While the present experiments did not yield satisfactory results below 10 ms, the problems in this time region are technical and amenable to improvement. Second, the relatively long path lengths of the reflection mode permit weak chromophores to be monitored, in contrast to most OTE experiments. Third, extensive time averaging is not usually necessary, again in contrast to OTE methods. Many reactions of electrogenerated species are irreversible, and a long period of time must elapse between runs to re-establish initial conditions. In many cases this is impractical, so time averaging is commonly applied to mechanisms involving reversible coupled reactions, such as redox reactions between an electrogenerated component and a solution species. The present technique will be useful where time averaging is not permissible or is very time-consuming. Fourth, a wider range of concentrations may be used with the present method compared to OTE experiments. Lower concentrations are usable because of the longer optical path length, and higher concentrations are possible because of the lower resistance of the working electrode relative to thin-film electrodes.

The concept of using reflected light for absorbance measurements on electrogenerated species is not a new one. However, when the reflection angle is very small with respect to the electrode surface, significant enhancements in path length are observed and important experimental advantages result.

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RECEIVED for review December 6, 1978. Accepted January 25, 1979. This work was supported in part by grant 28412 from the National Institute of Mental Health.

Electrochemical Study of the Degradation of Vitamin K_3 and Vitamin K_3 Bisulfite

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Polarography is used to follow the degradation and the products of vitamin K_3 bisulfite (I) and vitamin K_3 (II). The degradation scheme is outlined in Figure 2. The predominant degradation of I in alkaline solution is to II. In neutral solution, gradual isomerization to the naphthoquinone sulfonate (VIII) becomes significant. II is degraded primarily via rearrangment to the epoxynaphthohydroquinone (VI) in the absence of oxygen, except in very alkaline medium (pH 12) where formation of the naphthohydroquinone (VII) becomes significant. Small amounts of the epoxnaphthoquinone (III) occur by reaction of II with O_2 or H_2O_2 formed with VII. VI and IV degrade to the phthiocol (IV) and the dinaphthalenetetrone dimer (V), the former becoming more dominant at higher pH. I is more stable than II at pH $\geq$ 7. The relative rates of degradation are reported.

K vitamins are present in vegetables where they seem to play an active role in photosynthetic mechanisms (*1*). They also play a role in cellular respiration as electron transporters (*2*) and in oxidative phosphorylation (*3*). These properties result from the reversible character of this quinone/hydroquinone redox system.

In the human body, they are synthesized by microorganisms in the intestine to provide required physiological quantities and are implicated in synthesis of four blood coagulation factors in the liver (*4*, *5*). Deficiencies of vitamin K in the body are infrequent, but may occur if obstruction of the bile or intestine occurs and in cases of liver disease such as cirrhosis (*4*, *5*). Deficiencies are generally accompanied by a decrease in the prothrombin level with an increase in blood coagulation time. At advanced stages, the capillaries may become fragile and hemorrhage. Symptoms are reversed by incorporation of K vitamins in the diet, except in cases of some liver diseases.

In recent spectrophotometric studies of naphthoquinones of the important K group vitamins, we have observed several properties related to complex formation with titanium(IV) (*6*). We have also studied the electrochemistry of vitamin K_3 (menadione) and its bisulfite derivative (*7–10*). We were interested in the stability of the active forms of these compounds in aqueous solution. The present work was undertaken to determine both the rates of degradation and the degradation products of vitamin K_3 and vitamin K_3 bisulfite in neutral and alkaline media.

EXPERIMENTAL

Direct current (dc), alternating current (ac), and differential pulse (dp) polarographies were used to identify and monitor the compounds resulting from the degradation of these two vitamins. Polarograms were obtained with solutions of pH 6–12 at regular intervals during periods ranging up to between 20 and 50 days. These periods correspond to stabilization of the developed chemical degradation processes. The solutions were stored in the dark. They were deaerated with nitrogen and stored in Teflon-stoppered volumetric flasks to exclude oxygen. Control experiments with electrolyte solutions demonstrated no po-

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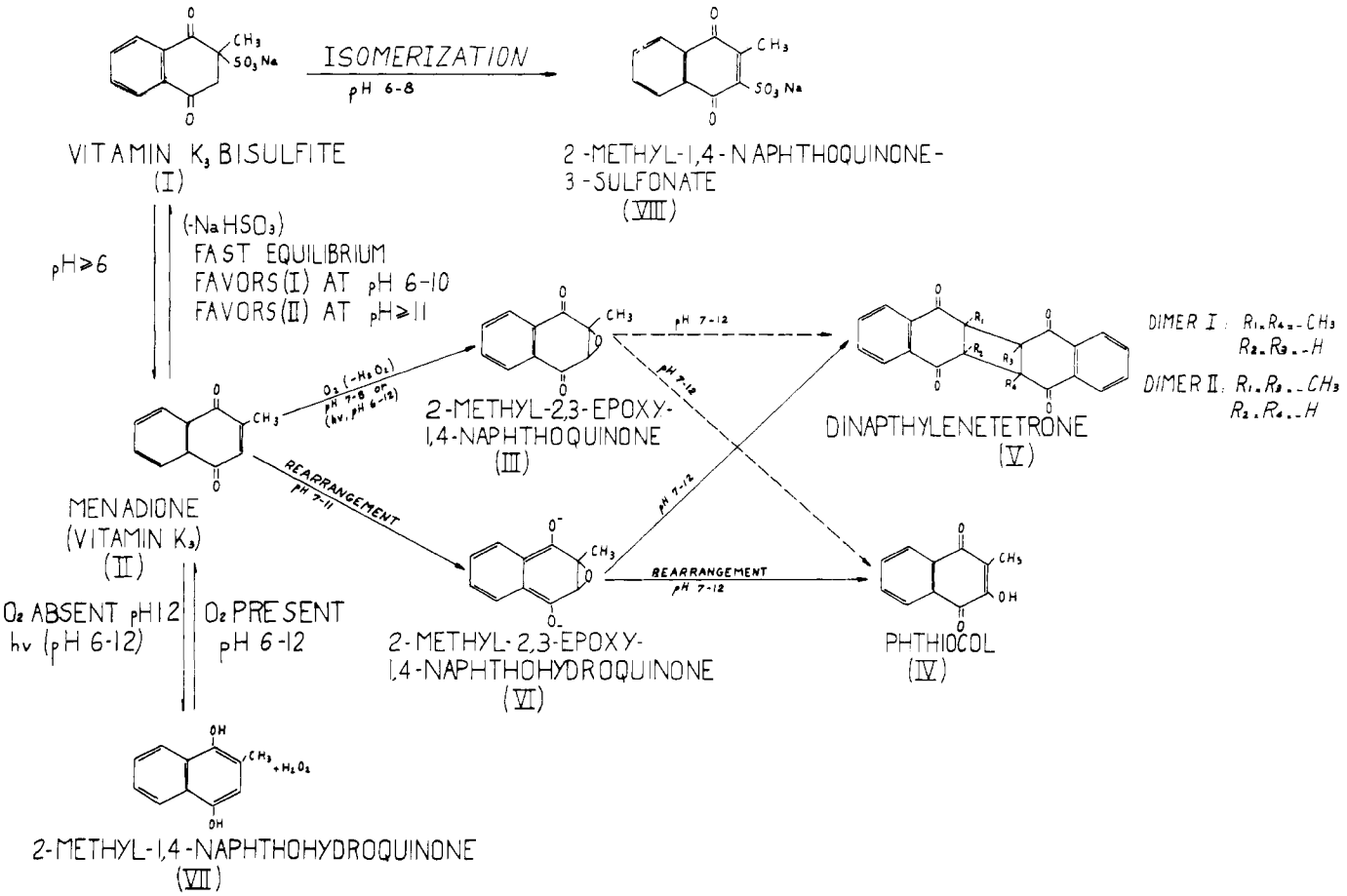


Figure 1. Degradation scheme for vitamin K_3 and vitamin K_3 bisulfite

larographically measurable oxygen entered the solutions stored in the flasks.

Apparatus and Reagents. Polarograms, dc, ac, and dp, were obtained on a Tacussel polarograph type PRG 34, using a saturated calomel reference electrode. The following parameters were used unless otherwise stated.

Alternating current polarography: 10 mV superimposed, frequency 90 Hz., drop time 1.1 s; dp polarography: sweep rate 2 mV/s, drop time 2 s, current sampled 40 ms, starting 1.9 s after initiating the drop, pulse amplitude 40 mV.

Direct current polarography: drop time 1.1 s. A nitrogen atmosphere was maintained above solutions during recording of polarograms. Nitrogen was initially bubbled in the solution for a few minutes to remove any traces of oxygen that may have been introduced during transfer of solutions.

The photochemical degradation of menadione was studied in a quartz polarographic cell with the aid of a 100-W high pressure mercury vapor lamp. A Hanovia high voltage dc power supply was used for the lamp. The lamp was placed 60 cm from the solution.

Menadione (Merck 5793) and Vitamin K_3 bisulfite (PCB c 813930) were used without further purification. Solutions were prepared immediately before initiation of the measurements, and final solutions contained 10% methanol (Merck 6008). Buffers used were phosphate/HCl and phosphate/NaOH at 0.05 M.

RESULTS AND DISCUSSION

General Reaction Scheme. As many as eight different polarographic waves or peaks were observed in these studies, corresponding to the reduction of eight different compounds, some with multiple waves. Some of the waves overlapped. In order to facilitate discussions, a summary of the degradation reaction scheme as evolved from the electrochemical studies is presented in Figure 1, and a dp polarogram identifying most of the peaks is shown in Figure 2. We shall refer to the various peak numbers as waves throughout the discussion.

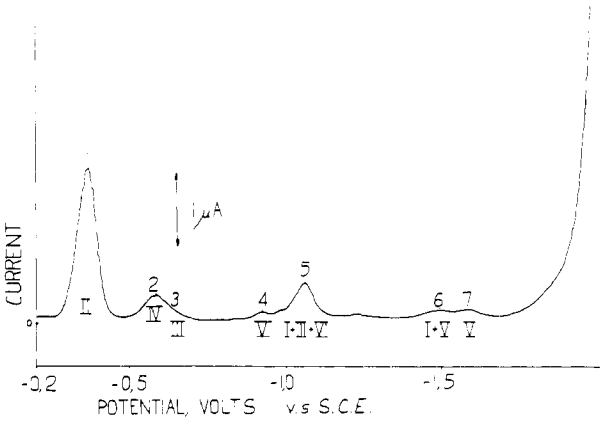


Figure 2. Differential pulse polarogram of a 5×10^{-4} M solution of menadione at pH 9 after 600 h. The waves (peaks) are due to reduction of compounds identified by roman numerals as follows. Wave 1, reduction of carbonyl groups of menadione (II). Wave 2, reduction of carbonyl groups of phthiocol (IV). Wave 3, reduction of carbonyl groups of 2-methyl-2,3-epoxy-1,4-naphthoquinone (III). Wave 4, reduction of first carbonyl groups of dinaphthylenetetrone (V). Wave 5, reduction of first carbonyl group of vitamin K_3 bisulfite (I), and of epoxy groups of 2-methyl-2,3-epoxy-1,4-naphthoquinone (III) and hydroquinone (VI). Wave 6, reduction of second carbonyl group of vitamin K_3 bisulfite (I) and of second carbonyl groups of dinaphthylenetetrone (V). Wave 7, reduction of cyclobutane groups of dinaphthylenetetrone (V)

Vitamin K_3 Bisulfite Degradation. At pH 6, vitamin K_3 bisulfite (I) is degraded, and the rate of degradation progressively increases with increased pH (Figure 3). One observes a simultaneous decrease of the two waves of this compound and the appearance of another wave at more positive potentials as the compound degrades. The half-wave

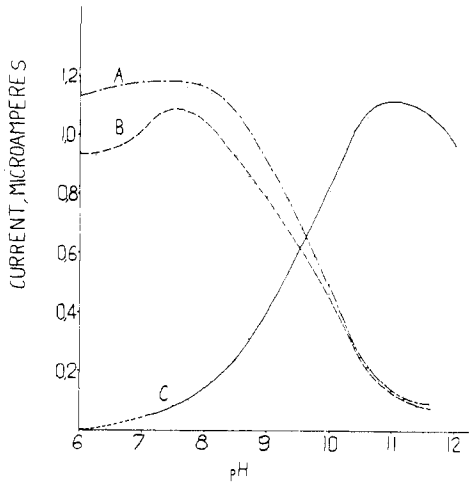
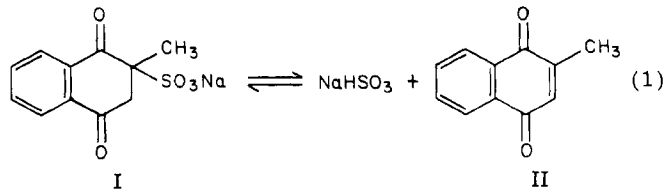


Figure 3. Equilibrium concentrations of vitamin K_3 bisulfite and menadione as function of pH measured 30 min after preparation of vitamin K_3 bisulfite solution. (A) First wave of vitamin K_3 bisulfite (5). (B) Second wave of vitamin K_3 bisulfite (6). (C) Menadione wave (1)

potential and the pH dependence of this wave, and its adsorption characteristics identify it as being due to menadione (wave 1) (*8*, *9*). This compound is formed by a β cleavage mechanism which eliminates the bisulfite ion. This reaction confirms the results of Carmack et al. (*11*). Although degradation begins at pH 6, the new wave cannot be detected until pH 7. It diminishes starting at pH 11 because this molecule is rapidly decomposed in very alkaline media.

Except at very alkaline pH, the vitamin K_3 bisulfite decomposes slowly. For example, at pH 9, a freshly prepared solution preserved in a nitrogen atmosphere for 8 h and measured at regular times showed no significant degradation. The same experiment at pH 11 showed about 40% degradation, but greater for the menadione product (about 60%) than for the parent dioxotetraline form. So it appears that the dioxotetraline form is a more stable form in alkaline medium than the naphthoquinone structure. A polarographic study of the reaction between menadione and sodium bisulfite in neutral (pH 7) and alkaline (pH 9) media at different ratios of reactants indicated that at equal concentrations of bisulfite and menadione, the reaction was incomplete and that it was favored at the lower pH (Figure 4). Extrapolation of the curves in Figure 4 indicates that complete reaction should be obtained at ratios of about 0.26 and 0.85 menadione/bisulfite, respectively, for pH 9 and 7. These results suggest that there is an equilibrium reaction as follows



I II (1)

which is governed by the concentrations of menadione and bisulfite, and the pH. This reaction is reversible in neutral and alkaline media. In acidic media, no reaction is observed between bisulfite and either menadione or vitamine K_3 bisulfite.

The vitamin K_3 bisulfite derivative quickly degrades by β cleavage to give equilibrium concentrations of menadione and bisulfite via reaction (*1*), producing the menadione wave and a diminished vitamin K_3 bisulfite wave. The equilibrium lies toward compound I at pH 6–10 and toward compound II at pH 11 or above.

The produced menadione degrades to produce new waves, causing a further shift in the equilibrium and a decrease in both the vitamin K_3 bisulfite wave and the menadione wave. The kinetics of these decompositions are considered below.

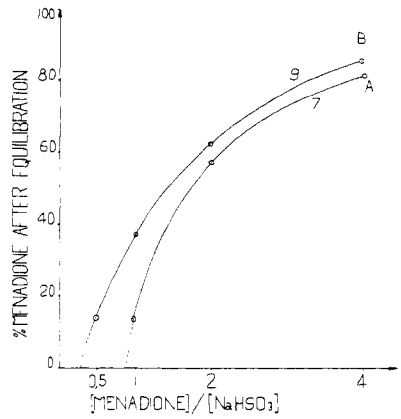


Figure 4. Percentage of menadione remaining at equilibrium after reaction with $NaHSO_3$, as a function of the ratio of the initial concentrations of menadione/$NaHSO_4$ measured after 30 min. (A) pH 7, (B) pH 9

For the bisulfite, another peak appears between pH 6 and 8 at a potential more positive than the menadione (wave 1) peak. Its intensity goes through a maximum with time, being reached sooner at the higher pH. The peak is attributed to the formation of an isomer of the vitamin K_3 bisulfite: 2-methyl-1,4-naphthoquinone-3-sulfonate (VIII). The formation of this isomer is small compared to the formation of menadione in alkaline solution, but it can become significant at neutral pH over long periods of time when the equilibrium between menadione and the bisulfite lies towards the bisulfite. This isomer has been demonstrated by Ksenzhek et al. (*12*) using voltammetry with a carbon paste electrode. The relatively positive potential of its peak is due to the inductive effect of the sulfonate group on the quinone structure of the isomer. The dc polarographic wave of this isomer shifts in a cathodic direction with increased pH from 6 to 7 (near –0.15 V vs. SCE).

Menadione Degradation. Several authors (*13–16*) have studied the photochemical decomposition of menadione in various media. Results obtained can be described as follows. In the absence of oxygen, light promotes reduction of vitamin K_3 (II) to 2-methyl-1,4-naphthohydroquinone (VII) with simultaneous formation of hydrogen peroxide. In the presence of oxygen, the naphthohydroquinone is reoxidized to menadione. This oxidation is reported to be accompanied by formation of 2-methyl-2,3-epoxy-1,4-naphthoquinone (III), which is said to then be transformed into phthiocol (IV) or a dimer (V) which has the structure of dinaphthylenetetrone.

Asahi (*14*, *17*) proposed the use of polarography for the study of the photochemical decomposition of vitamine K_3. We have employed polarography to study the chemical degradation.

Figure 5 shows a dc polarogram (curve A) of menadione at pH 9 after 600 h. Curve B shows a dc polarogram obtained at the same pH, but for a fresh solution irradiated for 1 h with UV radiation. (A fresh solution exhibits primarily only the menadione wave.) The similarity of the two polarograms is evident, the only difference being at the menadione wave. In the first instance only the cathodic wave of the quinone form is seen, but, after irradiation, about two thirds of the menadione present is in the reduced hydroquinone form (VII), as seen from the anodic wave. This anodic wave is also seen at pH 12 upon standing in the presence of nitrogen. The conclusion is that chemical and photochemical decomposition result in the same degradation products. Note also the similarity of the dc polarograms with the dp polarogram in Figure 2. The dp polarographic technique is more useful in

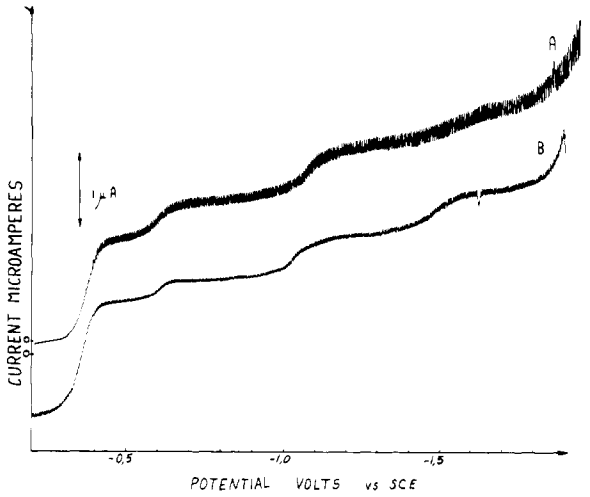
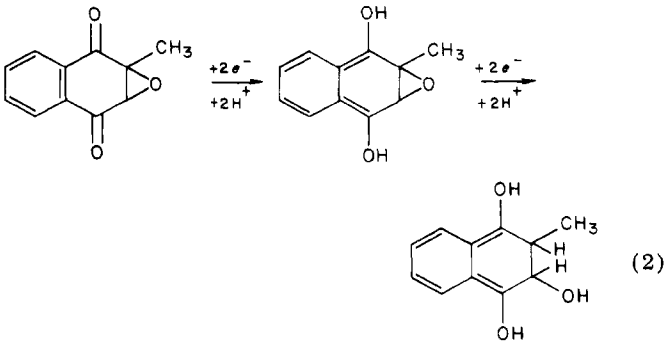


Figure 5. Direct current polarograms of 5×10^{-4} M menadione at pH 9. (A) after 600 h. (B) After UV irradiation for 1 h

resolving new waves at low concentrations.

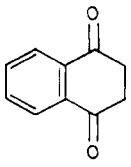
Both menadione and its degradation product phthiocol are reduced at the DME to the corresponding naphthohydroquinones.

In addition, the chemical degradation intermediate 2-methyl-2,3-epoxy-1,4-naphthoquinone (III) is reduced in two 2-electron steps at the DME. The reduction of the epoxy form may be represented by



(2)

The first wave (3) occurs at -0.6 V vs. SCE and corresponds to the reduction of the two carbonyl groups. This reduction potential is due to the fact that the epoxy form is intermediate in structure between the quinone form, which is conjugated (and which is reduced at -0.3 V), and the *o*-benzenedicarbonyl form



which is not conjugated (and is reduced at -1.0 V). The second wave (5) is due to reduction of the epoxy function to an alcohol, and appears between -1.00 and -1.13 V for pH 7 to 11. The hydroquinone epoxy intermediate (VI) also exhibits this wave (see below).

The dimer produced from the epoxy intermediate (probably the hydroquinone (VI) is reduced in two 2-electron steps in a manner similar to the reduction of the epoxy form, with half-wave potentials of -0.80 to -1.05 V for the first wave (4) and -1.45 to -1.55 V. for the second wave (6) at pH 7 to 11. The waves for the two different dimers occur at identical potentials and can not be resolved (*14, 17*). But dimer I also exhibits a third ill-defined wave (7) at -1.6 V which corresponds to the electrochemical opening of the cyclobutane ring,

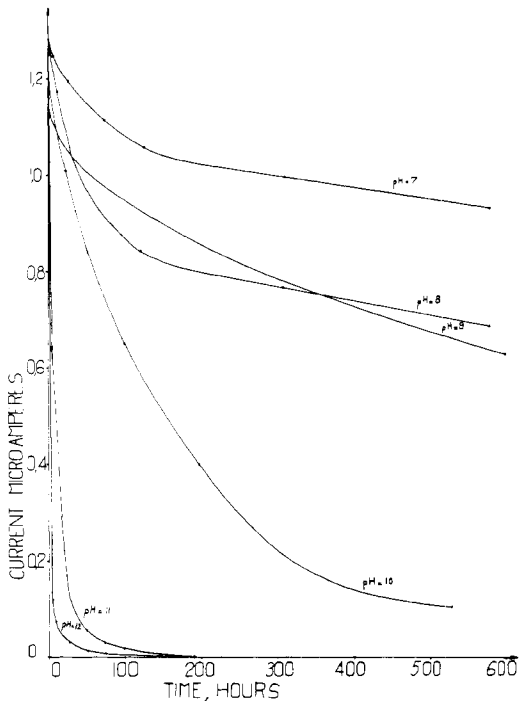


Figure 6. Intensity of menadione (5×10^{-4} M) dc polarographic wave (1) as a function of time at different pH values

Table I. Time Required for 50% of the Menadione Initially Present to Disappear

	pH					
	7	8	9	10	11	12
$T_{1/2}$, h	1500[a]	700	550	110	2	*b*

[a] Obtained by extrapolation. [b] Not measurable after 2 h, less than 10% remains.

with addition of two protons (*14, 17*). This wave can only be resolved from the second wave (6) using dp polargraphy.

From the above electrochemical properties, it is possible to interpret the degradation of vitamin K_3 in alkaline solution. Figure 6 represents the change in the menadione concentration as a function of time at different pH values, obtained from dc polarograms. The decomposition of this compound is initially rapid and then slows down after 150 h at pH 7 and 8. Above pH 8, the behavior of the curves changes somewhat, indicating more gradual degradation throughout. This behavior could be due to the fact that the buffering capacity of phosphate is small at pH 9. Above pH 10, the degradation is very rapid. Table I lists the times required for decomposition of one half the menadione at the different pH values. Note the short half-life above pH 10.

Similar rate curves are obtained using dp polarography with improved sensitivity. But ac polarography gives irregular curves because the intensity of the peaks is modified by adsorption which accompanies the reduction of menadione. This adsorption phenomenon also results in an anodic shift in the dc half-wave potential of the menadione wave as a function of time (due to decreased concentration of menadione with time).

When the degradation is very fast, it is not possible to observe significant quantities of 2-methyl-1,4-naphthohydroquinone (VII), except at pH 12 with the solution maintained under a nitrogen atmosphere. It is not observed if the solution is contacted with oxygen between measurments, and increased amounts of III are observed at pH 7-8.

The first dp polarographic peak (5) appears at pH 7 after 24 h, corresponding to the reduction of the epoxy function

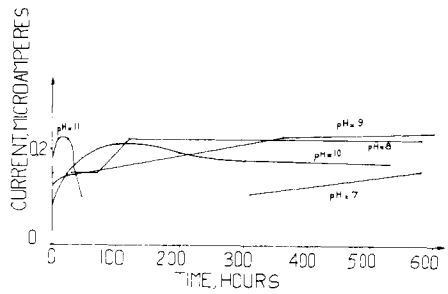


Figure 7. Intensity of second epoxy derivative wave (5) as a function of time at different pH values. Starting solution 5×10^{-4} menadione

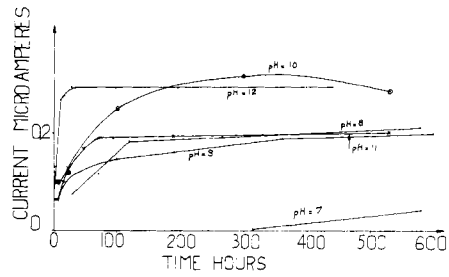


Figure 8. Intensity of phthiocol wave (2) as a function of time at different pH values. Starting solution 5×10^{-4} M menadione

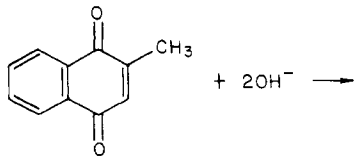
of either compound III or VI. A DC polarographic wave is observed only after 300 h. But a dc wave appears rapidly at pH 8 and above due to compound VI (Figure 7). At pH 7 and 8, the wave remains stable after 120 h, and at pH 9 after 360 h. However, at pH 10 and 11, the epoxy form goes through a maximum, indicating rapid degradation.

A second dp polarographic peak of the epoxy form can be observed at more positive potentials after 28 h at pH 7, and is due to the reduction of the two carbonyl functions (wave 3). It increases with time, but its height is never more than one fifth of the other wave. It also appears at pH 8, but is smaller, and is not seen at higher pH.

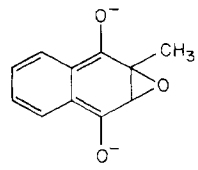
These results indicate the intermediate character of the epoxy derivative in the degradation of vitamin K_3, especially at pH 10 and 11.

The predominance of the more negative potential peak over the other and the fact that the more positive one is not present above pH 8 indicates that the carbonyl form of the epoxy compound exists at pH 7 and 8 only, while the hydroxy form can exist up to pH 11.

These results suggest that menadione undergoes chemical oxidation, by two different pathways depending on the pH. At pH ≥7, an internal oxidation occurs by rearrangement of the molecule as follows:



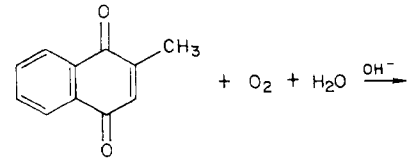
II



(3)

VI

The corresponding hydroxy form of the epoxy product is electrochemically reduced (wave 5). This compound can only exist in the ionic form at these pH values because its pK_a is about 5 (*17*). In neutral and weakly alkaline solution, a second oxidation can occur by reaction with oxygen:



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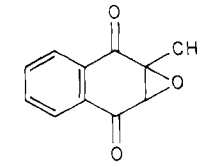
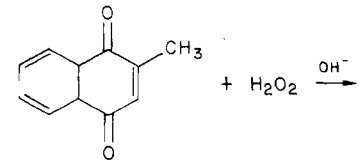


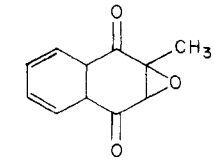
(4)

III

The hydrogen peroxide in turn can oxidize menadione:



II



(5)

III

However, this reaction would be a minor one. The quinone epoxy compound results in the more positive wave (3) observed, only at pH 7 and 8. The hydroxy epoxy compound produced in reaction 3 can also react with oxygen to give the carbonyl compound at pH 7 and 8, but this is only a minor side reaction.

The 2-methyl-2,3-epoxy-1,4-naphthoquinone (III) isomerizes at pH 7 to produce a small amount of phthiocol (IV). This isomerization increases at pH 8 and above (Figure 8). The abnormally high current (wave 2) at pH 10 is resolved into two peaks using dp polarography indicating the presence of another compound at this pH. As seen in the figure, the phthiocol is stable in alkaline medium and does not undergo further degradation.

The dimerization reaction of the epoxy form starts at pH 7 (very small) and increases with increasing pH. The first dimer wave (4) is generally only detected by dp polarography. It can be seen by dc polarography only at pH 11 because it coincides with the epoxy wave and is not resolved; at this pH, the epoxy wave is diminished (Figure 7).

The dimerization reaction can be monitored more easily with the second appearing wave of the dimer (wave 6). It appears immediately at pH 8 with dp polarography, and after 300 h at pH 9 with dc polarography. It increases regularly up to pH 11 (Figure 9), as the rate of degradation of menadione increases. At pH 12, it diminishes, suggesting further degradation of the dimer in very alkaline solution.

A third dimer peak appears between pH 9 and 10 (the last peak (7) in Figure 2), and is probably due to electrochemical breaking of the cyclobutane ring of the dimer. The intensity remains small.

Rate of Degradation of Vitamin K_3 Bisulfite. As we have indicated, the principal decomposition product of vitamin K_3 bisulfite following the β cleavage reaction is menadione, which is then further degraded by the reactions given above.

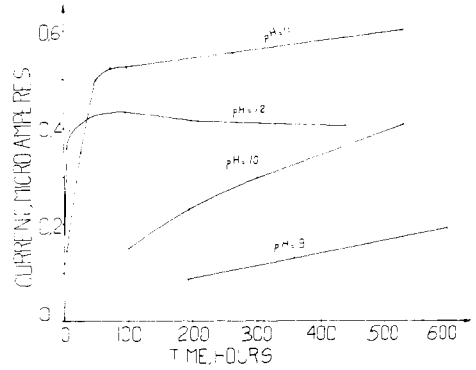


Figure 9. Intensity of second dimer wave (6) as a function of time at different pH values. Starting solution 5×10^{-4} M menadione

The degradation of vitamin K_3 bisulfite as a function of time was investigated using dp polarography. The degradation products exhibited many polarographic waves which could be neither resolved nor detected by dc polarography.

The first vitamin K_3 bisulfite wave is overlapped with the second wave of the epoxy derivative (wave 5) at pH 9 to 10, while its second wave is fused with the second wave of the dimer (wave 6) at pH 7–10.

The concentration of vitamin K_3 bisulfite decreased regularly starting at pH 6. The degradation rate increased progressively with increased pH, but decreased with time. The two peaks of the compounds disappeared after 100 h at pH 11, and did not appear at pH 12 because of immediate decomposition.

The menadione peak increases as the concentration of vitamin K_3 bisulfite decreases, passing through a maximum between pH 7 and 9, at which time the vitamin K_3 bisulfite and its menadione product are decaying at equal rates. At pH 6, there is no degradation of menadione for up to 50 days.

The reduction peak of the epoxy function (wave 5) develops starting at pH 7, and its intensity remains significant at pH 11 after 30 days. In contrast, when starting with menadione, this peak disappears after 2 days at pH 11, indicating the slower rate of decay of the bisulfite compound. The phthiocol peak can be detected only at pH 9 or above in traces when starting with vitamin K_3 bisulfite, but with menadione it is seen starting at pH 7. So the rate of formation of menadione from the bisulfite is sufficient to produce only traces of phthiocol from the menadione degradation. This is in contrast to the results of Kallmayer (*18*) and Fieser (*19*) who reported that phthiocol could come directly from the bisulfite degradation.

The two reduction peaks of the dimer form appear starting at pH 7 for both the bisulfite and menadione, and their evolution with time is similar at the different pHs.

This different behavior from the phthiocol compound may be due to differences in the rates of formation of each at different pHs, with the dimer formation rate being independent of pH and the phthiocol formation rate accelerated with increased pH.

CONCLUSION

The predominant degradation of vitamin K_3 bisulfite (I) in alkaline solution is to menadione (II), but in neutral solution, significant degradation to the naphthoquinone sulfonate (VIII) gradually occurs since the equilibrium between I and II lies towards I. The degradation of menadione is primarily via rearrangement to the epoxynaphthohydroquinone (VI) in the absence of oxygen, except in very alkaline medium (pH 12) where formation of the naphthohydroquinone (VII) becomes significant. Formation of the epoxynaphthoquinone (III) can be accounted for from either the presence of traces of oxygen in solution or from reaction of II with hydrogen peroxide or oxygen produced from the hydrogen peroxide formed with VII.

The end products of degradation are significant quantities of either the phthiocol (IV) or dinaphthylenetetrone dimer (V), primarily from VI, the formation of the phthiocol becoming more dominant with increasing pH. Vitamin K_3 bisulfite is more stable than menadione at pH $\geq$ 7.

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RECEIVED for review November 13, 1978. Accepted January 19, 1979. Thanks are expressed to the Fonds National de la Recherche Scientifique (F.N.R.S. Belgium) (G.J.P.) and the Commission for Educational Exchange between the United States of America, Belgium, and Luxembourg (G.D.C.) for support of this research.