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Polarographic determination of anticancer drug vitamin K_3 with direct and indirect methods/application to serum and K_3 injection solution

Ümmihan TAŞKOPARAN YILMAZ[1], **Güler SOMER**[2,*]
[1] *Nevşehir University, Chemistry Department, 50300, Nevşehir- TURKEY*
[2] *Gazi University, Chemistry Department, 06500, Ankara-TURKEY*
e-mail: gsomer@gazi.edu.tr

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In this work, direct and indirect methods for determination of vitamin K_3 were developed using differential pulse polarography. The reaction between sulfite and the quinone (Q) form of vitamin K_3 was used for indirect determination, since the sulfite peak at -0.7 V is sharp and very reproducible in 0.1 M HAc NaAc (pH = 5.5). It was found that at pH 4.5-5.5, this reaction was quantitative and very fast when the temperature was 45 °C. For its direct determination, on the other hand, vitamin K_3 was standardized by the indirect method using standard sulfite as the reducing agent. The calibration graph for vitamin K_3 (in Q form), using the peak at -1.0 V in a HAc-NaAc medium with a pH of 5.5, was linear for concentrations ranging from 5×10^{-7} to 3×10^{-5} M, and the limit of detection (LOD) was 1.5×10^{-7} M. The proposed methods were successfully applied to the determination of vitamin K_3 in a clinical injection solution and in blood serum.

Key Words: Vitamin K_3, sulfite, direct, indirect, differential pulse polarography

Introduction

Vitamins are important organic compounds for biological activities. Deficiencies in several vitamins are risk factors for chronic diseases such as cardiovascular disease, cancer, and osteoporosis[1]. They can be divided into 2 categories according to their solubility in water or fat. Water-soluble vitamins are vitamin C and several varieties of vitamin B. The fat-soluble vitamins are K, D, E, and A.

*Corresponding author

Vitamin K plays an important role in blood coagulation and in the bone mineralization process.[2–5] It also may play a variety of health-promoting roles. Vitamin K reduces the risk of heart disease, kills cancer cells, and enhances skin health, and may have antioxidant properties. As can be seen, determination of trace vitamin K has vital importance.

Vitamin K_3 contains a naphthoquinone ring, and its basic structure is 2-methyl-1,4-naphthoquinone. These vitamins are named according to their side chains by forming new derivatives. Vitamin K_3 is obtained synthetically without a side chain and is named as menadione. Menadione (vitamin K_3, 2-methyl-1,4-naphthoquinone) is a synthetic vitamin K analog, which has been used chiefly as an antihemorrhagic agent.[6] There are 2 versions of water soluble vitamin K_3; one is menadione sodium bisulfite and the other is menadiol sodium diphosphate. The physiological activity of menadione is the strongest among the K group vitamins. It shows antitumor and antiinflammatory activity because of the quinone group in its structure. In a series of in vitro and in vivo animal studies, menadione (vitamin K_3) showed significant antineoplastic activities against both malignant cell lines and a variety of human tumor cells.[7]

There are several methods for the separation and determination of vitamin K_3. For the clinical tests, sensitive and rapid analytical methods are needed. The common methods for determination of vitamin K analogs include spectrophotometric,[8–12] chromatographic,[13–15] and spectroscopic[16] methods, which are frequently used but have lower sensitivity when compared with polarographic methods.

Cloud point extraction has been used for the preconcentration of vitamin K_3 and 1,4-naphthoquinone after their reaction by aniline. Preconcentration of 15 mL of sample solution permitted the detection of 0.05 and 0.08 μg mL^{-1} for vitamin K_3 and 1,4-naphthoquinone, respectively.[11]

Vitamin K derivatives (vitamin K_1, phylloquinone, menaquinones) were determined with the HPLC fluorescence determination method using post-column reduction and internal standards.[13] Vitamin K_3 was extracted from the plasma samples with n-hexane, and it was determined by reverse-phase high performance liquid chromatography. The recovery was 82.4 $\pm$ 7.69% (n = 7).[15]

As compared with the generally used chromatographic and spectrophotometric methods, the polarographic method has inherent advantages concerning speed, simplicity, and sensibility. Determination of vitamin K_3 has also been made using electrochemical methods such as voltammetry[17,18] and polarography.[19–23] A cathodic stripping procedure was described for determination of vitamin K_3 using sodium sulfate as the supporting electrolyte at pH 1.70-1.85.[17] Square wave adsorptive anodic stripping voltammetry was used in a $HClO_4$ medium and, with 10 min of deposition time, a detection limit of 1.3×10^{-10} M was achieved.[18] A catalytic wave for menadione determination has been proposed in 0.2 M HAc-NaAc (pH = 4.7) electrolyte in the presence of 4×10^{-3} M KIO_3, and a limit of detection (LOD) of 2×10^{-9} M was obtained.[19] In one work, menadione was determined using differential pulse polarography (DPP) in a methyl alcohol medium using 0.2 M borate buffer (pH = 6.8) with a LOD of 5×10^{-7} M.[21]

In our former studies,[22] direct and indirect methods were developed in which the reaction between vitamin K_3 and Ti(III) was used. After reaction between Ti(III) and vitamin K_3, the quantity of Ti(IV) formed was determined by standard addition and, from stoichiometric relations, vitamin K_3 content could be calculated. In both methods, the LOD was found to be 7×10^{-7} M. These methods can be applied for human fluids without any extraction or preconcentration procedure.

Electrochemical methods offer useful alternatives since they allow faster, cheaper, and safer analysis.

The results obtained with the DPP method are very reproducible, since with the use of a dropping mercury electrode, the behavior of the electrode is independent of its past history.[23,24]

All of these methods need reference standard solutions. However, since the solutions on the market are only 95%-98% pure and very expensive, a standard solution of vitamin has to be prepared using an indirect method. In nearly no investigations mentioned above was this fact taken into account.

The purpose of this investigation was to establish a simple polarographic method for the determination of vitamin K_3 indirectly so that standard solutions could be prepared, which would enable direct determination of the vitamin. For this purpose, the quantitative reaction between sulfite and vitamin K_3 was used. Direct and indirect polarographic methods were developed, whereby it was possible to determine very low concentrations of vitamin K_3. These methods were used for the determination of vitamin K_3 contents in clinical injection solutions and in serum. The results indicate that both of these methods can be used safely for its determination.

Experimental

Apparatus

A polarographic analyzer (PAR 174 A) equipped with a PAR mercury drop timer was used. The drop time of the electrode was in the range of 2-3 s (2.3 mg s^{-1}). A Kalousek electrolytic cell with a saturated calomel electrode (SCE), separated by a liquid junction, was used in the 3-electrode configuration. All potentials in the manuscript are given versus SCE. The counter electrode was platinum wire. The polarograms were recorded with a Linseis (LY 1600) X-Y recorder under the conditions of a drop life of 1 s, a scan rate of 2-5 mV s^{-1}, and a pulse amplitude of 50 mV.

Reagents

All of the chemicals used were of analytical-reagent grade (proanalysis). Triply distilled water was used in the preparation of all solutions. Solutions of 10^{-3} M and more diluted ones were prepared before every use in order to avoid the aging process of the solution.

By adding 6 g of NaOH washed with distilled water, in order to remove the carbonate formed, to 57 mL of HAc and diluting the mixture to 1 L, 1 M HAc/Ac buffer was prepared.

The mercury used in the dropping mercury electrode was obtained from Merck (Darmstadt, Germany). Contaminated mercury was cleaned by passing it successively through dilute HNO_3 (3.0 M) and water columns in the form of fine droplets by using a platinum sieve. The collected mercury was dried between sheets of filter paper. Before use, a differential pulse (DP) polarogram of this mercury was recorded in order to confirm the absence of impurities.

Preparation of 10^{-3} M vitamin K_3 solution

Vitamin K_3 (276.24 g mol^{-1}) (menadione sodium bisulfite) was obtained from Libavit K. It contains, in each 2-mL vial, 0.02 g (about 0.036 M) of menadione sodium bisulfite, 0.006 g of potassium metabisulfite ($K_2O_5S_2$), 0.0126 g of sodium chloride, and 2 mL of water. For the preparation of a 10^{-3} M vitamin K_3 solution, 0.28 mL

from the clinical injection solution was taken and diluted with distilled water into 10 mL.[22] Air or oxygen gas was passed for about 20 min to oxidize all of the vitamin and the sulfite present (see section 3.2.). Nitrogen gas was then passed to purge the oxygen from the solution. This procedure was repeated for each solution before use.

Preparation of 0.1 M standard thiosulfate solution

In 1 L of distilled water, 26.8 g of $Na_2S_2O_3$ was dissolved. For its standardization, KIO_3 had to be used. The primary standard of KIO_3 (0.64 g) was dissolved in a 250-mL flask with distilled water, 50 mL of it was taken, and 2 g of KI and 10 mL of 1.0 M HCl were added and titrated with the thiosulfate solution until the color of the solution became light yellow. Then 3 mL of freshly prepared starch was added, and the titration was ended when the solution was colorless.

Preparation of iodine solution and standardization

In 20 mL of distilled water, 20 g of KI and 6.7 g of I_2 were dissolved and then diluted to 500 mL. From the dilution, 25 mL was taken and titrated with standardized thiosulfate solution in the presence of starch.

Preparation of 0.2 M sulfite solution and standardization

In 50 mL of deaerated distilled water, 1.26 g of oven-dried Na_2SO_3 was dissolved. To protect it from air oxidation and to reduce the sulfate present, mercury amalgamated zinc granules were added. For the standardization of the solution, 50.0 mL of 0.048 M I_2, 2.3 mL of HCl, and 10.0 mL of Na_2SO_3 solutions were mixed; the iodine used by sulfite was determined by titrating the excess iodine with standardized thiosulfate in the presence of starch. This solution was standardized after certain periods, when the zinc peak from the Jones reductor appeared in the polarogram.

Preparation of standard vitamin K_3 solution

A 10 mL solution of approximately 10^{-3} M vitamin K_3, in hydroquinone (H_2Q) form (from Libavit K), was prepared. Oxygen was passed for about 20 min to oxidize it into quinone (Q) form. The oxidation may be controlled by taking DC polarograms (see section 3.1.). Nitrogen was passed for about 20 min to expel oxygen. A known portion was added to 10 mL of acetate buffer (pH = 5.5) for a concentration of about 10^{-5} M. To this solution, a known amount (more than 2.5 times the amount of vitamin) of standardized sulfite was added and then warmed up to 45 °C. After it cooled down, it was taken into a polarographic cell and its DP polarogram was taken. The peak height of sulfite was measured at -0.7 V. The quantity of sulfite, which remained after the reaction with vitamin K_3, was determined by additions of standard sulfite. To determine the quantity of the sulfite reacted, the remaining sulfite had to be subtracted from the sulfite added at the beginning. This number was equal to the vitamin K_3 quantity.

Preparation of Jones reductor

About 50 g of zinc granules were first cleaned in 1 M HCl, and then they were left in 0.25 M $Hg(NO_3)_2$ for about 2-3 min so that they were amalgamated with mercury.[25] This amalgam was used for the reduction of $CrCl_3$ into $CrCl_2$, for the elimination of trace oxygen in nitrogen. It was also used to protect the sulfite solution from air oxidation.

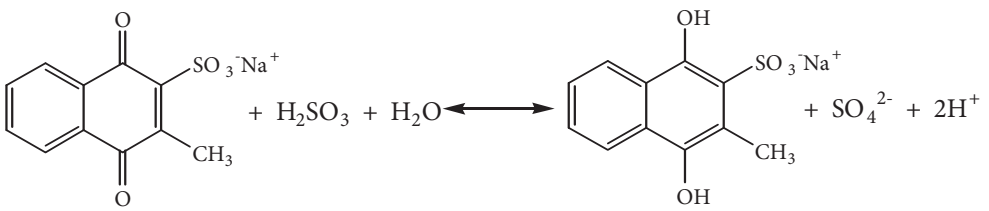
Procedure for indirect determination of vitamin K_3

Through an unknown sample of vitamin K_3, oxygen was passed for about 20 min in order to oxidize both vitamin K_3 (if in hydroquinone form, H_2Q) and sulfite. Excess sulfite was then added and warmed to 45 °C. A certain amount of it was added to a polarographic cell containing acetate buffer (pH = 4.5-5.5) and a known amount of sulfite. The peak height was measured, and standard sulfite was added. From the peak height of sulfite, the vitamin K_3 content was calculated.

Results and discussion

Electrochemical behavior of vitamin K_3

As given in the experimental section, vitamin K_3, in clinical injection solution, contains potassium metabisulfite $K_2S_5O_2$. This enables the vitamin to be kept in a reduced and water-soluble form, and thus it is possible to obtain concentrated solutions of the vitamin. With the addition of sulfite, the quinone (Q) form turns into the hydroquinone (H_2Q) form, as given in Eq. (1). To protect it from air oxidation in the quinone form, sulfite has to be added in excess.



Q H_2Q (1)

For polarographic determination, the peaks must first be identified. For this purpose, 1×10^{-3} M solution was prepared from the Libavit K in H_2Q form, containing sulfite in excess (see section 2.2.1.). In acetate buffer (pH = 4.5-5.5), its DP polarogram was taken and 4 peaks were observed (Figure 1). The first one, at -0.67 V, belonged to sulfite,[26] and the others, at -1.0, -1.2, and -1.45 V, belonged to H_2Q. The peak at about -1.0 V was used throughout the work.

As is known, the nature (cathodic or anodic) of a polarographic peak cannot be evaluated by DPP. For this purpose, direct current (DC) polarography has to be used. The DC polarographic wave for 2×10^{-4} M vitamin K_3 (H_2Q, hydroquinone), taken in the positive direction, had an oxidation wave with a half-wave potential of $E_{1/2}$ = -0.94 V in an acetate buffer with a pH of 5.5 (Figure 2). The DC polarogram of Q, on the

other hand, had a cathodic wave with a half-wave potential of $E_{1/2}$ = -0.94 V, indicating that the oxidation of vitamin K_3 was reversible[27] (Figure 3). Using these polarograms, it is possible to follow the presence of Q and H_2Q individually in solution.

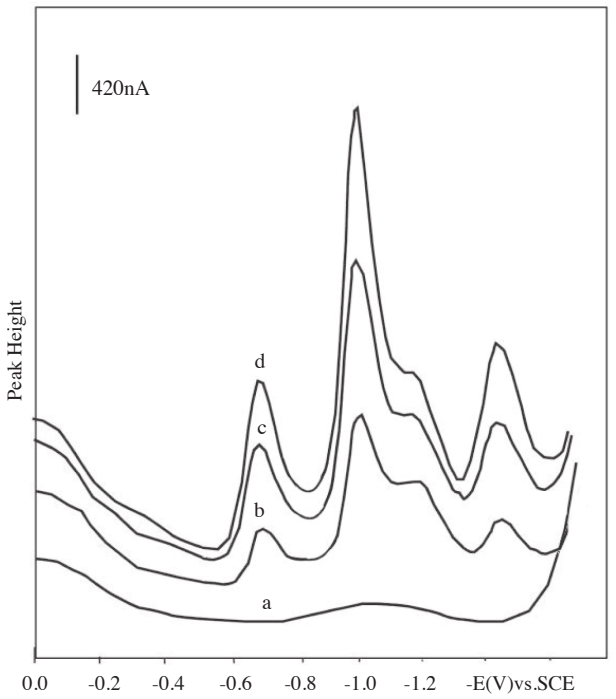


Figure 1. Electrochemical behavior of Libavit K, a) pH = 5.5, 10 mL HAc-NaAc, b) a + 0.1 mL 1 × 10^{-3} M vitamin K_3 (H_2Q), c) b + 0.1 mL 1 × 10^{-3} M vitamin K_3, d) c + 0.1 mL 1 × 10^{-3} M vitamin K_3.

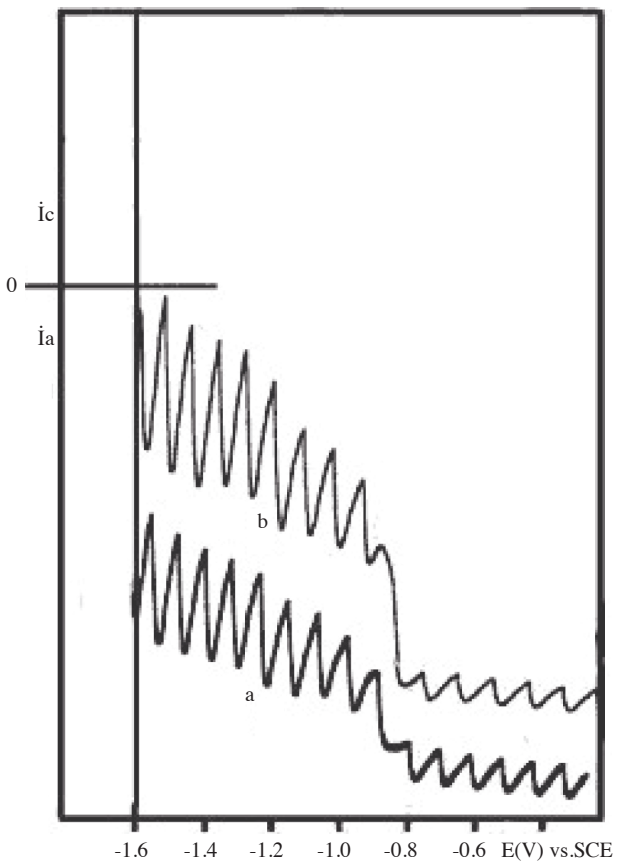


Figure 2. DC polarogram of vitamin K_3 (H_2Q, hydroquinone), a) pH = 5.5, 10 mL HAc-NaAc + 2 × 10^{-4} M vitamin K_3 (H_2Q, hydroquinone), b) a + 2 × 10^{-4} M vitamin K_3 (H_2Q).

Optimum conditions for the oxidation of vitamin K_3

The purity of vitamins found on the market is about 95%-98%, and it is not possible to prepare a standard solution. A standard solution of vitamin K_3 can be prepared using the quantitative reaction between standard sulfite and vitamin K_3 (in quinone form, Q). For this purpose, vitamin K_3 has to be in its oxidized form. As given before, vitamin K_3 was in H_2Q form in a vial (Libavit K); thus, it had to be oxidized into its quinone form. Sulfite in a vial also has to be oxidized, or otherwise it may create problems as a reducing agent. Since the standard reduction potential of sulfate is E^0 = 0.172 V, the reduction potential of Q is E^0 = 0.7 V, and that of oxygen is E^0 = 1.23 V, oxygen can be used for their oxidation quantitatively (Eqs. (2) and (3)). Oxygen

gas has been used for the oxidation of both sulfite and vitamin K_3 in H_2Q form.

$$O_{2(g)} + 2H_2Q \leftrightarrow 2H_2O + 2Q\Delta E = 0.53V \tag{2}$$

$$O_{2(g)} + 2H_2SO_3 \leftrightarrow 2SO_4^{2-} + 4H^+\Delta E = 1.06V \tag{3}$$

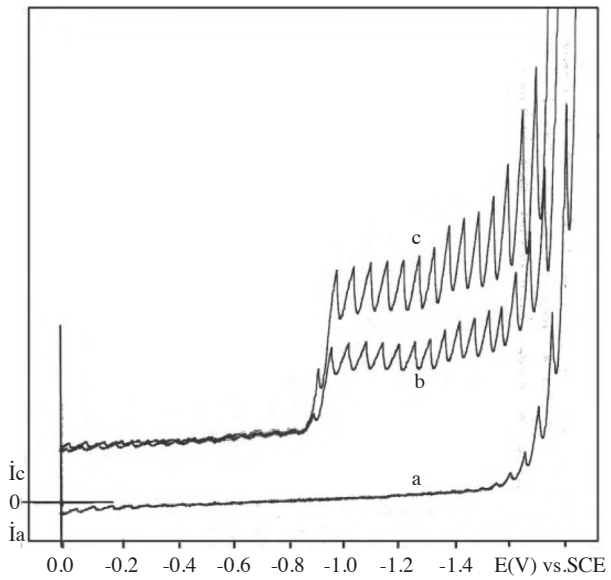


Figure 3. DC polarogram of vitamin K_3 (Q form). a) pH = 5.5, 10 mL HAc-NaAc, b) a + 5 × 10^{-5} M vitamin K_3 (Q), c) b + 5 × 10^{-5} M vitamin K_3 (Q).

To estimate the oxygen gas passing period for the conversion to be complete, 5, 10, 15, 20, or 30 min of passing time were tested in a solution containing 10 mL of 1 × 10^{-3} M vitamin K_3. After each period of time, a DP polarogram was taken in acetate buffer (pH = 4.5-5.5) to observe whether sulfite (from its -0.67 V peak), which was present in the vial, was still present (nitrogen gas was passed before each experiment). It was found that sulfite was completely oxidized when oxygen was passed for 10 min. To follow the oxidation of vitamin K_3, a DC polarogram (see section 3.1.) of this solution was taken in the positive direction. It was found that some H_2Q was still present after 10 and 15 min of oxygen passing time, but after 20 and 30 min of oxygen passing time, no oxidation wave (DC) for vitamin K_3 was observed, indicating that all H_2Q was oxidized. Thus, it was decided to pass oxygen for about 20 min throughout the work.

Optimum conditions for the reaction between sulfite and vitamin K_3

Vitamin K_3 in an unknown sample can be determined by direct and indirect methods. As is known, it is not possible to find a standard solution of this vitamin. Therefore, an indirect method, in which a substance reacts with the vitamin quantitatively, has to be provided. After it is standardized, this solution can be used for direct determination safely. Although the standardization will take a longer time, after it is standardized, the vitamin K_3 content of an unknown sample can be determined in a very short time directly by standard additions, and it may be applied for routine analysis. Since the standard reduction potential of sulfate is E^0 = 0.172 V and

the reduction potential of Q is $E^0 = 0.7$ V, the reaction between sulfite and vitamin K_3 should be quantitative. By following the peak of sulfite in DPP polarograms, it was shown that vitamin K_3 was reduced by sulfite. According to Eq. (1), 1 mol of vitamin K_3 uses 1 mol of sulfite.

To find the optimum conditions, the reaction had to be followed under various conditions. For this purpose, DP polarograms of vitamin K_3 containing sulfite were taken in varying concentrations (4, 3, 2, 1, and 0.1 M) of HCl. It was observed that in these acidic solutions, SO_2 was escaping,[22,26,28] and thus indirect determination of vitamin K_3 with sulfite was not possible in acidic solutions. After testing various electrolytes, it was found that the best medium for the indirect determination of vitamin K_3 was acetate buffer (pH = 4.5-5.5). At higher pH values, the reaction between vitamin and sulfite was not quantitative.

To follow the reaction between sulfite and vitamin K_3, 3.2×10^{-7} M vitamin K_3 (in Q form) and 8.4×10^{-7} M sulfite were added to a polarographic cell containing acetate buffer with a pH of about 4-5. The sulfite concentration was taken to be 2.5 times larger than the stoichiometric amount according to our preliminary studies. In order to accelerate the reaction by mixing, nitrogen gas was purged for 5, 10, 15, 20, and 30 min, and polarograms were taken after each time interval.

As can be seen from Figure 4, when vitamin K_3 was added to sulfite ions, the sulfite peak height at about -0.7 V decreased, and 3 new peaks, at -1.0, -1.2, and -1.45 V, were observed. These peaks belonged to the H_2Q formed from the reaction between sulfite and vitamin K_3 in Q form. The remaining sulfite quantity after the reaction could be determined by standardized sulfite additions. The vitamin K_3 concentration, on the other hand, was calculated using the stoichiometric relation given above in Eq. (1). It was found that under these conditions, the reaction was not quantitative even when 30 min were given. However, when the solution temperature was increased to 45 °C, the reaction was quantitative within seconds. Thus, in all reactions throughout the work, this temperature was used.

Determination of vitamin K_3 in a synthetic sample using indirect method

A synthetic sample containing 0.32 mL of 1×10^{-3} M vitamin K_3 (Q) (3.2×10^{-5} M in cell) was warmed to 45 °C after the addition of 0.6 mL of 1.4×10^{-3} M sulfite (8.4×10^{-5} M in cell) and added to a polarographic cell containing acetate buffer (pH = 4.5-5.5). The polarograms taken are shown in Figure 5. While the vitamin K_3 (Q) peaks were observed at -1.0, -1.2, and -1.45 V, the sulfite peak appeared at about -0.7 V. The quantity of sulfite that remained after the reaction with vitamin K_3 could be determined by the addition of standard 1.4×10^{-5} M sulfite. The vitamin K_3 (Q) concentration, on the other hand, was calculated from the stoichiometric relation between sulfite and vitamin K_3. Using this method, 3.2×10^{-5} M vitamin K_3 (Q) could be determined with high precision as $(3.3 \pm 0.5) \times 10^{-5}$ M for $n = 4$ and a 90% confidence interval. The limit of quantification found was 2×10^{-6} M with indirect determination using sulfite.

According to our former studies,[26] there will only be interference from cadmium, because of the peak overlap with sulfite. However, this interference could be eliminated by the addition of EDTA, whereby the cadmium peak shifts to more negative potentials because of the complex formation.

Direct determination of vitamin K_3 using its standard solution

Standardized vitamin K_3 solution (in Q form) can be used for direct determination in routine analysis with a very simple and fast method, using the peak at about -1.0 V in acetate buffer (pH = 5.5).

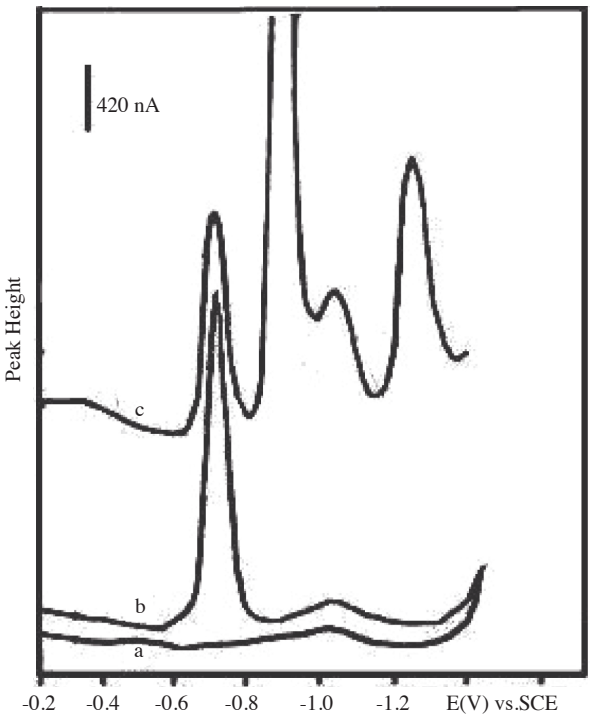


Figure 4. Investigation of the reaction between sulfite and vitamin K_3 (Q). a) pH = 5.5, 10 mL HAc-NaAc, b) a + 8.4 × 10^{-5} M SO_3^{2-}, c) b + 3.2 × 10^{-5} M vitamin K_3 (Q) + 5 min N_2.

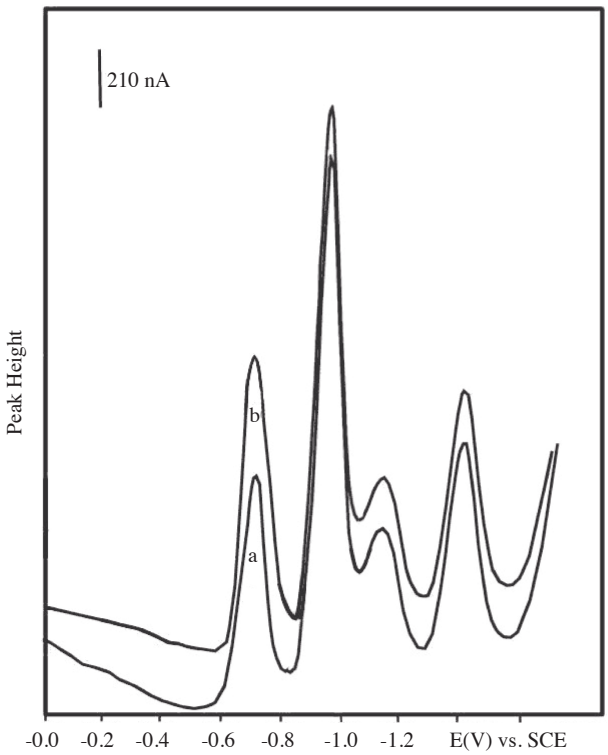


Figure 5. Indirect determination of vitamin K_3 (Q) using sulfite. a) pH = 5.5, 10 mL HAc-NaAc + 8.4 × 10^{-5} M SO_3^{2-} + 3.2 × 10^{-5} M vitamin K_3 (Q) (warmed to about 45 °C and cooled), b) a + 1.4 × 10^{-5} M SO_3^{2-}.

After the same oxidation process (section 2.2.5.) of the unknown sample, a DP polarogram was taken in 0.1 M acetic acid (pH = 5.5) (Figure 6). Vitamin K_3 (Q) had 3 peaks, at -1.0, -1.2, and -1.45 V, in this medium. From the peak at -1.0 V, the quantity of vitamin K_3 was calculated with standard additions using the standardized solution of the vitamin K_3 prepared in section 2.2.5. Using this method, 7.9 × 10^{-5} M vitamin K_3 (quinine, Q) could be determined with high precision as (7.8 ± 0.7) × 10^{-5} M for n = 4 and a 90% confidence interval.

Under the optimal conditions chosen, 0.1 M acetic acid with a pH of 5.5, the cathodic peak current at -1.0 V was linearly proportional to vitamin K_3 concentrations from 5 × 10^{-7} to 3 × 10^{-5} M.

The linear regression equation was:

$$I_p/\mu A = 2.0 \times 10^4 C + 0.0883 (R^2 = 0.998)(n = 4). \tag{4}$$

The LOD and limit of quantification (LOQ) were obtained as 1.5 × 10^{-7} and 5 × 10^{-7} M, respectively, according to the relation $k\times$ SD/b, where k = 3 for the LOD and k = 10 for the LOQ, SD is the standard deviation of the intercept, and b is the slope of the calibration curve. The high sensitivity of DPP is accompanied by very good repeatability. This analytical performance was evaluated with 4 repeated measurements.

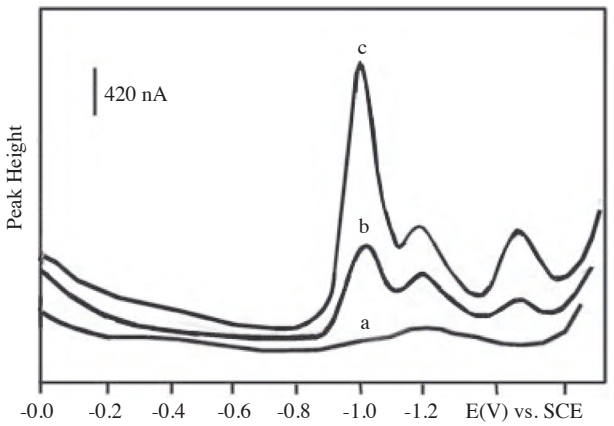


Figure 6. Direct determination of unknown concentration of vitamin K_3 using standardized vitamin K_3. a) pH = 5.5, 10 mL HAc-NaAc, b) a + 0.4 mL unknown vitamin K_3 sample, c) b + 9.92 $\times$ 10^{-4} M vitamin K_3 solution.

Application to real samples using direct method

Direct determination of vitamin K_3 in clinical injection solution

The direct determination method was applied for vitamin K_3 in the clinical injection solution. The vitamin K_3 obtained from the market (Libavit K) contained, according to the formula, 0.02 g of the vitamin in each 2 mL sample. From this, 10 mL of 10^{-3} M solution was prepared, oxygen gas was passed for 20 min, and then nitrogen gas was passed for about 20 min to expel oxygen from the solution. An aliquot of 0.1 mL was taken and added to 10 mL of 0.1 M acetic acid (pH = 5.5) in a polarographic cell. From the peak at -1.0 V, the quantity of vitamin K_3 was calculated with standard additions using the standardized solution of the vitamin. The result found was 0.019 $\pm$ 0.005 g 2 mL^{-1} with a 90% confidence interval (n = 4).

Determination of vitamin K_3 in blood serum

A blood serum (centrifuged) was taken from the medical center of Gazi University. For determination of the vitamin K_3 in the serum, 0.5 mL of blood serum was added to a polarographic cell containing 9.0 mL of HAc-NaAc electrolyte with a 5.5 pH, and a polarogram was taken. As can be seen from Figure 7, no peak for the vitamin was observed, indicating that the level of K_3 was under the detection limit.

In order to demonstrate that vitamin K_3 in low concentrations can be determined in serum medium accurately, a stock serum solution spiked with vitamin K_3 was used. For this purpose, 1.0 mL of blood serum and 0.3 mL of 9.69 $\times$ 10^{-4} M vitamin K_3 were mixed, such that the vitamin K_3 concentration was 2.23 $\times$ 10^{-4} M.

As can be seen from Figure 7, when 0.5 mL of serum sample spiked with vitamin K_3 was added to a polarographic cell, a peak at -1.0 V appeared (curve b). The quantity of vitamin K_3 in this serum was determined by standard additions (Figure 7, curve c), using the standardized solution of the vitamin prepared as in section 2.2.5. The results obtained for the determination of spiked vitamin K_3 in blood serum samples

are given in the Table. As can be seen there, high recoveries for vitamin K_3 were obtained by DPP for blood serum samples.

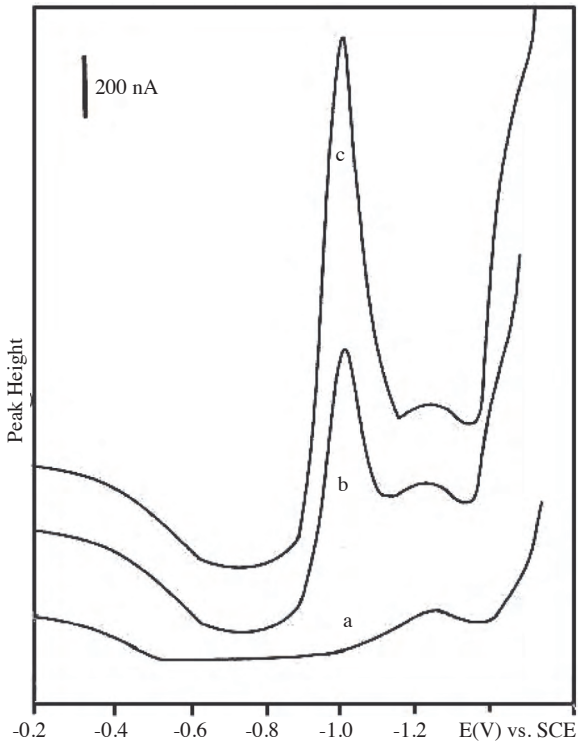


Figure 7. Determination of vitamin K_3 in spiked blood serum, a) 9.0 mL 0.1 M HAc-NaAc electrolyte (pH = 5.5) + 0.5 mL blood serum, b) a + 0.5 mL stock serum solution spiked with vitamin K_3 (2.24×10^{-4} M K_3), c) b + 0.1 mL 9.69×10^{-4} M K_3 (concentration was determined using standard sulfite).

Table. Determination of spiked vitamin K_3 in blood serum.

Sample	Spiked vitamin K_3 (M)	[a]Found vitamin K_3 (M) $\overline{X} \pm$ ts/$\sqrt{N}$	Recovery (%)
Blood serum	1.11×10^{-5}	$(1.09 \pm 0.04) \times 10^{-5}$	98

[a]95% confidence interval (n = 4)

These optimized procedures were also successfully applied for the determination of vitamin K_3 spiked to serum. Recovery experiments were performed in order to evaluate the interference of organic and inorganic components in blood serum. A calibration curve was obtained for vitamin K_3 in serum. The relationship between peak current (I_p) and concentration of vitamin K_3 was rectilinear for the serum. Linear regression analysis of the data gave the following equation:

$$I_p/\mu A = 1.1 \times 10^4 C + 0.128 (R^2 = 0.999)(n = 4), \quad (5)$$

where C is the concentration in mol L^{-1} and I is the peak current in μA. The LOD and LOQ were obtained as 3×10^{-7} and 9×10^{-7} M, respectively. The straight line had a slope of $1.1 \times 10^{-4} \mu$A / mol L, an intercept of 0.128 μA, and a correlation coefficient of 0.999. The high sensitivity of DPP was accompanied by very good repeatability.

Conclusions

The vitamin K_3 solutions on the market are only 95%-98% pure and very expensive. Thus, for direct determination of the vitamin, a standard solution of vitamin has to be prepared using an indirect method. In nearly no investigation mentioned above was this fact taken into account.

In this work, direct and indirect methods for the determination of vitamin K_3 were developed. For indirect determination, the quantitative reaction between sulfite and vitamin K_3 (quinone form, Q) was used. For this purpose, first the optimum reaction conditions were investigated. It was found that at a pH of 4.5-5.5, the reaction between the quinone (Q) form of the vitamin and sulfite was quantitative and very fast when the temperature was 45 °C. The sulfite concentration had to be at least 2.5 times that of the vitamin. A known quantity of sulfite was added to an unknown vitamin K_3 (Q form) solution, and from the peak of sulfite at about -0.7 V, the vitamin K_3 (Q) concentration was calculated. With this method, it was possible to prepare a standard vitamin K_3 solution, and using this solution the vitamin content of the unknown solution could be determined directly. The LOD for direct determination was 1.5×10^{-7}, which was better than the LOD obtained with the indirect method.

The DPP method presented for the quantitative determination of vitamin K_3 allowed accurate determination and was found to be rapid, simple, and highly sensitive. The main advantage of such a procedure is the possibility of determining the concentration of the active component directly from the pharmaceuticals and natural samples without any previous treatment, such as extraction, clean-up, derivatization, or preconcentration, which are tedious, time consuming, and polluting.

The proposed methods were successfully applied to the determination of vitamin K_3 in a clinical injection solution and in blood serum. Contrary to modified electrodes, the results obtained in both methods are very reproducible since, with the use of a dropping mercury electrode, the electrode surface is always new and the behavior of the electrode is independent of its past history.

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