass through the axis.

Drop time.

The limiting current depends on the surface area of the electrode together with the two-third power of drop time and the mass of mercury. According to theoretical considerations (14) the adsorption phenomena can be examined by evaluating the limiting current versus $t^{2/3}$, where t is the drop time.

In order to examine the change of drop time it was varied in the 0.6 and 2 s range. An increase in the height of the limiting current was observed as the drop time was increased between these two limits.

By plotting the limiting current versus $t^{2/3}$, a linear relation was observed as shown in Figure 3 with a correlation coefficient of 0.9897. These results are in accordance with those of the pressure effect on the mercury reservoir.

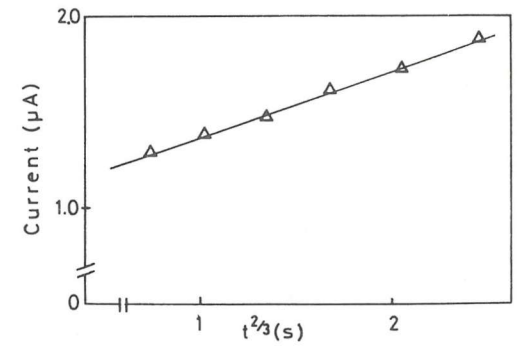

Figure 3. Variation of the limiting current as a function of $t^{2/3}$.

Scan rate.

An increase of the scan rate does not affect either the potential or the limiting current between the limits of 2 and 20 mV.s^{-1}. Well-defined and morphologically good polarograms were obtained in solutions of pH 6.

Electrocapillary curves.

In order to examine the possibility of adsorption, electrocapillary curves were drawn for solutions with and without cytarabine. The curve for cytarabine exhibits a slight shift in the electrocapillary curve. The result confirms the existence of adsorption phenomena to some degree.

Reversibility of the reduction process.

The reversibility of the process was studied at the same conditions but by using differential pulse mode. The potential was scanned anodically and cathodically, respectively. $E_p^a - E_p^c$ and I_p^a / I_p^c values were evaluated to decide on the reversibility of the process(15). After the evaluations, it was concluded that the process was quasi-reversible.

Effect of cytarabine concentration.

After the optimization of polarographic and analytical conditions, the effect of cytarabine concentration ($1.015\text{-}5.075 \times 10^{-4}$ mol·L^{-1}) on the limiting current was examined in a supporting electrolyte consisting of 0.2 M KCl and 0.2 M phosphate buffer at pH 6, using different polarographic modes such as direct current, superimposed increasing amplitude pulse and differential pulse. The original calibration polarograms are shown in Figure 4.

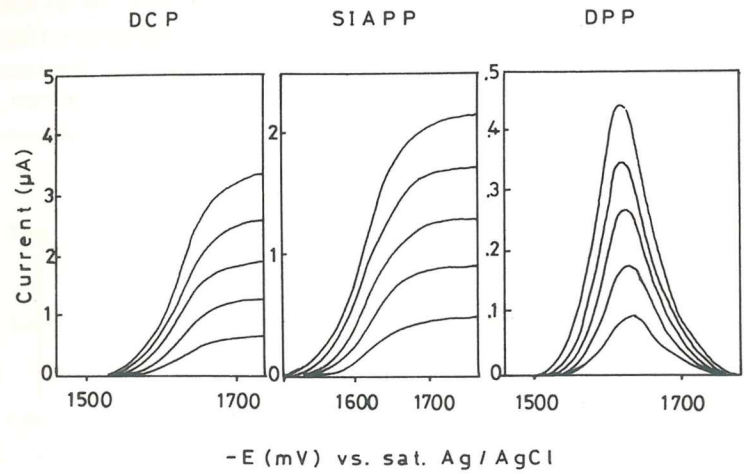

Figure 4. Calibration polarograms of DC, SIAP and DP polarographic modes.

The statistical evaluation of the calibration parameters and the correlation coefficients of the polarographic modes are shown in Table I.

Table I. Statistical evaluation of the calibration parameters.

		DCP	SIAPP	DPP
	Degree of Freedom	4	4	4
	R-Squared	0.99985	0.99861	0.99923
	Standart Error	0.000913	0.07188	0.00723
	Coeff. Variance	0.7188	2.3299	1.61053
intercept	Value	0.055	-0.08	0.020
intercept	Standart Error	0.00957	0.07539	0.00758
intercept	Variance	0.00009	0.00568	0.00006
intercept	T-value	5.74	-1.06	2.73593
slope	Value	3990.15	10242.71	1385.92
slope	Standart Error	28.44	220.68	22.20
slope	Variance	808.88	48700.78	492.89
slope	T-Value	140.29	46.41	62.42

According to the statistical results, it can be concluded that all the modes are usable for the determination of cytarabine in this concentration range with good correlation coefficients.

The analysis of cytarabine in aqueous dosage forms.

For the determination of cytarabine in Alexan® Ampoules, polarography was used. Once the polarograms of sample taken from ampoules were recorded the limiting currents were calculated after subtracting the residual currents. Using this limiting current and the calibration curve of DC polarographic mode the amounts of cytarabine were determined. UV Spectrophotometry was employed as a comparison method. The determination of cytarabine was done from the calibration equation at 280.5 nm. The corresponding results and the statistical evaluations are given in Table II. Statistical evaluations were computed by IBM PC 20 using Statgraphic Program.

Table II. The cytarabine determination in Alexan Ampoules by DC polarography and UV spectrophotometry and their statistical evaluations.

No of experiments	DC polarographic (mg/ml)	UV spectrophotometric (mg/ml)
1	19.15	19.66
2	19.57	19.61
3	19.74	19.66
4	19.74	19.92
5	19.74	19.87
6	19.74	19.92
7	19.74	19.71
8	19.96	20.02
Average	19.67	19.80
Standart Deviation	0.24	0.15
Rel. Std. Dev.	1.20	0.78
Confidence Limit	19.67±0.16	19.80±0.10
Variance	0.06	0.02

#declared amount of cytarabine is 20 mg/ml

The results obtained are in agreement with the official requirements to be followed (17) and the polarographic techniques presented in this study have proven to be accurate, rapid and practical for the determination of cytarabine in ampoules.

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ÉTUDE DES PROCESSUS CINETICO-CHIMIQUES DE LA METALLISATION AVEC DU CUIVRE DANS ABS (ACRONITRILE-BUTADIENE-STIRÈNE).

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Resumé.- *Fait le rapport de conclusions experimentales sur le procèssus cinetico-chimique de la métallisation chimique sur la surface du ABS, un des materiaux plastiques les plus utilisés. On décrit aussi les détails de chaque étape du procèssus.*

Summary.- *The experimental conclusions on the chemical-kinetic process of the chemical plating on ABS surface, one of the plastics materials more widely used, are presented, describing each step of the process.*

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