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Determination of Flucytosine in Tablets by Differential Pulse Polarography

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Abstract □ A differential pulse polarographic assay was developed for determining flucytosine in tablets. The drug is extracted from the sample with water and hydrochloric acid and, after the pH is adjusted, an aliquot is added to the cell and the solution is polarographed at the dropping mercury electrode *versus* the saturated calomel electrode with 0.066 *M* Sørensen phosphate buffer (pH 5.6) as the supporting electrolyte. The polarographic peak height enables precise quantitative determination. The E_p value for flucytosine is −1.54 v *versus* the saturated calomel electrode. The mean recovery of the drug is 101.5% ± 1.9 (*SD*). The method is simple, rapid, and precise.

Keyphrases □ Flucytosine—differential pulse polarographic analysis in dosage forms □ Polarography, differential pulse—analysis, flucytosine in dosage forms □ Antifungal agents—flucytosine, differential pulse polarographic analysis in dosage forms

Flucytosine, 5-fluorocytosine (I), is a fluorinated pyrimidine that has shown *in vitro* and *in vivo* antifungal activity. Microbiological (1), fluorometric (2, 3), and high-pressure liquid chromatographic (4) procedures were reported for the estimation of I in biological fluids. The USP (5) employs a spectrophotometric method for the estimation of I in capsules. The mechanism of polarographic reduction of pyrimidine derivatives was reported previously (6–8). This paper reports a simple, convenient, and rapid differential pulse polarographic analysis of I in tablets.

EXPERIMENTAL

Apparatus and Conditions—Polarograms were obtained using a polarographic analyzer[1] equipped with a drop timer[2]; the differential pulse mode was used. A three-electrode cell (5–50 ml) was comprised of a dropping mercury electrode, a saturated calomel electrode (SCE), and a platinum wire auxiliary electrode. The drop time t, was 2 sec, the drop mass was 1.4 mg/sec, and the capillary characteristic was $m^{2/3}t^{1/6} = 1.3$, measured in 0.066 *M* Sørensen phosphate buffer (pH 5.6) with an open circuit and at a mercury column height of 95 cm. The current range was either 5 or 10 μamp for a peak response of full-scale deflection, the scan range was from −1.2 to −1.95 v, the peak potential was −1.54 v, the modulation amplitude was 25 mv, and the scan rate was 2 mv/sec. The polarogram was recorded on an x–y recorder[3].

A pH meter[4], fitted with a combination glass–saturated calomel electrode electrode pair, was used to monitor the pH of all solutions.

Reagents and Chemicals—All chemicals were analytical reagent grade. The Sørensen buffer was prepared by mixing stock solutions A (9.073 g of monobasic potassium phosphate/liter) and B (11.87 g of dibasic sodium phosphate dihydrate/liter) in varying proportions to produce the desired pH (9). Flucytosine[5] was used as a standard.

Calibration Curve—An aqueous stock solution of I was prepared at 1×10^{-2} *M*. A range of 1×10^{-3}–1×10^{-4} *M* was employed for the preparation of the diffusion current *versus* concentration calibration curve. All standard solutions were prepared by pipetting an aliquot of the aqueous stock solution and adding 13.0 ml of Sørensen phosphate buffer (pH 5.6) and sufficient distilled water to a final volume of 20 ml.

These solutions were transferred to the cell and deoxygenated with pure nitrogen for 10 min prior to obtaining the polarograms in quiescent solution. A layer of nitrogen was maintained over the solution surface during the electroreduction. Polarograms were obtained using the differential pulse polarographic mode.

[1] Princeton Applied Research (PAR) model 174A.
[2] Princeton Applied Research (PAR) model 172A.
[3] Houston Omnigraphic model 2000.
[4] Fisher, Accumet model 230 pH/ion meter.
[5] Hoffmann–La Roche lot I-898.

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Table I—Effect of Supporting Electrolyte Concentration on the Peak Height of Flucytosine

Molarity of Buffer	Current, μamp
0.200	4.52
0.100	4.46
0.066	4.15
0.050	3.97
0.040	3.78

Tablet Analysis—Twenty tablets were weighed and finely crushed. A portion of the powder equivalent to 200 mg of I was weighed accurately and transferred to a 250-ml volumetric flask. About 50 ml of dilute hydrochloric acid (1:100) was added, and the flask was shaken for 30 min on a mechanical shaker and diluted to volume with the dilute hydrochloric acid. The contents were mixed, and the excipients were allowed to settle.

A 10-ml aliquot was adjusted to pH 5.6 using dilute sodium hydroxide, quantitatively transferred to a 25-ml volumetric flask, and diluted to volume with water. An aliquot of the solution sufficient to give a peak height in the middle of the calibration curve was pipetted into the polarographic cell. The remainder of the procedure was identical to that described for the preparation of the calibration curve.

RESULTS AND DISCUSSION

Preliminary experiments revealed that I exhibits a single sharp differential pulse polarographic peak in Sørensen phosphate buffer over pH 5–8. In the dc mode, a single well-defined reduction wave was obtained (10) having a half-wave potential of −1.49 v *versus* the saturated calomel reference electrode (Fig. 1). That this electrode process was diffusion controlled was evident from a linear plot of the diffusion current *versus* the square root of the mercury column height corrected for back pressure (10). The fact that this plot did not quite pass through the origin on extrapolation may indicate that some small additional process such as adsorption or a rate-limiting reaction may occur at the surface of the dropping mercury electrode (11).

To select optimal conditions for the quantitative determination of I, the effects of both the concentration and pH of the buffer on the peak potential, E_p, and the corresponding peak current were investigated (Table I and Fig. 2). The E_p value shifted to more negative values with

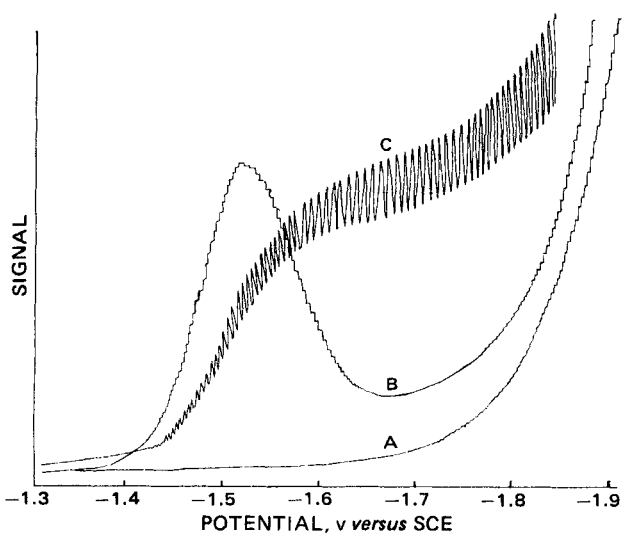

Figure 1—*Polarograms of 6 × 10⁻⁴ M I in 0.066 M Sørensen phosphate buffer (pH 5.6); $m^{2/3}t^{1/6}$ = 1.29; mercury column height = 95 cm; potential scan rate = 2 mv/sec; and drop time = 2 sec. Curves A and B represent the supporting electrolyte and I, respectively, in the differential pulse mode with a modulation amplitude of 25 mv. Curve C represents the drug run in the dc mode. The current sensitivity (full-scale deflection) for A and B was 10 μamp; for C, it was 5 μamp.*

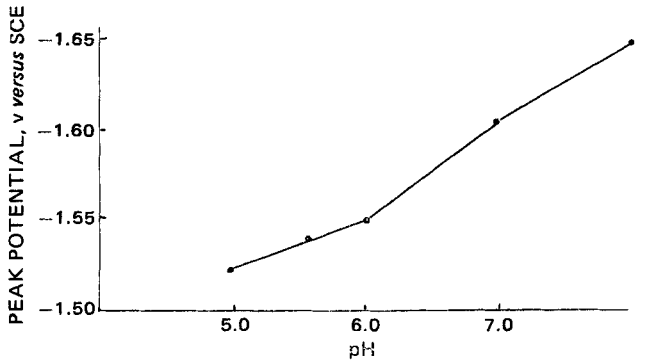

Figure 2—*Effect of pH on peak potential for the electroreduction of I at a concentration of 6 × 10⁻⁴ M in Sørensen phosphate buffer (0.066 M).*

increasing pH. The I concentration did not affect the E_p value in this buffer at pH 5.6. Increasing molarity of the buffer caused a slight increase in the current measured at the E_p (Table I). The final supporting electrolyte used in the assay of I was 0.066 *M* Sørensen phosphate buffer (pH 5.6).

A linear relationship existed between the current measured at an E_p of −1.54 v (*versus* the saturated calomel electrode) and the concentration of I in the range of 1×10^{-3}–1×10^{-4} *M*. This plot had a correlation coefficient of 0.9976, a *y*-intercept of 0.0604, and a slope of 0.6605 (10). The failure of this calibration curve to pass through the origin is not important since the size of the aliquot of the sample was selected to give a current peak height reading falling in the middle portion of this plot.

The mean assay result of 10 replicate determinations of I in commercial tablets[6] was 101.5% ± 1.9 (*SD*) of the labeled amount. Tablets from the same bottle were assayed by the USP XIX method for I capsules, involving UV spectrophotometry at 285 nm. The plot of absorbance *versus* concentration of the same reference material (I) used for the polarographic method resulted in a straight line with a slope of 0.0699, a *y*-intercept of −0.007, and a correlation coefficient of 0.9997. The mean result of three replicate determinations of I in the same lot of tablets was 102.2%. This excellent agreement of results obtained by two unrelated methods indicates that the tablet excipients in this formulation do not interfere with either assay.

Filtration of the tablet sample suspension was not required. This differential pulse polarographic method is simple, rapid, and convenient for analysis of flucytosine in commercial tablets.

The quantitative determination of I in biological fluids by differential pulse polarography (10) will be reported elsewhere.

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