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ELECTROCHEMICAL CHARACTERISTICS OF 2-METHYL-1,4-NAPHTHOQUINONE (VITAMIN K_3)

A COULOMETRIC MICROMETHOD OF DETERMINATION

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The importance of 2-methyl-1,4-naphthoquinone (vitamin K_3, menadione) and its action in relation to blood coagulation processes is well known.

The increased use of this compound in polyvitamin preparations and the fact that its electrochemical characteristics have not been studied critically led us to this investigation.

FIESER AND FIESER[1] were the first to show that it is possible to reduce naphthoquinones at a dropping mercury electrode. ONTRUST AND WOSTMANN[2] determined menadione, using a solvent mixture of isopropanol–petroleum ether and a buffer composed of ammonia and ammonium chloride as supporting electrolyte. In this medium the reduction wave shows a maximum suppressed only by methylene blue. JONGKIND *et al.*[3] used a solvent mixture of benzene and methanol with 0.2 *M* lithium chloride and made alkaline with ammonia; they also employed methylene blue as a maximum suppressor. The alkaline media proposed by these authors are unsatisfactory because of the instability of vitamin K_3 at pH above 8. BURGER[4] employed 0.5 *M* potassium chloride as a supporting electrolyte but without buffering the medium.

The purpose of this work was to investigate the electrochemical characteristics of menadione and to develop a micro-coulometric method of determination of this compound. We were interested in several electroanalytical aspects and the investigations were carried out by different techniques such as d.c. polarography, coulometric titration, controlled potential coulometry, and cyclic voltammetry.

EXPERIMENTAL

Polarograms were recorded in the usual manner with a Leeds and Northrup Electrochemograph at 25° ±0.1°.

Cyclic voltammetry was performed with a hanging mercury drop electrode, at a voltage sweep of 20 mV per sec.

The controlled potential coulometry was carried out, with a potentiostat, in an H-cell with a mercury pool cathode and a platinum wire auxiliary electrode.

The coulometric titrations with electrogenerated cerium(IV) ion were performed in an H-type glass cell with a fine sintered glass disk in the cross arm. The solution

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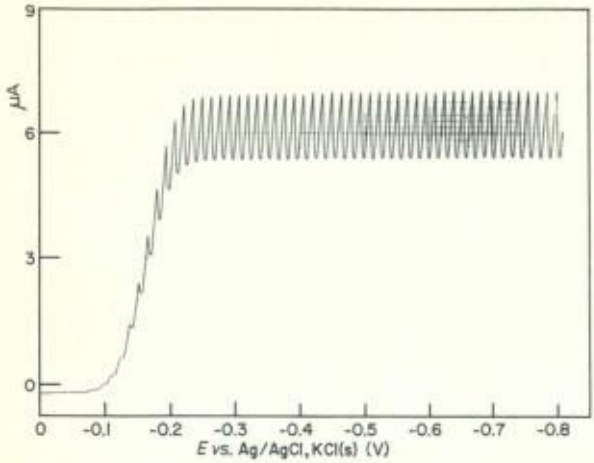


Fig. 1. Polarogram of 1 m*M* vitamin K_3 in an acetate buffer of pH = 5.9.

TABLE I

HALF-WAVE POTENTIALS OF VITAMIN K_3 AS A FUNCTION OF pH

pH	$E_{\frac{1}{2}}$ *vs. Ag/AgCl, KCl (s) (V)*
4.30	−0.040
4.75	−0.075
4.98	−0.085
5.35	−0.120
5.70	−0.132
5.90	−0.150
6.25	−0.175
8.00	−0.285

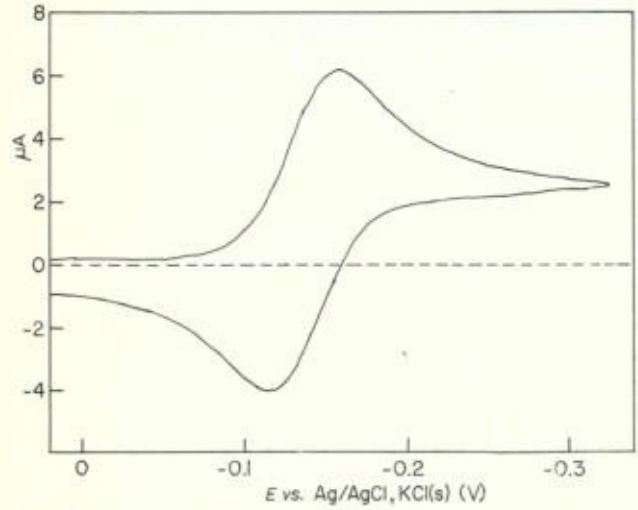


Fig. 2. Cyclic voltammetry of 1 m*M* vitamin K_3 in acetate buffer of pH = 5.9. Voltage sweep: 20 mV/sec. Hanging mercury drop electrode.

level in the auxiliary electrode compartment was kept above that in the working electrode compartment to avoid backflow of sample. The volume of the test solution was 75–100 cm^3 and rapid magnetic stirring was employed.

As discussed in detail elsewhere[5], the current efficiency for the oxidation of cerium(III) to cerium(IV) ion at a platinum anode approaches more closely to 100%, the greater the concentrations of both cerium(III) ion and sulfuric acid. However, because the solubility of cerium(III) sulfate is not very large, and decreases with increasing concentration of sulfuric acid, a compromise is necessary. An optimal compromise is 3 *F* sulfuric acid and 0.05 *F* $Ce_2(SO_4)_3 \cdot 8\ H_2O$, and this supporting electrolyte was used in all of the titrations. The same supporting electrolyte was used in the auxiliary electrode compartment.

A platinum foil generator anode with an area of 5 cm^2 (total of both sides) was used to provide a current-density of 2–3 mA/cm^2 in the optimal range. A coiled platinum wire served as auxiliary electrode and its area was not critical.

The electrolysis current was supplied from a conventional line-operated power supply. Since the current was only approximately constant ($\pm 1\%$), the Ether Ltd. integrator previously described[6] was used to measure precisely ($\pm 0.1\%$) the number of coulombs passed during the coulometric titrations.

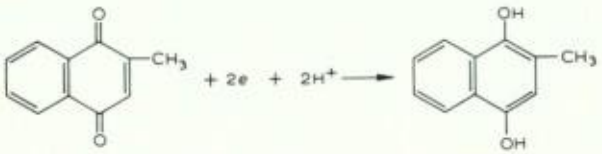
Potentiometric end-point detection with a platinum indicator electrode and an Ag/AgCl, KCl (s) reference electrode, was employed.

Standard solutions of vitamin K_3 (U.S.P. Standard) were freshly prepared in methanol.

DATA AND DISCUSSION

Because vitamin K_3 is very unstable in alkaline medium, an alkaline supporting electrolyte was avoided, and acetate–acetic acid and phosphate buffers of pH below 8 were employed.

In these two media, the 2-methyl-1,4-naphthoquinone shows a well shaped wave, whose limiting current is controlled by diffusion and is without a maximum. The reduction involves two electrons to produce the corresponding hydroquinone:



A typical polarogram is shown in Fig. 1.

The observed half-wave potentials in various buffers of different pH values are listed in Table I. The values are referred to the Ag/AgCl, KCl (s) reference electrode whose potential is +0.197 V *vs.* N.H.E.

The slope of the wave indicates that the 2-electron reduction is nearly but not perfectly reversible. Values of $E_{\frac{3}{4}}-E_{\frac{1}{4}}$ from many polarograms averaged 34 ± 2 mV (even though correction for iR drop was considered), compared to the theoretical value of 28 mV.

When plotted against pH, the values of $E_{\frac{1}{4}}$ produce a straight line whose slope is 68 mV, which is 8.9 mV greater than the theoretical value. Extrapolated to pH 0,

the straight line gives a value of +0.26 V *vs.* Ag/AgCl, KCl (s) which is close to the standard oxidation–reduction potential.

The near reversibility of the reaction is also demonstrated by the cyclic voltammogram in Fig. 2. The difference in potential between the cathodic and anodic peaks is 43 mV which is only 13.8 mV greater than the theoretical value of a 2-electron reaction. The quasi-reversibility of the system is further shown by the fact that the anodic half-wave potential of the hydroquinone (prepared by controlled potential reduction at a mercury cathode) is only a few millivolts more positive than the cathodic $E_{\frac{1}{2}}$.

The limiting current for the reduction of vitamin K_3 is diffusion-controlled and thus directly proportional to the concentration. In a buffer composed of 0.1 *M* sodium acetate and 0.08 *M* acetic acid, and 3–10 vol. % methanol (necessary to dissolve the vitamin originally), the observed value of $i_d/Cm^{\frac{2}{3}}t^{\frac{1}{6}}$ was 3.88 ± 0.04 with a dropping electrode whose $m^{\frac{2}{3}}t^{\frac{1}{6}}$ value was 1.60.

The usual method of determining menadione is based on its preliminary reduction to the hydroquinone by zinc dust in acid medium, followed by titration with standard cerium(IV) solution[7]. This method is only suitable with relatively large samples (150 mg) and because of the low levels of this vitamin in pharmaceuticals, biological fluids, and foods, it is highly desirable to have a micromethod for its determination.

We have developed such a method based on preliminary reduction to the hy-

TABLE II

CERI-COULOMETRIC DETERMINATION OF 2-METHYL-1,4-NAPHTHOQUINONE AFTER REDUCTION BY METALLIC ZINC IN A MIXTURE OF ACETIC AND SULFURIC ACIDS

Taken (mg)	*Found (mg)*	*Difference (mg)*
0.300	0.297	−0.003
0.628	0.619	−0.009
1.000	1.010	+0.010
1.256	1.250	−0.006
1.500	1.520	+0.020
1.820	1.800	−0.020
2.50	2.49	−0.01
2.50	2.46	−0.04
3.00	3.00	0.00
3.03	3.00	−0.03
3.20	3.24	+0.04

TABLE III

CERI-COULOMETRIC DETERMINATION OF 2-METHYL-1,4-NAPHTHOQUINONE AFTER REDUCTION BY CONTROLLED POTENTIAL COULOMETRY AT −0.60 V *vs.* Ag/AgCl KCl (s)

Taken (mg)	*Found (mg)*	*Difference (mg)*
0.855	0.860	+0.004
0.855	0.848	−0.007
1.710	1.690	−0.020
3.42	3.39	−0.03

droquinone by controlled potential electrolysis, or by zinc, followed by coulometric titration with electrogenerated cerium(IV) ion.

Table II summarizes results obtained by coulometric titration with electrogenerated cerium(IV) ion after reduction by zinc metal in a mixture of 2 *M* acetic and 1 *M* sulfuric acid.

Table III summarizes results obtained by coulometric titration with electrogenerated ceric ion after reduction by controlled potential electrolysis at —0.60 V *vs.* Ag/AgCl KCl (s).

In all cases potentiometric end-point detection with a platinum indicator electrode was employed. The potential change at the end-point is large (about 150 mV per 1% titrated).

It should be easily possible to determine considerably less than the quantities of vitamin K_3 listed in Tables II and III simply by employing a smaller volume of supporting electrolyte in an appropriately smaller cell. This would be desirable, for example, after separating small amounts of 2-methyl-1,4-naphthoquinone by solvent extraction from foods and biological samples.

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SUMMARY

Electrochemical characteristics of 2-methyl-1,4-naphthoquinone (vitamin K_3) were studied by polarography, controlled potential coulometry, and cyclic voltammetry. A polarographic procedure in acetate and phosphate buffers and a microcoulometric method of determination of this compound with electrogenerated cerium(IV) ion are described.

RÉSUMÉ

Les caractéristiques électrochimiques de la 2-methyl-1,4-naphtoquinone ont été déterminées à l'aide de la polarographie, de la coulométrie à potentiel contrôlé et par voltamétrie cyclique. Les auteurs proposent une microméthode coulométrique de dosage à l'aide de l'ion cerium(IV) électrogénéré de même qu'une méthode polarographique de dosage en milieux acétique et phosphorique tamponnés.

ZUSAMMENFASSUNG

2-Methyl-1,4-naphthochinon (Vitamin K_3) wurde polarographisch, coulometrisch mit kontrolliertem Potential und mit der zyklischen Volametrie untersucht. Die mikrocoulometrische Bestimmung dieser Verbindung mit elektroerzeugtem Cer(IV)-Ionen wird beschrieben.

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