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Voltammetric determination of cholecalciferol at glassy carbon electrode performed in water-ethanol mixture

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ABSTRACT

To confirm or disprove previous hypotheses, cyclic voltammetry of 0.5 mM cholecalciferol (vitamin D_3) at glassy carbon electrode (GCE) and platinum disk electrode (PtE) in pure acetonitrile and water-ethanol mixture at 50 $mV \cdot s^{-1}$ has been used to investigate the oxidation mechanism. The oxidation occurs in two one-electrone steps. According to calculation of the highest electron density in cholecalciferol molecule which is evidently delocalized over carbon atoms of the three conjugated double bonds (C19, C10, C5-C8) points to part of the molecule involved in oxidation processes. An oxidation peak (at +0.925 V vs. Ag/AgCl) was used to develop direct voltammetric method based on differential pulse voltammetry for the vitamin D_3 determination at GCE performed in 40% ethanol containing 0.1 M $LiClO_4$. Under optimization of analytical procedure, it was found that a composition of the supporting electrolyte used significantly affects a current response of oxidation peak obtained. Satisfactory sensitivity was achieved in the 1:1 water-ethanol mixture containing 0.05 M lithium perchlorate as as supporting electrolyte. The linear range for vitamin D_3 determination was $2.4 \times 10^{-6} - 3.5 \times 10^{-4}$ M with the detection limit of 8.0×10^{-7} M. This work demonstrates a fact that the GCE is suitable electroanalytical device for analysis of various food supplements and medicaments.

Keywords: cholecalciferol; anodic differential pulse voltammetry; glassy carbon electrode; vitamin food supplements

INTRODUCTION

As known, vitamin D belongs to the group of lipophilic vitamins **(Webster, 2012)**. In a narrower sense, it is included into the group of fat-soluble steroid substances liable for increasing of intestinal absorption of calcium and other minerals **(Wang, 2013)**. Vitamin D occurrs in two different forms **(Thacher et al., 2009)** such as vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Generally, cholecalciferol is naturally generated in human body during exposure of skin to UV radiation **(Wacker and Holick, 2013)**. In contrast, the ergocalciferol present in muschrooms is produced by activation of ergosterol at the same radiation **(Bikle, 2014)**.

In case of avitaminosis, sufficient intake of vitamin D-rich food is necessary. Due to low content of vitamin D in foodstuffs usually µg per 100 g **(Sabolová et al., 2016)**, it can be ingested from commercial supplements which were one of the targets of this work. Already mentioned avitaminosis has a direct influence on the absorption of calcium and phosphorus in the body, especially in the proximal section of the small intestine **(Christakos et al., 2014)**. It is related with symptoms of several diseases such as bone thinning and rhinitis in children **(Kutluğ et al., 2017)**. From the medical environmental point of view, several reports appeared in scientific papers refering the fact that deficiency of vitamin D may also cause cardiovascular disease, immunological abnormalities, and cancer **(Zittermann et al., 2012; Lappe et al., 2007)**. From previous paragraphs, it is evident that development of sensitive analytical methods for determination of cholecalciferol is important in several branges such as food quality control, medicine, and pharmacy.

Nowadays, standard analytical method for determination of ergocalciferol and cholecalciferol in foodstuffs ČSN EN 12821 (560047) is used. After saponification from selected foodstuff, vitamins are extracted with a suitable solvent. Determination of these vitamins in suitable sample extracts is usually performed by normal phase semipreparative high-performance liquid chromatography (HPLC) followed by reverse phase analytical HPLC. Subsequent detection is spectrometric in the UV-area. Nevertheless, it is necessary to state that the existing standard method is characterized by some disadvantages such as time analysis including complex sample preparation, high consumption of organic solvents, and high acquisition costs.

Especially, the last mentioned disadvantage represents the main problem for small routine laboratories. For that

reason, the aim of this work was to develop a simple electroanalytical procedure to determine cholecalciferol based on direct anodic oxidation at glassy carbon electrode (GCE) performed in water-ethanol mixture using pulse voltammetric technique, namely different pulse voltammetry (DPV). The main objective of this work was to verify whether the direct voltammetric method, if applied, can achieve the necessary sensitivity required for quantitative analysis of food supplements and become therefore an alternative way.

Scientific hypothesis

Two different reaction mechanisms of anodic oxidation of cholecalciferol were proposed in the literature. Probably just one of these two versions is true. Older hypothesis argues that cholecalciferol is anodically hydroxylated on C8 and C7 which are part of a conjugated system of three double bonds **(Webster, 2012; Canevari et al., 2014)**. In contrast, a completely different reaction mechanism is proposed in a recent study **(Filik and Avan, 2017)**. Authors claim that hydroxylation of C25 located in the aliphatic happens. One of the aims of this work was to select one of these claims as more credible.

MATERIAL AND METHODOLOGY

Chemicals and reagents

Analytical standard of crystalline cholecalciferol was purchased from Merck (Darmstadt, Germany). Anhydrous lithium perchlorate with 99.9% acetonitrile (ACN) from Sigma Aldrich (Prague, Czech Republic), 96% ethanol from Lach-Ner, s.r.o. (Neratovice, Czech Republic), and demineralized water (ρ = 18.3 MΩ·cm; Milli-Q system, Millipore) and were used for preparation of supporting electrolytes (50% ethanol containing 0.05 M $LiClO_4$ and 0.1 M $LiClO_4$ in pure ACN). Due to relatively high chemical stability, only the 0.01 M cholecalciferol stock solution in pure ethanol was stored in a refrigerator at 5 C.

Instrumentation

All electrochemical experiments were carried out in the 20 mL supporting electrolyte at 25 °C. Typical three-electrode system consisting of solid GCE (or platinum disk electrode; PtE), Ag/AgCl/3.0 M KCl (reference) and platinum wire (auxiliary) electrodes was connected to potentiostat Autolab PGSTAT101 from Metrohm (Prague, Czech Republic) operating with software Nova version 1.11.

Pretreatment of glassy carbon electrode

Due to the fact that products of cholecalciferol electrochemical oxidation remain adsorbed on the surface of the GCE, it was necessary to renovate the electrode surface by rinsing with pure hexane and subsequently by polishing on a furry pad with presence of wet alumina powder (particle size 1.0 μm) for 10 s. This time duration is also recommended by the accompanying manual (Metrohm). After subsequent rinsing of the surface by distilled water, the GCE was ready for further electrochemical experiments.

Sampling

Two different food supplements Optisana (calcium + vitamin D) available in Tesco Stores Czech Republic ČR a. s. and PRO formula (calcium, D_3 & K) from Lidl Czech Republic v.o.s. were analysed. Usually eight tablets were dissolved in the supporting electrolyte in a 50 mL volumetric flask and subsequently sonicated for 30 minutes at laboratory conditions. The resultant dispersion was filtered using stacked filter paper. After that, a volume of 10 mL resulting filtrate was inserted into voltammetric cell and analysed by standard addition methods where three 50 μL aliquots of 0.001 M cholecalciferol were used. Analyses of each sample were repeated at least three times.

Procedure

Cyclic voltammetry (CV) of 500 μM cholecalciferol at GCE and PtE in 0.1 M $LiClO_4$ in pure ACN as supporting electrolyte was performed for study of its electrochemical behaviour. Potential window from -0.2 to +1.6 V (or +0.2 to -0.6 V), step potential (E_{step}) 5 mV, scan rate (v) 50 mV·s^{-1}, and five repetitive cycles were chosen as working conditions. Differential pulse voltammetry (DPV) of cholecalciferol at GCE was used under following conditions: the applied potential from 0 to +1.2 V, E_{step} = 5 mV, potential of amplitude (E_{ampl}) 140 mV, interval time (t_{int}) 0.5 s, and v = 50 mV·s^{-1}. All voltammetric measurements were repeated at least 5 times. If not stated, otherwise all changes in the experimental conditions are shown in the legends below the corresponding figures.

Validation of differential pulse voltammetry

Each analytical method is defined by three main parameters, namaly sensitivity, accuracy, and precission. The sensitivty is defined as the lowest detectable concentration of an analyte (limit of detection; LOD) and is given by a slope of corresponding calibration curve (k). From a practical point of view, limit of quantification (LOQ) is a more important parameter than the theoretical value of LOD because it usually represent the lowest value of the calibration curve. Values of LOQ and LOD were calculated according to the following equations LOQ = 10σ/k and LOD = 3σ/k, respectively, where σ is the standard deviation of measurement (n = 5) of the concentration for lowest concetration of the calibration curve.

The accuracy is usually defined as a consistency of repeated measurements under the same conditions and it can be calculed as RSD = σ/$\bar{x}$*100, where $\bar{x}$ is the arithmetic mean of minimally five repetitions.

The precission represents an accordance between the real concentration of analyte and that found by an analytical method used. This analytical parameter is often verified using a model sample (recovery), declared amount, or by comparison with a reference method based on another physicochemical principle.

Statisical analysis

Within the optimization, calculation of p-value (X) to determine statistical significance of evidence was used. The p-value is widely used in statistical hypothesis H testing, specifically in null hypothesis H_0 significance testing. Before performing an experiment, the null hypothesis is chosen for threshold value which is called the significance level of the test, traditionally 5% and

denoted as α. The smaller the p-value, the higher the significance because it tells the investigator that the *H* under consideration may not adequately explains the observation. The H_0 is rejected if any of these probabilities is less than or equal to a small, fixed but arbitrarily pre-defined threshold value α which is referred to as the level of significance.

Within analysis of vitamin supplements, a standard reference analytical method ČSN EN 12821 (560047) did not used for comparison. Due to analysis of only two samples (n = 2), normality test, ANOVA or t-test were not required to apply. In this case, calculation of the recovery (%) for model sample was found to be sufficient.

RESULTS AND DISCUSSION

Electrochemical behaviour of cholecalciferol

One of the main objectives of this study was to confirm or refute the previous allegations regarding to cholecalciferol oxidation mechanism due to the major inconsistencies previously published. Two different electrode reaction mechanisms are listed in the literature, namely hydroxylation of C25 located in the aliphatic chain **(Filik and Avan, 2017)** or hydroxylation of C8 and C7 located in a conjugated system of double bonds **(Canevari et al., 2014; Webster, 2012)**.

Theoretically, the mechanism of the electrode reaction can be predicted according to quantum chemical calculations because every redox process is generally associated with an electron exchange. Knowledges of the distribution of the electron charges in certain molecule can help to determine individual reaction centres. It means that molecular orbitals with an excess of electrons are more easily oxidized than those with a deficiency. Thus, the prediction of cholecalciferol electrochemical behaviour can be facilitated by theoretical calculation of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). However, steric hindrances, especially accessibility of the reaction center to the surface, should be taken into account. Calculated orbitals for cholecalciferol are shown in Figure 1.

Program Gaussian 09 with density functional method B3LYP was used for calculation. Based on the results obtained, it can be predicted that an electrochemical oxidation is delocalized over carbon atoms of the three conjugated double bonds (C8-C19), where the highest current density has been found. Due to this delocalized electron system, it is not possible to decide with certainty which carbon of the conjugate system is oxidized primarily. It is also evident that carbon atoms of the side aliphatic chain (C20-C27) are not energy efficient for electrochemical oxidation.

Cyclic voltammetry as a frequently used analytical technique for study of electrochemical processes has been preferred. Cholecalciferol provided two oxidation peaks at +1.05 and +1.15 V and two reduction peaks at +0.13 and -0.02 V in the back-cathodic scan at PtE in non-aqueous supporting electrolyte (pure ACN). It was confirmed that theese two cathodic signals corresponded to the electrochemical reduction of the resulting oxidation products because no reduction peak was observed for cathodic scan form +0.2 to -0.6 V (see Figure 2). Very similar electrochemical behaviour of cholecalciferol was obtained at GCE at the same conditions. Thanks to the worst charge transfer, only one oxidation (at +1.18 V) and one reduction peaks (at -0.14 V) were observed (not shown). Moreover, it is very important to mention that no reduction peak was obtained in water-organic mixures.

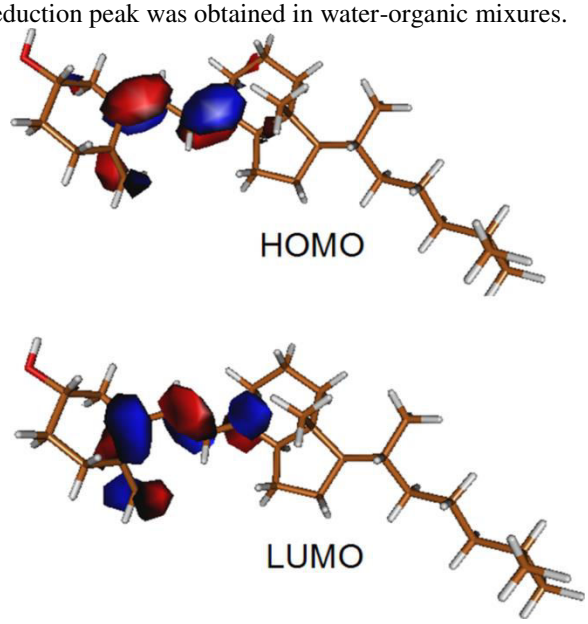

Figure 1 Calculated orbitals for cholecalciferol.

Within scan rate study (not shown), it was found that oxidation peak current linearly ($R^2 = 0.9996$) increased with the square root of the scan (*v*). Moreover, slope (*k*) value 0.419 for linear dependency of the peak current logarithm on log *v* was obtained. For this finding, diffusion-controlled electrochemical oxidation reaction can be accepted.

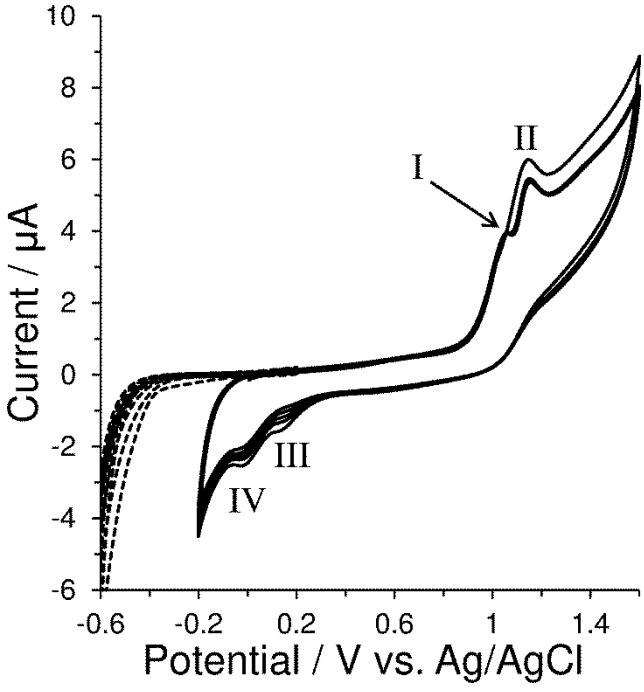

Figure 2 Cyclic voltammetry of 0.5 mM cholecalciferol at PtE performed in 98% ACN containg 0.1 M $LiClO_4$ at 50 $mV \cdot s^{-1}$.

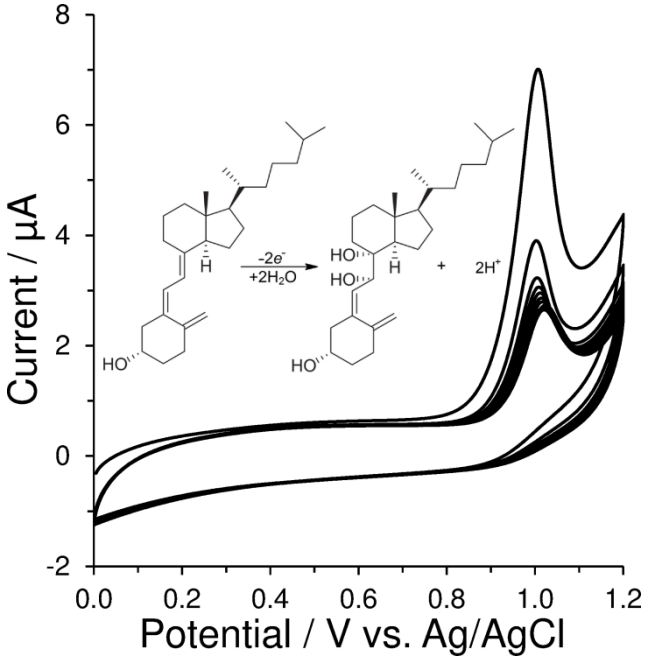

Figure 3 Repettive cyclic voltammetry (10 cycles) of 0.2 mM cholecalciferol at GCE performed in 50% ethanol containg 0.1 M $LiClO_4$ at 50 $mV \cdot s^{-1}$.

Thus, it can be concluded that the cholecalciferol undergoes chemically irreversible anodic oxidation processes at GCE in used water-ethanol mixture (see Figure 3), which corresponds to previously published results **(Chan et al., 2014; Sýs et al., 2016)**.

Thus, it can be concluded that a reaction mechanism (see Figure 3 insert) proposed by **Webster (2012)** is more probable than that from **Filik and Avan (2017)**. However, it seems that electrochemical techniques do not represent sufficient tools to determine the cholecalciferol oxidation mechanism. This should be considered as the identification of the voltammetric oxidation product is a necessary way to design a mechanism with certainty.

Optimization of supporting electrolyte composition

Selection of the working electrode, supporting electrolyte, and setting of the electrochemical teqnique represent the main factors influencing the final analytical parameters of the direct voltammetric method. Due to insolubility of cholecalciferol in strictly aqueous solutions, it was necessary to find optimum content of ethanol and $LiClO_4$. The highest value of anodic peak current (I_p) was obtained when 50% content of ethanol was used. For the higher content of ethanol, evidently lower constant peak current responses were observed (see Figure 4). For that reason, 50% content of ethanol was chosen as the optimum.

Generally, solubility of the lipophilic substance in the water-organic mixture decreases with a higher salt content due to the increasing of ionic strength (I) defined as the sum of all electrically charged particles (positive and negative ions) present in the solution. Nevertheless, it is important to mention that the conductivity (G) of the supporting electrolyte increases with higher salt content. Concentrations higher than 50 mM did not cause peak heights to increase. The above salt content was therefore used as the optimum (see Figure 5).

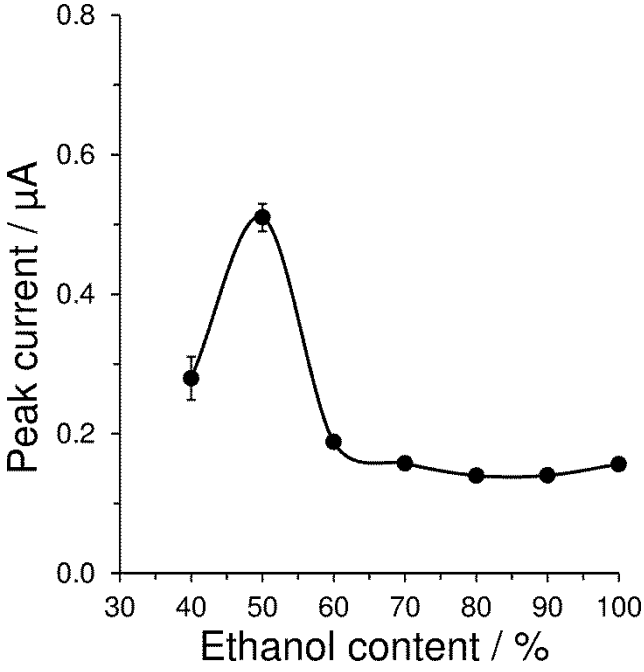

Figure 4 Dependency of peak current on amount of ethanol in supporting electrolyte containing always 0.1 M $LiClO_4$. For concetration of 100 µM cholecalciferol measured at GCE by DPV at E_{step} = 5 mV, E_{ampl} = 25 mV, t_{int} = 0.5 s, and v = 50 $mV \cdot s^{-1}$.

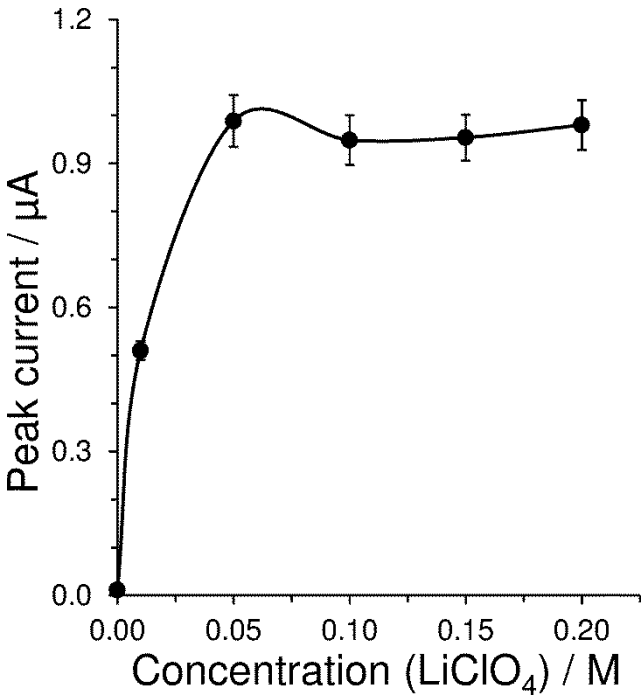

Figure 5 Dependency of peak current on amount of $LiClO_4$ in supporting electrolyte containing always 50% ethanol. For concetration of 100 µM cholecalciferol measured at GCE by DPV at E_{step} = 5 mV, E_{ampl} = 25 mV, t_{int} = 0.5 s, and v = 50 $mV \cdot s^{-1}$.

Electrochemical detection of cholecalciferol using DPV

The optimization of differential pulse voltammetric detection focused on finding the proper pulse amplitude and scan rate, which influence the peak current the most importantly. It was found that setting pulse amplitude higher than 140 mV did not significantly increase the peak current response, because it was calculated that values of corresponding peak current belonged to the interval for *p*-value 0.05.

Analogous behaviour was found for the effect of the scan rate higher than 50 mV·s^{-1}. Typical records of voltammograms together with the corresponding calibration curve are shown in Figure 6.

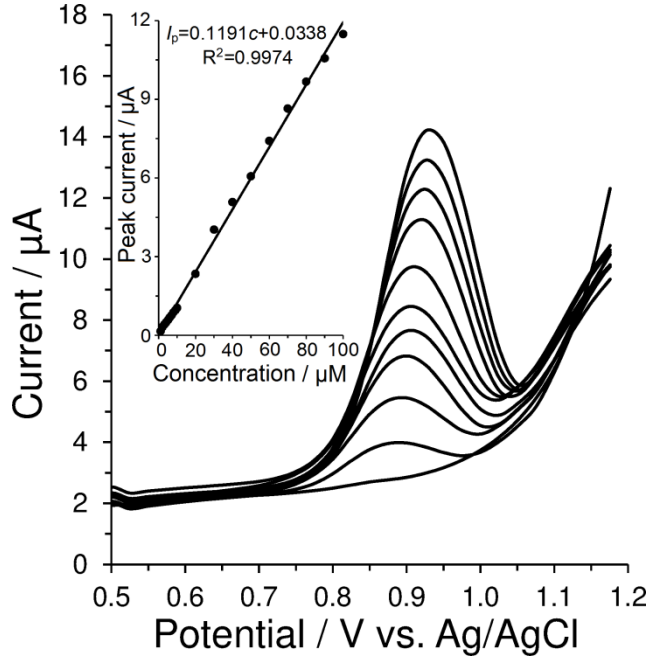

Figure 6 Voltammograms for 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µM cholecalciferol with corresponding calibration curve obtained at GCE. Measured by DPV at E_{step} = 5 mV, E_{ampl} = 140 mV, t_{int} = 0.5 s, and v = 50 mV·s^{-1}.

All analytical parameters are demonstrated together with those of previously published direct voltammetric method (see Table 1). Evidently, this voltammetric method based on direct anodic oxidation in water-ethanol mixture is sensitive for analysis of samples (fish oils, food supplements, pharmaceutical products like Vigantol etc.) with relatively high content of vitamin D_3. Unfortunately, it cannot be applied in the analysis of milk and products derived from it, clinical samples, etc.

Herein, it can be concluded that voltammetric method developed by **Cincoto et al. (2014)** based on anodic oxidation at GCE in water-ethanol mixtures was thoroughly verified. Fortunately, significant progress in linear range of calibration curve was achieved after setting completely new optimum working conditions. Therefore, it seems that some improvement has been achieved in the already published method (**Cincoto et al., 2014**).

Using repeated voltammetric measurements of 10 µM cholecalciferol, value of RSD was calculated. Values 3.2% from peak height and 3.9% from peak area were found. Both these values indicate a satisfactory repeatibility of voltammetric measurements because they are lower than 5%, and are therefore considered optimal at a significance level $\alpha = 0.05$.

Voltammetric determination of cholecalciferol in vitamin food supplements

First, the developed voltammetric method was validated by analysis of 10 mL model 5.0 µM cholecalciferol sample. It was analyzed by the standard addition method (three 10 µL consecutive additions of 0.01 M cholecalciferol stock solution in 98% ethanol). A recovery of 94.9 % was calculated from three repettions.

Two different types of food supplements such as Optisana (calcium + vitamin D) available in Tesco Stores Czech Republic ČR a. s. and PRO formula (calcium, D_3 & K) from Lidl Czech Republic v.o.s. were analyzed by this developed voltammetric standard addition method

Corresponding analyses of food supplements were repeated minimally five times, the evaluated results showed correlation coefficients (R^2) of minimally 0.9992 (not shown). The final results from DPV measurements of these food supplements on the content of vitamin D were compared with declared amounts listed by distributors (see Table 2). With pure consciency, it can be stated that the developed voltammetric method provides satisfactory results, and could be used for routine food analysis in more simply equipped laboratories.

Table 1 Comparison of conventional voltammetric methods developed for determination of cholecalciferol.

Sensor	Techniq ue	Supporting electrolyte	Linearity (µM)	LOD (µM)	References
rotating GCE	DPV	75 mM $LiClO_4$ in Me-OH	2.0 to 200	2.0	**(Hernández Mendez et al., 1988)**
GCE/SiO_2/GO/Ni(OH)$_2$	DPV	0.1 M NaOH	5.0 to 50	0.003	**(Canavari et al., 2014)**
GCE	DPV	0.1 M $LiClO_4$ in 40% Et-OH	0.3 to 4.3	0.12	**(Cincoto et al., 2014)**
GCE/poly(ARS)MWCNTs	SWV	0.05% SDS in 1.5 M AAS	8.0 to 160	5.0	**(Filik et al., 2017)**
GCE	DPV	50 mM $LiClO_4$ in 50% Et-OH	2.4 to 350	0.8	**(Present work)**

Note: AAS; ammonium acetate solution, DPV; differential pulse voltammetry, Et-OH; ethanol, GCE/poly(ARS)MWCNTs; poly (Alizarin red S)/multi-walled carbon nanotubes modified glassy carbon electrode, GCE/SiO_2/GO/Ni(OH)$_2$; amorphous nickel (II) hydroxide particles onto a hybrid material composed of silica and graphene oxide modified glassy carbon electrode, LOD; limit of detection, Me-OH; methanol, SDS; sodium dodecyl sulfate, SWV; square wave voltammetry.

Table 2 Analysis of selected food supplements.

Food supplements	Distributor	DPV (μg per tablet)	Declared amount (μg per tablet)	Recovery (%)
Optisana (calcium + vitamin D)	Tesco Stores Czech Republic ČR a. s.	5.8 ±0.3	5.0	116
PRO formula (calcium, D_3 & K)	Lidl Czech Republic v.o.s.	5.3 ±0.4	5.0	106

Note: Values given as arithmetic means with appropriate standard deviations for five analyses.

CONCLUSION

In this contribution, the cyclic voltammetry of cholecalciferol using GCE as well as disk PtE in water-ethanol mixture and in pure ACN was performed and evaluated to clarify its electrochemical reaction mechanism. Under this study, it was found that cholecalciferol is electrochemically oxidized in which probably correspond to oxidation in delocalized electron system of conjugated double bonds. This is confirmed by the results obtained with quantum chemical calculations.

This paper also represents a secondary study on already developed electroanalytical method to determine cholecalciferol, commonly known as vitamin D_3. In this case, it can be concluded that that certain improvements in analytical parameters were achieved, especially evident extension of the linear range. On the other hand, an analysis of more complex samples can not be done without a time-consuming sample preparation consisting a hydrolysis and subsequent extraction into a suitable organic solvent as it is as is the case with standard analytical method ČSN EN 12821 (560047) based on HPLC.

REFERENCES

Bikle, D. D. 2014. Vitamin D metabolism, mechanism of action, and clinical applications. *Chemical Biology*, vol. 21, no. 3, p. 319-329. https://doi.org/10.1016/j.chembiol.2013.12.016

Canevari, T. C., Cincotto F. H., Landers, R., Machado, S. A. S. 2014. Synthesis and characterization of a-nickel (II) hydroxide particles on organic-inorganic matrix and its application in a sensitive electrochemical sensor for vitamin D determination. *Electrochimica Acta*, vol. 147, no. 1, p. 688-695. https://doi.org/10.1016/j.electacta.2014.10.012

Chan, Y. Y., Yue, Y., Webster, R. D. 2014. Voltammetric studies on vitamins D_2 and D_3 in organic solvents. *Electrochimica Acta*, vol. 138, no. 20, p. 400-409. https://doi.org/10.1016/j.electacta.2014.06.133

Christakos, S., Lieben, L., Masuyama, R., Carmeliet, G. 2014. Vitamin D endocrine system and the intestine. *Bonekey Reports*, vol. 3, no. 1, p. 496-453. https://doi:10.1038/bonekey.2013.230

Cincotto, F. H., Canevari, T. C., Machado, S. A. S. 2014. Highly sensitive electrochemical sensor for determination of vitamin D in mixtures of water-ethanol. *Electroanalysis*, vol. 26, no. 12, p. 2783-2788. http://dx.doi.org/doi:10.1002/elan.201400451

Filik, H., Avan, A. A. 2017. Simultaneous electrochemical determination of vitamin K_1 and vitamin D_3 by using poly (Alizarin red S)/multi-walled carbon nanotubes modified glassy electrode. *Current Analytical Chemistry*, vol. 13, no. 5, p. 350-360. http://dx.doi.org/doi:10.2174/1573411013666170105143113

Hernández-Méndez, J., Sánchez-Pérez, A., Delgado-Zamarreño, M., Hernández-Garcia, M. L. 1988. Voltammetric determination of vitamin D_3 with a rotating glassy carbon electrode. *Journal of Pharmaceutical and Biomedical Analysis*, vol. 6, no. 6-8, p. 737-741. https://doi.org/10.1016/0731-7085(88)80085-2

Kutluğ, S., Kılıç, M., Bilgici, B., Paksu, Ş., Yıldıran, A., Sancak, R. 2017. An evaluation of vitamin D levels in children with seasonal allergic rhinitis during pollen season. *Pediatric Allergy and Immunology,* vol. 28, no. 5, p. 446-451. https://doi.org/10.1111/pai.12728

Lappe, J. M., Travers-Gustafson, D., Davies, K. M., Recker, R. R., Heaney, R. P. 2007. Vitamin D and calcium supplementation reduces cancer risk: Results of a randomized trial. *The American Journal of Clinical Nutrition,* vol. 85, no. 6, p. 1586-1591. PMid:17556697

Sabolová, M., Adámková, A., Kouřimská, L., Chrpová, D., Pánek, J. 2016. Minor lipophilic compounds in edible insects. *Potravinarstvo*, vol. 10, no. 1, p. 400-406. https://doi.org/10.5219/605

Sýs, M., Žabčíková, S., Červenka, L., Vytřas, K. 2016. Adsorptive stripping voltammetry in lipophilic vitamins determination. *Potravinarstvo,* vol. 10, no. 1, p. 260-264. https://doi:10.5219/587

Thacher, T. D., Obadofin, M. O., O'Brien, K. O., Abrams, S. A. 2009. The effect of vitamin D_2 and vitamin D_3 on intestinal calcium absorption in nigerian children with rickets. *The Journal of Clinical Endocrinology & Metabolism,* vol. 94, no. 9, p. 3314-3321. https://doi.org/10.1210/jc.2009-0018

Wacker, M., Holick, M. F. 2013. Sunlight and Vitamin D a global perspective for health. *Dermato-Endocrinology*, vol. 5, no. 1, p. 51-108. https://doi.org/10.4161/derm.24494

Wang, Ch. 2013. Role of vitamin D in cardiometabolic diseases. *Journal of Diabetes Research*, vol. 2013, no. 1, p. 1-10. https://doi.org/10.1155/2013/243934

Webster, R. D. 2012. Voltammetry of the liposoluble vitamins (A, D, E and K) in organic solvents. *The Chemical Record*, vol. 12, no. 1, p. 188-200. https://doi.org/10.1002/tcr.201100005

Zittermann,A., Borgermann, J., Gummert, J. F., Pilz, S. 2012. Future directions in vitamin D and cardiovascular research. *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 22, no. 7, p. 541-546. https://10.1016/j.numecd.2012.02.004

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Conflict of interest:

All authors declare no conflict of interest.

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