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Direct and indirect methods for the determination of vitamin K_3 using differential pulse polarography and application to pharmaceuticals

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ABSTRACT

Two methods for the determination of vitamin K_3 have been developed. Vitamin K_3 in its oxidized form is determined by direct and indirect methods. Its standard solution was prepared by the indirect method using Ti(III) as reducing agent. For this purpose vitamin K_3 (menadion) in a clinical injection solution, which is in its hydroquinone form in the presence of sulfite, is oxidized with oxygen. In 0.2 M HAc and 0.02 M HCl electrolyte vitamin K_3 and Ti(IV) have reduction peaks at −0.58 V at −0.82 V respectively. The reaction between Ti(III) and vitamin takes place quantitatively in a medium of 0.2 M HAc and 0.002 M HCl. After the reduction, the reaction product Ti(IV) is followed from its polarographic peak at about −0.82 V. The most important result in this work is that, with this method vitamin K_3 can be standardized and after standardization this solution can be used for the direct determination in routine analysis with a very simple and fast method, using only the peak at −0.71 V in 0.2 M HAc medium. Both direct and indirect methods have been used for the determination of Vitamin K_3 in a clinical injection solution. The limit of quantification (LOQ) was 1.5×10^{-6} M and in both methods the detection limit found was 7×10^{-7} M.

1. Introduction

Vitamin K is a cofactor for an enzyme which converts some glutamyl residues in several proteins. These vitamin K dependent proteins play an important role in calcification, and homeostasis [1]. Several studies suggest that vitamin K may reduce bone loss in osteoporotic people and decrease fracture risk.

There are two forms of vitamin K in nature, vitamin K_1 is produced by plants and vitamin K_2 is synthesized by bacteria. Vitamin K_3 contains a naphtoquinone ring and its basic structure is 2 methyl-1,4 naphtoquinone. 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. There are two water soluble vitamin K_3, one is sodium menadiol diphosphate, the other is menadione sodium bisulphite. The physiological activity of menadione is the strongest among the K group vitamins. It shows antitumour and anti-inflammatory activity because of the quinone group in its structure.

Several methods for the separation and determination of vitamin K have been proposed. For the clinical tests sensitive and rapid analytical methods are needed. Spectroscopic, colorimetric and chromatographic methods which are frequently used [2–4] have lower sensitivity when compared with polarographic methods. The electrochemical behavior of menadione has been the subject of various investigations [5–7]. The degradation of vitamin K_3 and vitamin K_3 bisulfite has been studied by electrochemical methods [8–10]. Several polarographic and voltammetric methods have been proposed for the determination of the vitamin. A catalytic wave for menadion determination has been proposed in the presence of KIO_3 [8] and a detection limit of 2×10^{-9} M was obtained. By using cathodic stripping voltammetry [9] it was possible to obtain a detection limit of 1×10^{-8} M. On the other hand by applying differential pulse polarography (DPP) K group vitamins could be determined in CH_3OH and in CH_3Cl_3 solutions [10,11]. A square wave adsorptive anodic stripping voltammetry was used in $HClO_4$ medium and with 10 min of deposition time a detection limit of 1.3×10^{-10} M was achieved [12].

All of these methods need reference standard solutions. However, since the solutions in the market are only 95–98% pure and very expensive, a standard solution of vitamin has to be prepared using an indirect method.

The main purpose of this investigation was to determine vitamin K_3 indirectly so that standard solutions could be prepared and this determination had to be made in the presence of sulfite since it is present in nearly all clinical injection solutions. Sulfite is present in these solutions to protect the vitamin from air oxidation and to keep the vitamin in its water-soluble form. But its presence may create problems during the determination because of its reducing effect. This

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problem has to be solved for the determination of vitamin. In this work differential pulse polarograpy was preferred to use since it is the most reproducible method [13–15] among all electrochemical methods. With the use of dropping mercury electrode the electrode surface is always new and the behavior of the electrode is independent of its past history.

Vitamin K_3 contents in clinical injection solutions were determined polarographically with direct and indirect methods and the results indicate that both of these methods can be used safely for its determination. After preparation of the standard solution, which will take longer time, the direct method can be used for routine analysis in a very short time. These methods do not need any extraction or separation process, they are simple, rapid, accurate and sensitive.

2. Experimental

2.1. Apparatus

For the polarographic measurements a PAR Model 174 A polarographic analyzer system equipped with a PAR mercury drop timer, was used. A Kalousek electrolytic cell with reference saturated calomel electrode (SCE), separated by liquid junction, was used in three electrode configuration. The natural drop time of mercury electrode was in the range 2–3 s (2.2 mg/s). Counter electrode was platinum wire. The polarograms were recorded with a Linseis (Selb, Germany) LY 1600 X-Y recorder, under the conditions of 1 s, a scan rate of 2–5 mV/s and a pulse amplitude of 50 mV.

2.2. Reagents

All chemicals were reagent grade chemicals. Triple distilled water was used in preparation of all solutions.

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

Vitamin K_3 (276.24 g/mol) (Menadione Sodium Bisulfite) was obtained from Libavit K. It contains in each 2 mL vial 0.02 g (0.036 M) Menadione Sodium Bisulfite, 0.006 g potassium metabisulfite ($K_2O_2S_5$), 0.0126 g sodium chloride and 2 ml water. For the preparation of 10^{-3} M vitamin solution, 0.28 mL from the clinical injection solution is taken and diluted with distilled water into 10 mL. Air or oxygen gas is passed for about 20 min to oxidize the vitamin and some of the sulfite present. Then nitrogen gas is passed to purge oxygen from solution. This procedure is repeated for each solution before use.

2.2.2. Preparation and standardization of 0.1 M $TiCl_4$ solution

It is prepared from 15% v/v, d=1.12 g/mL solution, BDH Limited Pool England. From this solution 6.3 mL is taken and it is diluted into 50 mL with distilled water. Since Ti(IV) forms some insoluble oxides in aqueous solution with time it has to be standardized. For this purpose it is titrated with standard $FeCl_3$, after it is reduced into Ti(III) form since there is no direct method for Ti(IV) standardization. The reduction process of Ti(IV) is made with zinc–mercury amalgam and at the end of reduction the color of the solution was blue violet. The end of reduction was followed by taking polarograms with some intervals until there was no Ti(IV) peak. For the standardization of this solution, 5 mL 0.1 M $FeCl_3$ (standardized) in 0.1 M HCl solution is taken, 2 mL HCl is added, so that the acidity in solution was 2 M. The solution is warmed up to 50–60 °C and it is titrated with $TiCl_3$. When the color of solution became light yellow, 2–3 drops 10% KSCN was added. The titration was ended when the red color disappeared and the molarity of Ti(IV) was calculated.

2.2.3. Preparation of 0.1 M $TiCl_3$ solution

The commercial solution of $TiCl_3$ used was from Merck, (15% (v/v) d=1.2g/mL). This solution contains 10% HCl. From this solution 0.86 mL is taken and diluted with distilled water into 10 mL. This solution has to be standardized since $TiCl_3$ can be oxidized easily in the presence of oxygen. A polarogram of newly prepared $TiCl_3$ had a peak of Ti(IV). This solution is left in the presence of zinc–mercury amalgam (Section 3.6) so that all Ti(IV) was reduced and the polarogram had no Ti (IV) peak. This solution was standardized with standard 0.1 M $FeCl_3$ solution as given above. After standardization it has to wait in zinc–mercury amalgam to protect from air oxidation. In this medium it can be stored for months.

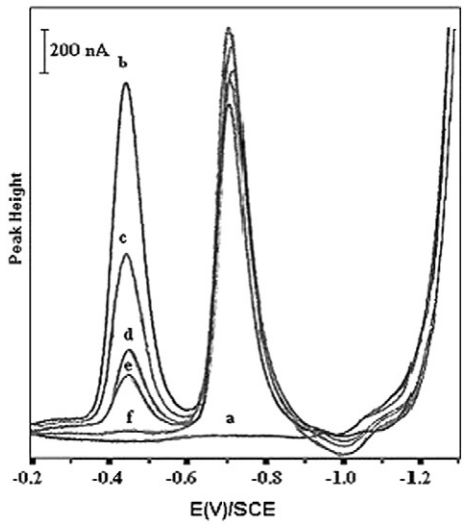

Fig. 1. Effect of nitrogen purging time on the polarographic peaks of vitamin K_3. a) 10 mL 0.2 M HAc and 1×10^{-5} M vitamin K_3 (oxidized). b), c, d, e and f each after 5 min of nitrogen purging.

3. Results and discussion

3.1. Oxidation of vitamin K_3

As given in the experimental section vitamin K_3 in clinical injection solution contains potassium meta bisulfite $K_2S_2O_5$. This enables to keep the vitamin in reduced and water-soluble form and thus it is possible to obtain concentrated solutions of the vitamin. If we try to use directly the polarographic oxidation peak of vitamin K_3 for its determination, the sulfite present as a reducing agent in solution will create problems. Therefore it was decided to oxidize hydroquinone form of vitamin into quinone structure and $SO_3^=$ to $SO_4^=$ with oxygen (Section 2.2.1). The reduction peak of vitamin (at −0.71 V, Section 3.2) can be used for its direct determination. Air, oxygen and hydrogen peroxide has been used for the oxidation of both sulfite and vitamin K. The excess of oxygen was purged out with nitrogen, but when H_2O_2 was used the solution had to be warmed up to purge the excess H_2O_2. Thus it was decided to pass either air or oxygen throughout the work for about 20 min.

3.2. Direct determination of vitamin K_3

A differential pulse polarogram of 10^{-5} M vitamin K_3 in quinone form (first oxidized) prepared as given above (Section 2.1) was taken in 0.2 M acetic acid electrolyte. As can be seen in Fig. 1 there were 2 peaks at −0.46 V and at −0.71 V. The first peak belongs to unoxidized sulfite since it increased with standard additions. By passing nitrogen it decreased and then disappeared after about 10 to 20 min, depending on sulfite concentration. According to our previous work (16), sulfite will form SO_2 in acidic solution (0.2 M HAc) and will be purged out while passing nitrogen.

Vitamin K_3 can be determined directly from the peak at about −0.71 V, after oxidation with oxygen into its quinone form, since it responds very

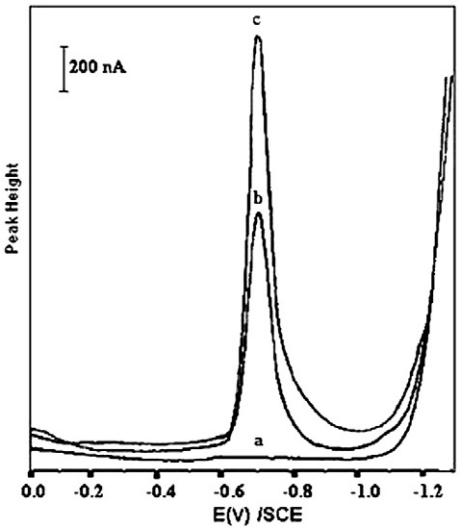

Fig. 2. Determination of vitamin K_3 by standard addition. a) 10 mL 0.2 M Hac. b) a + 1×10^{-5} M vitamin K_3 (oxidized). c) b + 1×10^{-5} M vitamin K_3 (oxidized).

well to the standard additions Fig. 2. The limit of quantification (LOQ) was 1.5×10^{-6} M and the detection limit (signal-to-noise ratio, $S/N = 3$) was 7×10^{-7} M.

The purity of vitamin found in the market (according to several catalogues) is about 95–98% and it is not possible to prepare a standard solution. Thus, an indirect method where a substance, that reacts with vitamin quantitatively, has to be established. After it is standardized using indirect method (Section 3.7) this solution can be used for its direct determination safely. Although the standardization will take longer time, after it is standardized the vitamin K content of an unknown sample can be determined in a very short time and it may be applied for routine analysis.

3.3. Indirect determination of vitamin K_3

According to the reduction potentials, Ti (III) can be used for the standardization of vitamin K, since it will reduce quinone into hydroquinone and itself will be oxidized into Ti (IV) which is electro active. This redox reaction will take place quantitatively because of the large difference in their reduction potentials as given below. According to Turkish codex and British pharmacopoeia, Ti(III) is used for titrimetric determination of menadione. Ti(III) is not electro active but Ti (IV) has a peak at about −0.67 V. Thus, after redox reaction between Ti(III) and vitamin K_3, the quantity of Ti (IV) formed can be determined by measuring its polarographic peak height and then vitamin K_3 content can be calculated.

The reduction potentials are as follows

$$Q + 2e + 2H^+ \leftrightarrow H_2Q \quad E^0 = 0.699V$$

$$TiO^{2+} + 2H^+ + e \leftrightarrow Ti^{3+} + H_2O \quad E^0 = 0.099V$$

And the redox reaction will be

$$2Ti^{3+} + Q + 2H_2O \rightarrow 2TiO^{2+} + 2H^+ + H_2Q$$

3.4. Polarographic determination of Ti (IV)

According to the proposed method, to an unknown vitamin K_3 solution a known amount of Ti(III) has to be added. The Ti(IV) formed from this reaction has to be determined from its peak height using standard additions. However, the polarogram of newly prepared Ti(III) solution contained a Ti(IV) peak which indicates that Ti(III) solution was partly oxidized by air. This peak disappeared when the solution was kept in the presence of zinc–mercury amalgam. Thus, care has to be taken during its preparation and storage.

Vitamin K_3 and Ti(IV) have peaks at about −0.7 V in 0.2 M acetic acid electrolyte so that they cannot be separated in this electrolyte medium. But by the addition of 0.05 mL HCl into 10 mL 0.2 M acetic acid solution in polarographic cell, the peak of vitamin shifted to −0.58 V and the peak of Ti(IV) shifted to −0.82 V enabling their separation and determination.

3.5. Reaction between Ti(III) and Vitamin K_3 in the polarographic cell

The reaction has been studied first in the polarographic cell. For this purpose 0.1 mL 10^{-3} M vitamin K_3, in oxidized form, was added into 10 ml 0.2 M acetic acid and 0.05 mL 12 M HCl (0.06 M HCl). Nitrogen gas was passed for about 10–20 min so that the first peak of sulfite disappeared and the polarogram was taken. Then 0.2 mL 10^{-3} M Ti(III) was added and nitrogen passed for about 5 min as given below, so that the reaction was finished. As can be seen the concentration of Ti(III) was taken two times of vitamin K_3. In later studies it was taken in larger quantities to be on the safer side. After 5 min of waiting period the polarogram was taken and the formation of the Ti(IV) peak at −0.82 V was followed. Its quantity found was smaller than it should be, indicating that under these conditions the reaction was not quantitative. Thus, optimum conditions such as proper acid concentration and waiting period have to be determined.

The acid concentration in polarographic cell had to be 0.06 M for the separation of vitamin and Ti(IV) peaks (Section 3.4). But it seems that this acidity was not proper for a quantitative reaction. Thus the effect of acidity is investigated in a separate reaction cell containing 2.0 mL 1×10^{-3} M vitamin K_3, and 0.05 mL 0.1 M Ti(III). The stock Ti (III) solution contains 10% (v/v) HCl and its concentration will be about 0.002 M in the reaction mixture. It was observed that with this acidity the reaction was quantitative. When more acid was added there was no reaction and as a result no Ti(IV) peak could be observed. On the other hand when the solution was made basic no reaction took place. Thus, it was decided to use the acidity (0.002 M) which is already present in Ti(III) solution.

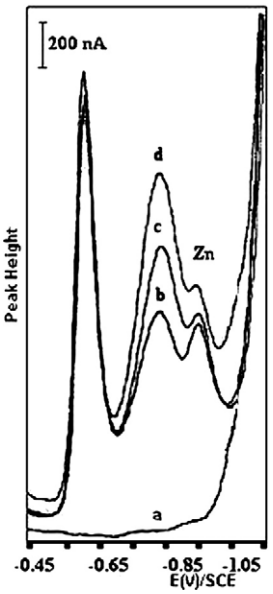

Fig. 3. Determination of vitamin K_3 by indirect method. (Reaction mixture: 2.0 mL 1×10^{-3} M vitamin K_3 + 0.05 mL 0.1 M Ti(III)). a) 10 mL 0.2 M HAc + 0.05 mL 12 M HCl. b) a + 0.1 mL reaction mixture (given above). c) b + 0.1 mL 1×10^{-3} M Ti(IV). d) c + 0.1 mL 1×10^{-3} M Ti(IV).

Table 1
Determination of vitamin K_3 in clinical injection solution using two different methods (labelled amount = 0.02g/2 mL)

Method	No. determinations	Vitamin K (g/2 mL; $\bar{x}\pm ts/\sqrt{N}$)
Present		0.020
Indirect method	5	0.019±0.001
Direct method	5	0.019±0.001

t, 90% confidence interval.

In order to establish the waiting period for the reaction to complete, 45, 35, 25, 15, 10 and 5 min of waiting time have been investigated in a solution containing 1×10^{-3}M vitamin K_3 and 2.5×10^{-3}M Ti(III) in a reaction cell. Each time for each solution 3 polarograms were taken and it was found that 5 min of waiting period was sufficient. Using the above given optimum conditions, the limit of quantification was 2×10^{-6} M and the detection limit was 7×10^{-7} M (signal-to-noise ratio, $S/N=3$).

3.6. Effect of amalgam

In polarograms taken after the reaction of vitamin K_3 and Ti(III) a peak at about −0.94V which belongs to zinc(II) ion was observed. Ti(III) solutions had to wait in zinc–mercury amalgam to protect it from its oxidation by air. The zinc ions are formed from the HCl acid in Ti(III) solution, and also during the reduction of Ti(IV) into Ti(III) solution. Although there was no problem with a small zinc peak, large zinc peak showed overlap with Ti(IV). Thus conditions have to be found so that zinc peak will not be too large. It was observed that with a thick mercury layer, zinc peak was small, but in this case the Ti(IV) formed in Ti(III) solution could be reduced in a long time such as 55–60 min. In order to reduce the time for reduction, zinc granules (not amalgam) have been added into titan (III) solution while nitrogen was passing. In this case the reduction of Ti(IV) was only 5–6 min but as expected zinc peak was very large. When for amalgamation 0.2 M Hg (I) chloride was used so that the mercury film was thinner, the time for reduction was about 8–10 min and zinc peak was smaller as can be seen in Fig. 3. In vitamin K_3 determinations the thin film prepared with Hg (I) chloride was preferably used.

3.7. Standardization procedure

Vitamin K_3, which has to be standardized, is prepared and oxidized as given in Section 2.2.1. The sulfite present is purged out after addition of acid (about 0.002 M) and passing nitrogen. A known amount of Ti(III) is added, the reaction medium is mixed by passing nitrogen. After 5 min of waiting period a small aliquot of this solution is taken and added into the polarographic cell containing 0.2 M acetic acid and 0.06 M HCl. The quantity of Ti(IV) formed is determined by standard additions. The concentration of vitamin K_3 can be calculated from the stochiometric relation.

3.8. Application to real sample using indirect method

Vitamin K_3 sample obtained from the market (clinical injection solution) contains according to the formula 0.020 g of vitamin in each 2 mL sample. A 6 ml solution of 10^{-3}M vitamin K_3 is taken, oxygen is passed through about 10 min to oxidize vitamin and sulfite present, then 2 ml from this solution is taken and nitrogen is passed about 10–20 min so that oxygen was purged out. To this solution 0.05 ml 0.1M Ti(III) (kept in the presence of amalgam) is added and nitrogen gas was passed for about 5 min to complete the reaction. From this solution 0.1 ml is taken and it is added into the polarographic cell containing 10 mL 0.2 M HAc and 0.06 M HCl. Vitamin K_3 concentration has been calculated indirectly from Ti(IV) formed. For five separately prepared samples the result found was 0.019±0.001 g/2 mL with a 95% confidence interval (Table 1). As can be seen there was a good agreement with the quantity given on the label.

3.9. Direct determination of vitamin K_3 in clinical injection solution

After the accurate determination of concentration of vitamin K_3 using the above given method, this solution can be used as a reference standard and direct determination of vitamin can be made for unknown concentration of vitamin K_3.

Direct determination method has been applied for Vitamin K_3 in the clinical injection solution which was used in Section 3.8. This time after the same oxidation process of the unknown sample, a DPP polarogram is taken in 0.2 M acetic acid. From the peak at −0.71 V the quantity of vitamin K_3 is calculated with standard additions using the standardized solution of the vitamin prepared as given in Section 3.7. The result found was 0.019±0.001 g/2 ml with a 95% confidence interval. Thus it was in good agreement (Table 1) with the result obtained using indirect method.

4. Conclusions

Two methods for the determination of vitamin K_3 have been developed. The limit of quantification (LOQ) was 1.5×10^{-6} M and the detection limit (signal-to-noise ratio, $S/N=3$) was 7×10^{-7} M. Direct method could be used if a standard solution was available. In this case the polarographic peak of the vitamin at −0.71 V had to be used and by standard additions its quantity could be determined. On the other hand if there was no standard solution available then the indirect method had to be used. For this purpose standard Ti(III) solution has to be added to unknown solution of vitamin K_3 and the reaction product of Ti(IV) has to be determined from its peak at −0.82 V by standard addition. From stochiometric relation between Ti(III) and vitamin, the quantity will be calculated. With this method it was possible to prepare standard vitamin K_3 solution, and using this solution the vitamin contents of unknowns could be determined directly. Above mentioned direct method does not need any extraction or separation procedure, it is simple, rapid, accurate and sensitive. The results obtained in both methods are very reproducible since with the use of 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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