The authors wish to express their thanks to Mr. Jack Riseman for assistance in connection with Experiment II.

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NEW YORK, N. Y. RECEIVED JANUARY 27, 1940

The Oxidation–Reduction Potential of Vitamin K_1

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Because of the increasing physiological importance of oxidation–reduction systems, the oxidation–reduction potential of pure synthetic vitamin K_1 has been determined. Such potentials are also important for elucidating the structures of quinones and for this reason, at the suggestion of Professor L. F. Fieser, we first measured the potential of the vitamin in potent alfalfa concentrates. This value, 358 mv., in conjunction with the C-H analysis and the absorption spectrum first published by Dam, Karrer, *et al.*,[2] provided strong evidence that vitamin K_1 was a 2,3-dialkyl-1,4-naphthoquinone. This was evident from the values of Table I.

TABLE I

	E_0 in mv.
1,4-Naphthoquinone	484[a]
2-Methyl-1,4-naphthoquinone	408[a] 422[b]
2,3-Dimethyl-1,4-naphthoquinone	340[a]

[a] L. F. Fieser and M. Fieser, THIS JOURNAL, **57**, 491 (1935).

[b] J. W. H. Lugg, A. K. Macbeth and F. L. Winzor, *J. Chem. Soc.*, 1457 (1936).

This information together with other known facts led to the prediction of the first specific structure[3] for vitamin K_1 which later proved to be correct.

Karrer and colleagues[4] have reported the oxidation–reduction potential $E_m = +5$ mv. for vitamin K_1. It is not absolutely clear, from their experimental data, how their value compares with other quinone potentials because they did not use the customary method. However, from a consideration of the *p*H of the solvent used it seems that their value of the oxidation–reduction potential E_0 is about 400 mv. This estimation does not take into account a possible junction potential.

In the fractionation of alfalfa extracts it was found that a stock solution of 3% vitamin K_1 concentration had the best keeping qualities and gave the most reliable potentials over a period of time. More highly refined concentrates showed considerable variation in both bioassay and potential upon storage. Because of these variations and the occasional presence of other substances which affected the oxidation–reduction curve it seemed advisable to delay publication until the determination could be made on the pure vitamin.

The length of time required to establish equilibrium (in some instances twenty-four hours for the complete titration) in the case of the refined concentrates made it necessary to employ a stable reference electrode. The Ag–AgCl electrode was found to be very suitable for this purpose since it is stable for at least several weeks.

The oxidation–reduction potential of pure vitamin K_1 was found to be 363 mv. at 20°.

Experimental

The preparation of the vitamin K active concentrates[5] from alfalfa has been described. The synthetic vitamin K_1 used in this work was prepared according to the method of Fieser.[6]

In the solvent used, 95% ethanol 0.2 *N* in hydrochloric acid and 0.2 *N* in lithium chloride, the positive potential of the Ag–AgCl electrode against the hydrogen electrode in the same solvent was approximately 200 mv. This value varies somewhat in different lots of the same solvent depending on the exact chloride ion concentration. However, it is necessary to determine the potential against the hydrogen electrode only once for any one lot of solvent. In the solvent used for the determination of the potential of the pure vitamin the Ag–AgCl electrode gave a value of +203 mv. against the hydrogen electrode. The solubility of the vitamin at room temperature was found to be about 5 mg. per ml. in the above solvent. This same solvent was used to prepare the very dilute solution of titanium trichloride used in the potentiometric titrations. Both platinized and bright platinum electrodes were used and gave the same results although the platinized electrodes seemed to reach equilibrium more rapidly.

Two independent determinations, checking within 1 mv., gave a potential of +160 mv. for the vitamin against the Ag–AgCl electrode. Thus the vitamin potential, E_0, against the hydrogen electrode is 363 mv. All measurements were made at 20°.

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EVANSTON, ILLINOIS RECEIVED FEBRUARY 23, 1940

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(4) P. Karrer and A. Geiger, *Helv. Chim. Acta*, **22**, 945 (1939).

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