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The application of differential pulse voltammetry at the glassy carbon electrode to multivitamin analysis

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Differential pulse voltammetry at the glassy carbon electrode has been applied to the determination of ascorbic acid, pyridoxine and folic acid in a multivitamin preparation. The individual vitamins all gave well-defined peaks in the anodic region with a linear response of peak current to concentration. The water-soluble vitamins were extracted into aqueous solution and folic acid into dibasic potassium phosphate solution. Before determination of pyridoxine, the ascorbic acid peak was depressed by reaction with formaldehyde. Iron (II) present in the multivitamin preparation did not interfere with the analysis. The voltammetric method compared favourably in terms of accuracy and precision with official methods: it was found to be much simpler, with sample manipulation kept to a minimum. The method was found to be generally applicable to the determination of the vitamins in several multivitamin preparations, or, in simplified form, to the determination of the individual vitamin preparations.

The problem of determining individual vitamins in multivitamin preparations is reflected in the wide variety of methods reported (Freed 1966). Interference by one vitamin in the determination of another, or by other components of the preparation, generally requires extraction procedures to be carried out before the analysis. Differential pulse voltammetry offers a relatively sensitive and selective method of vitamin analysis in which peak voltammograms of components no less than 40 mV apart may be resolved.

Folic acid, pyridoxine and ascorbic acid are often found in combination in multivitamin preparations. Microbiological methods for the determination of folic acid (Teply & Elvehjem 1945) are complex and time consuming: extraneous coloured material interferes with the U.S.P. (XVIII) procedure, other vitamins with the sensitive fluorometric method of Allfrey et al (1949). Since folic acid is reducible at the dropping mercury electrode (D.M.E.) it may be determined by classical d.c. polarography (Duncan & Christian 1948; Mader & Frediani 1948). The reduction is complex and the relationship peak height/concentration is linear only over a narrow concentration range (Kretzschmar & Jaenicke 1971). More recently, phase sensitive a.c. polarography has been used (Jacobsen & Bjornsen 1978) for the determination of folic acid in pharmaceutical preparations.

With pyridoxine hydrochloride, problems of reproducibility and standardization of microbiological and enzymatic assay methods have led to increased interest in chemical methods. Colorimetric methods have low sensitivity and specificity (Hashmi 1973), fluorescent procedures are difficult to apply (Fujita et al 1955) and gas liquid chromatography has only been used for simple solutions (Prosser et al 1967).

The polarographic wave of pyridoxine in 0·1 M tetramethylammonium bromide, (Lingane & Davis 1941), is not analytically useful due to its low half-wave potential and bad reproducibility. The catalytic wave observed in acidic buffer solutions can only be used for relatively pure solutions because of the critical experimental conditions and bad reproducibility (Knobloch 1963). Since pyridoxal gives a better-defined polarographic wave than pyridoxine, prior oxidation to the former compound is often a first step (Kruse 1971; Soderhjelm & Lindquist 1975). Interference by iron and ascorbic acid, present in multivitamin preparations, requires prior separation of pyridoxine by use of an ion-exchange procedure.

Numerous chemical methods for the determination of ascorbic acid are extensively reviewed by Freed (1966), and Higuchi & Brochmann-Hanssen (1961). Ascorbic acid may be determined by anodic oxidation at the D.M.E. (Brezina & Zuman 1958) although the limited anodic range of the D.M.E. limits the sensitivity. An alternative electrochemical oxidation using the tubular carbon electrode, has been described by Mason et al (1972): it is specific but applicable only to flow systems. Lindquist (1975) has described the anodic oxidation of ascorbic acid at the carbon paste electrode.

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In the present study folic acid, pyridoxine and ascorbic acid present in admixture can be assayed using differential pulse voltammetry at the glassy carbon electrode.

MATERIALS AND METHODS

Materials

Folic acid, pyridoxine and sodium ascorbate were of U.S.P. quality. All other reagents employed were of Analar grade.

Instrumentation

Voltammetric determinations were made with a PAR 174A Polarograph (Princeton Applied Research Corporation) and a three electrode system consisting of a glassy carbon working electrode (Metrohm Ltd.), platinum auxiliary and saturated calomel reference electrodes. The voltammetry cell was of amber glass, capacity 10 cm^3 and contained a suitable buffer as the supporting electrolyte. Voltammograms were recorded on a Bryans Southern Ltd XY Recorder.

Spectrophotometric determinations were made using a Perkin-Elmer 554 spectrophotometer.

Voltammetry conditions

Differential pulse voltammograms were recorded in a suitable supporting electrolyte, with an initial potential of +0·1 V vs SCE and a scan rate of 5 mVs^{-1} in the anodic direction. The pulse modulation was 50 mV and the current range (sensitivity) varied between 0·2 mA to 10 μA as required. The solution was degassed with nitrogen before analysis.

Extraction procedure for multivitamin preparation

(1) Extraction of pyridoxine hydrochloride and sodium ascorbate. Powdered sample (0·337 g) in distilled water (~100 cm^3) was stirred (5 min), the solution filtered and the filtrate made to volume (100 cm^3) with distilled water.

(2) Extraction of folic acid. The residue powder from the previous extraction in 3% dibasic potassium phosphate solution ($<$ 10 cm^3 g^{-1} residue used) was stirred at a temperature $>$60 °C until completely dispersed. The volume of the cooled dispersion was adjusted so that 1 cm^3 represented between 0·005 and 0·01 mg of folic acid: the solution was then filtered.

Analytical procedure

Analysis of sodium ascorbate. An aliquot (10 cm^3) of the pyridoxine/sodium ascorbate extract was diluted to 100 cm^3 with citric acid buffer pH 4 (citric acid 0·1 M, 62% and disodium phosphate 0·2 M, 38%), transferred to the voltammetry cell, the differential pulse voltammogram recorded and the peak current (i_X) at Ep sodium ascorbate noted. A standard addition (0·1 cm^3) of sodium ascorbate solution was made and the new value of i_T sodium ascorbate recorded. The concentration of sodium ascorbate was calculated thus:

$$\frac{\text{Molarity of unknown}}{\text{Molarity of standard}} = \frac{i}{i_X + (i_T - i_X)\,V/v}$$

where i_X = peak current of unknown; i_T = peak current of unknown plus standard; V = volume of unknown in cell; v = volume of standard addition.

Analysis of pyridoxine hydrochloride. A further 10 cm^3 aliquot of the above diluted solution was added to 10 cm^3 of 4% formaldehyde solution and stirred (5 min). An aliquot (10 cm^3) of this solution was placed in the voltammetry cell and the concentration of pyridoxine hydrochloride determined by the method of standard addition described previously (Ballantine & Woolfson 1979).

Analysis of folic acid. The dibasic potassium phosphate extract was made up to volume (100 cm^3) with dibasic phosphate. An aliquot (10 cm^3) was transferred to the polarographic cell, the differential pulse voltammogram obtained, and i_X folic acid determined. A standard addition (0·1 cm^3) of folic acid was made and i_T determined. The concentration of folic acid was calculated from the above equation.

RESULTS AND DISCUSSION

Ascorbic acid, pyridoxine hydrochloride and folic acid all possess electrochemically oxidizable phenolic hydroxyl groups. At the glassy carbon electrode, well-defined differential pulse voltammograms were obtained for all three individual vitamins. The supporting electrolytes were pH 4 citric acid buffer, for ascorbic acid and pyridoxine, and 3% dibasic potassium phosphate for folic acid, giving respective peak potentials (versus S.C.E.) of +0·23 V, +0·91 V and +0·63 V.

A linear relationship was established between peak current and concentration over the range 10^{-1} to 10^{-6} M for all three compounds. An extraction procedure was developed in which the water soluble components of the multivitamin blend were extracted into aqueous solution, leaving behind the water-insoluble folic acid to be extracted into dibasic potassium phosphate solution. None of the constituents of the blend (Table 1) interfered with the extraction procedure which was monitored by recovery experiments on spiked samples. In the

Table 1. Constituents of multivitamin preparation (per 337 mg of blend).

Ferrous fumarate	250 mg
Sodium ascorbate	56 mg
Nicotinamide	10 mg
Thiamine mononitrate	2 mg
Riboflavine	2 mg
Pyridoxine hydrochloride	1 mg
Folic acid	0·5 mg

voltammetric method only the three phenolic vitamins are electrochemically active in the anodic region. However, ascorbic acid is generally present in much greater concentration than pyridoxine (a ratio in excess of 50 to 1 in this case), resulting in masking of the smaller pyridoxine peak by that due to ascorbic acid. This may be overcome by depressing the ascorbic acid peak before the determination of pyridoxine by reaction with formaldehyde (Snow & Zilva 1943; Soderhjelm & Lindquist 1975). A 90% reduction in ascorbic acid concentration can be observed 5 min after adding 4% formaldehyde solution in pH 4 citric acid buffer.

Like ascorbic acid, iron (II) is generally present in large excess over pyridoxine, and being oxidized at a potential lower than pyridoxine, has been reported to interfere with the determination of pyridoxine at the carbon paste electrode (Soderhjelm & Lindquist 1975). However, no peak for iron (II) was observed within the voltage range for pyridoxine in the present study. Although the presence of iron (II) was observed to interfere with the reaction between formaldehyde and ascorbic acid, sufficient suppression of the ascorbic acid peak was achieved for the pyridoxine peak to be resolved (Fig. 1): this eliminated the need for a preliminary ion-exchange extraction of iron (II) (Soderhjelm & Lindquist 1975).

Following determination of ascorbic acid and pyridoxine in successive 10 cm^3 aliquots of aqueous filtrate, folic acid was determined on a 10 cm^3 aliquot of dibasic potassium phosphate extract. The determination of folic acid by direct anodic oxidation at the glassy carbon electrode represents a considerable improvement on the d.c. polarographic reduction procedure in which the polarographic wave is not well-defined. The differential pulse voltammogram of folic acid in dibasic potassium phosphate (Fig. 2) allowed rapid and reproducible determination of the vitamin by a standard addition procedure. This is a considerable advantage over the cumbersome U.S.P. XVIII procedure for folic acid.

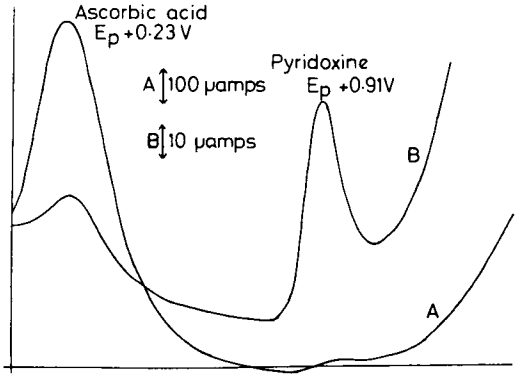

FIG. 1. Differential pulse voltammograms of ascorbic acid and pyridoxine. (A) Before formaldehyde treatment. (B) Resolution of pyridoxine following suppression of ascorbic acid peak by formaldehyde.

Six replicate analyses were carried out on aliquots of the multivitamin blend for each of the three vitamins of interest. The results, with a comparison of U.S.P. XVIII procedures, are given in Table 2. In all cases the voltammetric method gave satisfactory results with low standard deviations. For ascorbic acid, the high percentage recoveries

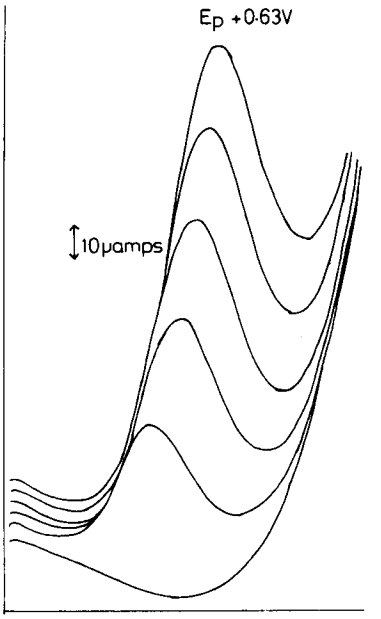

FIG. 2. Differential pulse voltammograms resulting from addition of successive 50 μl aliquots of folic acid (10^{-3} M) to 10 cm^3 of dibasic potassium phosphate as supporting electrolyte.

Table 2. Determination of sodium ascorbate, folic acid and pyridoxine in multivitamin preparation.

Vitamin	Method	% Recovery		% s.d.
Sodium ascorbate	Voltammetry	98·0	98·0	1·47
		98·0	98·0	
		101·6	98·0	
	U.S.P. XVIII	102·3	103·5	0·99
		103·5	101·9	
		100·9	102·3	
Folic acid	Voltammetry	98·0	96·1	1·30
		98·0	98·0	
		98·0	100·2	
	U.S.P. XVIII	97·9	95·2	2·57
		97·9	92·4	
		98·9	98·9	
Pyridoxine hydrochloride	Voltammetry	98·1	96·5	1·20
		98·1	99·7	
		96·5	98·1	
	U.S.P. XVIII	98·2	100·0	1·62
		95·2	98·2	
		98·0	96·7	

obtained by the official titrimetric procedure reflect the interference of iron (II), which is also oxidized by the titrant.

The present rapid method for ascorbic acid, pyridoxine and folic acid in a multivitamin preparation reduces interferences and extraction procedures are kept relatively simple. The method is generally applicable to determination of all three vitamins in various multivitamin preparations, or to preparations of the individual vitamins (using the appropriate extraction procedure). It is sensitive enough to be applied to single tablet or capsule assays.

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