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Simultaneous polarographic determination of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole, 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole and 2-methyl-5-nitroimidazole

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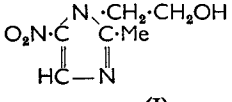
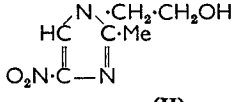
A method for the simultaneous quantitative polarographic determination of the three components in reaction mixtures has been proposed. The analysis is based on a separation of the half-wave potentials in strongly alkaline base solutions. The general polarographic behaviour of the three substances is also described.

THE nitroimidazole derivative 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole, (metronidazole) is used extensively as a specific agent against human trichomoniasis. Its determination, especially in reaction mixtures in the presence of the isomeric 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole and of 2-methyl-5-nitroimidazole, is most important when following the synthesis of the drug. Because of the ease of reduction of the nitro-group at the dropping mercury electrode, several methods (Danek, 1961; Kane, 1961; Cosar, Dubost, Dubost, Devoize & Pallière, 1962; Vignoli, Cristan, Gonezo & Fabre, 1963a,b) for the polarographic determination of metronidazole have been suggested, particularly for the analysis of biological materials. However, a simultaneous determination of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (I), 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole (II) and 2-methyl-5-nitroimidazole (III) has not been possible.



(I)



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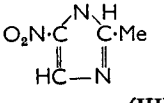

(III)

We now describe the general polarographic behaviour of these three compounds and propose the conditions for their simultaneous determination.

POLAROGRAPHIC BEHAVIOUR

The polarographic reduction of all three compounds was examined in aqueous Britton–Robinson universal buffers over the range pH 2 to pH 10·5, and in sodium hydroxide solution of various concentrations.

In acidic media metronidazole gives a 4-electron wave A deformed by a sharp maximum; wave A is followed by another wave B of about half the height of wave A. A small addition of surface-active agent, such as gelatin, suppresses the maximum on wave A (Fig. 1). With increasing pH values of the base solution, wave A increases at the expense of wave B (their sum remaining constant) until at pH 10 both waves almost coalesce

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to a single 6-electron wave. At higher pH values, a fall in the height of the combined wave A + B sets in. The time-dependence of the waves in strongly alkaline media will be dealt with later. The half-wave potentials of wave A shift to more negative voltage by −90 mV per pH unit ($E_{\frac{1}{2}} = -0{\cdot}16$ V at pH 3·10 and −0·60 V at pH 10·15) (Fig. 2). The number of electrons involved in the reduction process was determined by comparing the wave-heights with the wave-heights of substances in which the reduction mechanism is known. In addition to this, the diffusion coefficient D was calculated from the uncorrected Ilkovič equation

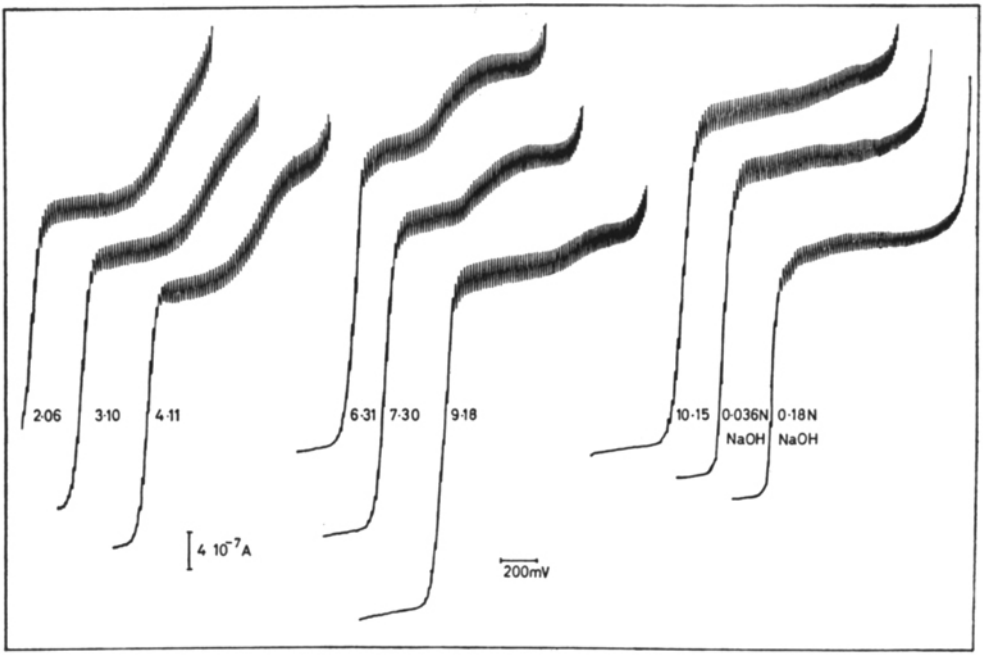

FIG. 1. Dependence of the reduction waves of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole 2×10^{-4}M on pH; Britton–Robinson buffers, pH values are given with the curves; 0·005% gelatin; all curves except the last start at zero applied voltage; the last curve starts at −0·3 V.

at suitable pH values and the plausible value of $8{\cdot}5 \times 10^{-6}$ cm sec^{-2} was obtained for pH 4·7 and $c = 4{\cdot}95 \times 10^{-4}$M (at pH 9·3, however, the much lower value of $5{\cdot}7 \times 10^{-6}$ cm sec^{-2} was calculated; this is obviously due to the fall in height of the original 6-electron wave of this pH). The log i/i_d–i against E plots of the metronidazole waves show distinctly that an irreversible electrode process is operative in both waves.

The next step was the investigation of 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole behaviour as a function of pH of the supporting electrolyte. In this instance the general pattern is characterised again by the appearance of two waves A and B. Wave A is deformed by a sharp maximum, the suppression of which requires higher gelatin concentrations than that of metronidazole. In acid media the ratio of wave-heights A:B is 2:1. With increasing pH values the height of B decreases and

the wave vanishes completely at pH > 9. In strongly alkaline solutions the waves are independent of time and no colouration of the solution appears upon adding sodium hydroxide or potassium hydroxide solutions. On average, wave A is about 100 mV more negative than the same wave for metronidazole ($E_{\frac{1}{2}}$ varies from −0·15 V at pH 2·06 to −0·73 V at pH 10·15) and shifts by +97 mV/pH to more negative potentials. The wave-heights point to the fact that 4 electrons are consumed for the reduction in wave A (from the Ilkovič equation $8{\cdot}4 \times 10^{-6}$ cm sec^{-2} is obtained for D at 4·7 and $8{\cdot}5 \times 10^{-6}$ cm sec^{-2} at pH 9·3).

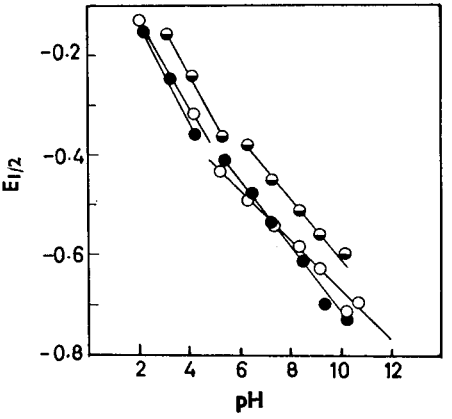

FIG. 2. $E_{\frac{1}{2}}$-pH plot for all three nitro-compounds. $E_{\frac{1}{2}}$ values are given against saturated calomel electrode. ◒ Metronidazole. ● 1-(2-Hydroxyethyl)-2-methyl-4-nitroimidazole. ○ 2-Methyl-5-nitroimidazole.

The 4-electron reduction wave of 2-methyl-5-nitroimidazole is deformed by a maximum up to pH 7·5 and, as with metronidazole, this maximum is removed by adding 0·005% gelatin. Wave A is followed by wave B which, originally, in acid media corresponds to the uptake of 2 electrons and finally disappears in sodium hydroxide solutions. Although the colour of the solution turns yellow on adding sodium hydroxide, the height of wave A is not time-dependent. The half-wave potential of wave A shifts by 90 mV per pH unit to more negative values with increasing pH (from −0·13 V at pH 2·1 to −0·72 V at pH 10·15).

The behaviour of 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole and of 2-methyl-5-nitroimidazole is in accordance with the general course of reduction for aromatic nitro-compounds (Volke, 1960). Here, e.g. in nitrobenzene, the nitro-group is reduced to phenylhydroxylamine; at more negative potentials, i.e. over the region of the second step B, a 6-electron reduction occurs leading to aniline. This mechanism is only restricted to acidic and slightly alkaline base solutions since a pre-protonation of phenylhydroxylamine is a necessary condition for the second reduction step (wave B). The mechanism for metronidazole reduction is somewhat different. Here the reduction, probably to the amine, takes place in a single 6-electron step (at higher pH values only). Similar behaviour has been found earlier, e.g. with some nitrophenols (Astle & McConnell, 1943).

In our case it could be ascribed to an influence of the vicinal $-CH_2CH_2OH$ group in the metronidazole molecule.

A strange phenomenon occurring with all three nitro-compounds should perhaps be mentioned: the waves A in the most acid buffers (pH 3) are somewhat lower than those at the other pH values. A similar discrepancy was observed earlier with 2-nitropyridine (Holubek & Volke, 1960).

It follows from the $E_{\frac{1}{2}}$-pH plot that only in strongly alkaline solutions (best in NaOH solutions of various concentrations where, e.g. in N NaOH the half-wave potentials have the following values: −0·57 V for metronidazole, −0·68 V for 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole, and −0·90 V for 2-methyl-5-nitroimidazole) are the half-wave potentials of the three compounds sufficiently apart to enable a simultaneous quantitative determination. This is also evident from the polarograms of equimolar mixtures of all three compounds at different pH values; only above pH 11 are three independent waves obtained. There is, however, a serious drawback when working with strongly alkaline solutions, as metronidazole decomposes upon adding sodium hydroxide. The originally colourless solution turns violet and this reaction is accompanied by a decrease in the height of wave A. Finally, after about 22 hr the solution becomes colourless again and the original reduction wave completely disappears. The character of the waves of the other two compounds is not influenced in this way by the alkalinity of the supporting electrolyte. Since the wave-height of metronidazole does not change rapidly during the first 15 min after the alkalisation, quantitative analysis of mixtures in this medium is rendered possible.

To this end the concentration dependences of all three compound were investigated in sodium hydroxide solutions and the wave-height was found to be a linear function of depolariser concentration for concentrations ranging from 5×10^{-5}M to 2×10^{-3}M.

At this instant the analysis of mixtures was attempted. The examples for metronidazole and for 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole, in each case with varying concentrations of the component to be determined, and with constant concentrations of the other two components are shown in Fig. 3A and B. The concentrations of metronidazole and 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole lie between $1{\cdot}66 \times 10^{-4}$ and $1{\cdot}66 \times 10^{-3}$M. The experiments were made in 0·63N sodium hydroxide and with $7{\cdot}5 \times 10^{-4}$M solutions of the other two components.

The sensitivity of the test is adequate and the separation of the three waves is clearly visible even with $2{\cdot}5 \times 10^{-6}$M solutions. At lower concentrations a quantitative determination would be dubious and unreliable.

ANALYTICAL PROCEDURE FOR THE SIMULTANEOUS DETERMINATION

A. *The wave of the sample.* Weigh accurately about 200 mg of the sample from the reaction mixture, dissolve in distilled water and make up to 100 ml. Transfer a 2 ml portion of this solution to a dry polarographic cell, and add water (3 ml) and N sodium hydroxide (5 ml). Deaerate the solution with pure nitrogen for exactly 5 min and thereafter

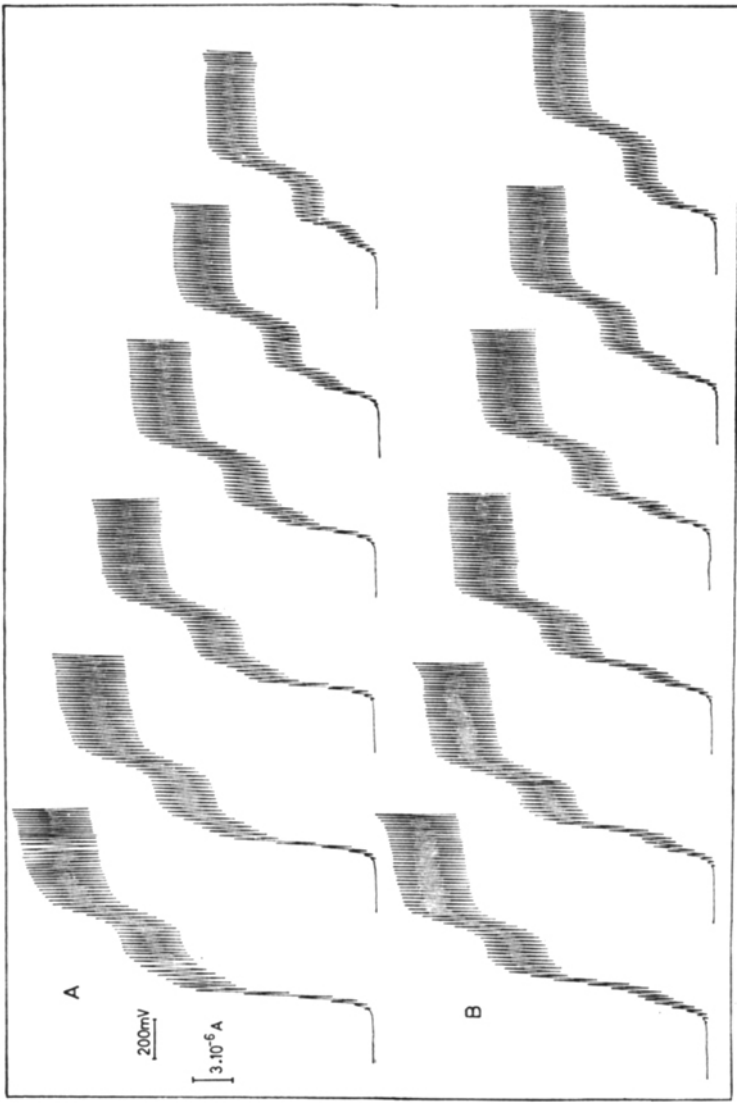

FIG. 3A. Dependence of wave-height of metronidazole on depolariser concentration in presence of 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole and 2-methyl-5-nitroimidazole. Composition of the solution: $3{\cdot}3 \times 10^{-4}$M 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole, $3{\cdot}3 \times 10^{-4}$M 2-methyl-5-nitroimidazole, 0·61N sodium hydroxide. The concentration of metronidazole varies from $1{\cdot}6 \times 10^{-4}$M to 10^{-3}M. Each curve starts at $-0{\cdot}3$ V and ends at $-1{\cdot}4$ V.

B. Dependence of wave-height of 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole on depolariser concentration in presence of metronidazole and 2-methyl-5-nitroimidazole. Composition of the solution: $3{\cdot}3 \times 10^{-4}$M metronidazole, $3{\cdot}3 \times 10^{-4}$M 2-methyl-5-nitroimidazole; 0·61N sodium hydroxide. The concentration of 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole varies from $1{\cdot}6 \times 10^{-4}$M to 10^{-3}M (equal additions). Each curve starts at $-0{\cdot}3$ and ends at $-1{\cdot}4$ V.

record the polarogram over the range from −0·3 V to −1·4 V against saturated calomel electrode.

B. *The wave of the sample with the standard addition.* Transfer a 1 ml portion of a $1{\cdot}0 \times 10^{-3}$M standard solution of metronidazole or 1-(2-hydroxyethyl)-2-methyl-4-nitroimidazole or 2-methyl-5-nitroimidazole, into a dry cell, add 2 ml of the sample solution, water (2 ml) and N sodium hydroxide (5 ml). Deaerate the solution for exactly 5 min and record the polarogram as above. To evaluate the polarograms the following formula is used:

$$\% = \frac{h \cdot c \cdot 100}{h' \cdot w}$$

% = percentage of the component to be determined in the reaction mixture,

h = wave-height for the sample,

c = concentration of the standard solution (mg in 1 ml),

w = weight of the sample (mg in 2 ml),

h′ = wave-height for the standard (i.e. wave-height for the solution with standard addition h″ minus the wave-height for the sample h).

The time-factor when working with the sodium hydroxide solution was eliminated by recording the polarograms exactly after a time interval of 5 min. The analyses were made with reaction mixtures containing about 10% of each component. In addition to this, the mixture contained the reagents necessary for converting 2-methyl-5-nitroimidazole to metronidazole. Since the reaction conditions and the composition of the solution varied for each case—the most favourable conditions for the synthesis were sought—calibration curves could not be used for evaluating the polarograms. Instead the above method of standard addition was used.

EXPERIMENTAL

All three nitro-compounds were prepared in the department of synthetic chemistry of the Galenika Laboratory and exhibited the properties described in the literature. Analytical grade reagents were used for preparing the buffer solutions and as supporting electrolytes.

The polarographic measurements were made with the Sargent Model XV Recording Polarograph and with a special thermostated cell containing a separated calomel electrode, and the dropping mercury electrode.

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