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Authors: Xu Wang, Kefeng Li, Liping Yao, Chunling Wang, Ann Van Schepdael

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Recent advances in vitamins analysis by capillary electrophoresis

Xu Wang[a], Kefeng Li[b], Liping Yao[c], Chunling Wang[a], Ann Van Schepdael[d]

[a] Key Laboratory of Food Nutrition and Safety, Ministry of Education, China ;College of Food Engineering and Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, China

[b] School of Medicine, University of California, San Diego, 92103, California, United States

[c] Department of Pharmacy, Xinjiang Production and Construction Corps Hospital, Urumqi, Xinjiang, China

[d] Pharmaceutical Analysis, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven - University of Leuven, Belgium

Correspondence: Professor Dr. Ann Van Schepdael, Pharmaceutical Analysis, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, O&N 2, PB 923, Herestraat 49, B-3000 Leuven, Belgium

Email: ann.vanschepdael@kuleuven.be

Phone: +32-16-323443

Fax: +32-16-323448

Abbreviations: carbon fiber micro-disk bundle electrode (CFMBE); dispersive liquid–liquid microextraction (DLLME); folic acid (FA); Glassy carbon (GC); niacinamide (NA); Riboflavin (RF);pentaerythritol diacrylate monostearate-ethylene dimethacrylate (PEDAS-EDMA); pressure-assisted field-enhanced sample injection (PA-FESI); tocopherols (TOHs).

Highlights

- Vitamin analysis by capillary electrophoresis reviewed from mid-2007 until mid-2017
- Overview of electrophoretic modes, detection and sample concentration approaches
- Table summary of pharmaceutical, dietary supplement and biological applications

Abstract

Vitamins are essential molecules required for human metabolism such as energy production, immune system and cell formation. Accurate vitamin analysis is critical for disease diagnosis, nutrients assessment, as well as food and drug production. This review gives an overview of the recent developments in the use of capillary electrophoresis combined with different detection techniques for the analysis of vitamins. The period covers mid-2007 until mid-2017. Different separation modes are discussed for the analysis of water-soluble vitamins and fat-soluble vitamins. On-line sample concentration for sensitive analysis is also described. The applications include the analysis of pharmaceutical, dietary supplement and biological samples.

Key words: Vitamins; Capillary Electrophoresis; Review

1 Introduction

As is well known, vitamins are a group of essential compounds for the development and normal growth, self-maintenance and functioning of the human and animal body. According to their solubility, they are classified into two main groups: water-soluble and fat-soluble vitamins. The group of water-soluble vitamins consists of eight vitamins collectively known as B-complex vitamins plus vitamin C (ascorbic acid) [1-3]. The fat-soluble vitamins include the vitamins A, D, E, K. These vitamins play specific and vital functions in metabolism, and their lack or excess can cause health problems. With the exception of vitamin D, the vitamins cannot be produced within the body and should be obtained from the diet or via pharmaceutical preparations. Therefore, health care products with vitamins are very common in modern food and pharmaceutical industry [4]. Some vitamins can also be used as biomarkers for certain diseases. For example, a deficiency of fat-soluble vitamins is typical for children with cystic fibrosis [5]. Therefore, a sensitive and reliable analysis of vitamins in different sample matrices is crucial for health care industry and disease control.

Internationally accepted conventional analytical methods for vitamins analysis mainly rely on microbiological assay and immunoassay. However, they are usually labor intensive and time consuming and mostly do not allow the simultaneous determination of multi-vitamins. Several analytical methods have been developed for the analysis of vitamins. HPLC is one of the most common techniques for separating vitamins in a variety of products due to its high selectivity and sensitivity [6-8]. However, this method usually requires a larger sample volume with much more solvent consumption. Nowadays, capillary electrophoresis (CE) as an alternative tool to HPLC offers unique advantages due to its short analysis time, low reagent cost, and minimal

sample requirement [9]. The sensitivity could be a disadvantage for CE in the analysis of vitamins due to the short light path. Currently, various detection formats can be coupled directly to CE, including UV absorbance, laser-induced fluorescence (LIF), electrochemical (EC) and MS detection, which makes CE a powerful tool to meet different requirements [10].The methods of vitamins analysis by CE have been covered in food, pharmaceutical and biological samples. Most of the developed methods based on CE-UV mainly concern the assessment of vitamins in pharmaceutical and food products due to the relatively high analyte concentration [11]. UV detection is also a good choice for simultaneous determination of several vitamins. However, the application of CE-UV was limited due to unsatisfactory detection sensitivity owing to its short light path and small sample volume, especially when biological samples are tested. To improve the detection sensitivity of CE, sensitive detectors and/or on-line preconcentration methods are usually applied in CE analysis [12].

LIF is an extremely sensitive detection method for CE, which is about 1000 times more sensitive than the traditional UV detector [13]. It is only suitable for analytes containing a fluorophore like riboflavin (vitamin B_2). Chemiluminescence (CL) detection is considered one of the most sensitive detection schemes. Its convincing advantage over fluorescence detection is that it does not require a bulky light source. The CL detector is limited in its applicability by the lack of CL reactions for many compounds. Electrochemical detection coupled with CE has been getting more attention due to its high sensitivity and good selectivity to electroactive analytes such as ascorbic acid (vitamin C). With electrochemical detection however, the high separation voltage could interfere with the detection of the electrochemical signal, and the contamination of the electrode surface might also pose problems in the analysis of real samples. MS detectors have a higher sensitivity and selectivity with the added function of structural identification. However, this requires expensive instrumentation, and method development in CE-MS is restricted to the use of volatile buffers [14]. MEKC can also be coupled with MS by using

volatile surfactants, as shown by Hernandez-Borges's group [15].

In the meantime, various types of extraction to enrich the analytes in the sample are employed to increase the sensitivity of the method [16]. However, these methods are usually time consuming and may lead to the loss of analytes during sample preparation steps. On-line pre-concentration techniques do not have these disadvantages and can be successfully used for electrophoretic separation, immediately after a parameters optimization procedure [17]. Highly sensitive detectors could thus be combined with on-line pre-concentration techniques for highly sensitive analysis.

Water-soluble vitamins such as vitamins B or C can be easily charged and can be separated using capillary zone electrophoresis (CZE) [18]. Micellar electrokinetic chromatography (MEKC) was mostly used for simultaneous separations of several water-soluble vitamins due to the improved selectivity [19,20].

Fat-soluble vitamins which are neutral and have poor water-solubility need to be separated by a chromatography-based method (MEEKC/CEC). On the other hand, the use of multiple separation modes is reported for the simultaneous separation of water and fat-soluble vitamins which have diverse properties.

This review, as a summary of vitamins analysis by CE, gives an overview of the recent developments and applications of CE combined with different detectors using various separation modes over the period from mid-2007 to mid-2017. The various types of samples in which vitamins have been determined, are listed in Table 1, together with the respective sample pretreatment, separation mode, buffer, detection type and obtained sensitivity.

A review regarding the application of capillary electrophoresis to the analysis of vitamins in food and beverages was presented by Trenerry [21].

2 Sample preparation

Sample pretreatment steps are very important for the analysis of vitamins.

In cells and tissues, vitamins can easily form complexes with phosphate groups (thiamine mono-, di- and triphosphate, nicotinamide adenine dinucleotide phosphate, etc.). Moreover, free thiamine, nicotinamide and nicotinic acid are often bound to proteins, and therefore acid hydrolysis with heat treatment is sometimes required to liberate them to the free form. Several procedures have been presented for the extraction of water-soluble vitamins. These are acid hydrolysis or enzymatic treatment, and their combination [22-24]. An alternative, in-capillary enzyme reaction method for vitamin extraction has been developed by H. Okamoto et al. [25]. Several extraction procedures including more drastic conditions (0.1 N HCl at 90 °C for 5 h) were used in order to obtain detectable levels of vitamin C and some vitamins from the B group using CE-UV in multivitamin preparations. There is no significant degradation effect on these compounds with high temperature under a N_2 atmosphere [26].

The determination of small amounts of vitamins in biological samples like human urine and serum is quite complex. Sample enrichment is necessary to extract the compounds from such a complicated matrix. Solid phase extraction (SPE) combined with protein precipitation with acetonitrile was developed for vitamin C extraction from human urine [27]. The optimal extraction conditions were reached using columns with hydrophilic–lipophilic balance (HLB) cartridges and elution with acidified water (pH 2.87).

Fat-soluble vitamins are a group of compounds fairly sensitive to high temperatures，and a liquid–liquid extraction (LLE) was developed for the extraction of fat-soluble vitamins from feed [28]. Basic ethanol was used to extract the vitamins and the supernatant was mixed with n-hexane. Subsequently, the hexane phase was evaporated to dryness in a water bath at 45 ºC and the residue was resuspended in 100 µL of microemulsion for MEEKC analysis. Considering the lower concentration of fat-soluble vitamins in human serum, a dispersive liquid–liquid microextraction (DLLME) was developed by the same group [27]. The optimal serum sample purification and

isolation of vitamins was obtained with the use of microextraction dispersion based on acetone as a dispersant and protein precipitant, and dichloromethane as the extracting reagent.

In some cases, enzymatic treatment would be required to liberate vitamins from the matrix. A single enzyme treatment with α-amylase was developed for folic acid (FA) in fortified instant fried Asian noodles with analysis performed by CZE. Traditionally, folate extraction has been performed using the tri-enzyme extraction technique with α-amylase, protease and folate conjugase (pteroylpoly-γ-glutamyl hydrolase) [29]. In fact, it is not always necessary to perform all three enzyme treatments for every food item and the conditions of the enzyme treatment will be different for each type of food [30]. In instant noodles, without the need for using either protease or conjugase, α-amylase alone was used and a complete recovery of all the folic acid was achieved. Sample extraction involved homogenization with running buffer for 1 h at ambient temperature. The mixture was adjusted to pH 7.0 prior to the addition of the enzyme solution and 1 h incubation at 65 °C.

3 Detection techniques used in the analysis of vitamins

3.1 Fluorescence detection

The analysis of vitamins by fluorescence detection can be achieved by direct analysis for compounds with a natural fluorophore or through a derivatization method. Riboflavin (RF), commonly called vitamin B_2, is a natural fluorophore. A new CE system with in-column optical fiber LIF detection was developed for the analysis of RF in beverage, green tea and urine samples. The optical fiber was inserted directly into the tail end of the capillary to avoid the light reflection and scattering from the capillary surface and decrease the background noise [31].

Cai et al [32] developed a CE-LIF method with simultaneous derivatization procedure for the analysis of FA and niacinamide (NA). Fluorescein isothiocyanate (FITC) was used as derivatization reagent and the reaction was

performed under basic condition according to [33]. In this study, NA and FA were derivatized by mixing vitamin solution with 5 mM FITC solution (1:1, v/v) at pH 9.3. A high molar ratio of reagent/sample (≥20) was required to maximize the yield. The reaction was performed in the dark at 43°C for 2 h and then 6 h at room temperature.

A microchip capillary electrophoresis (MCE) method with LIF detection combining field-amplified stacking (FASS) and reversed-field stacking was developed for efficient and sensitive analysis of niacinamide in functional drinks [34]. The sample derivatization was performed by mixing with 100 μM of sulfoindocyanine succinimidyl ester (Cy5) 1:1 (v/v) at room temperature in the dark. The maximum yield was obtained by using 4 h of reaction time and 20 mM borate buffer (pH 8.6) as the dilution buffer. The issue of the short separation distance in MCE was solved by using the reversed-field stacking conditions. In this condition, the concentrated sample zone was pushed back to the injection cross within an appropriate distance, which could give analytes a longer time for boundary enrichment and lengthen the effective separation distance, thus enhancing the enrichment effect of FASS and improving separation efficiency. The separation could be achieved within 4 min and the sensitivity was improved 236-fold for NA compared with the method without on-line concentration technique.

Non-aqueous capillary electrophoresis (NACE) has proven to be very useful in the analysis of compounds which are weakly soluble in water or of compounds with similar electrophoretic mobility in water. A NACE method with on-line UV and fluorescence detection was used to separate different tocopherols，namely α-, (β + γ)-, and δ-tocopherol in vegetable oil [35] (see Figure 1). Thanks to the fluorescence detection, the tocopherol signals are free of interferences, and are easily detected. However, no signals corresponding to tocopherols were observed in UV detection due to the low sensitivity. In this study, β- and γ-tocopherol appeared as a single peak due to their equal mobility.

3.2 Electrochemical and Electrochemiluminescence detection

Electrochemical detection (ECD) coupled with CE has gained more attention due to its high sensitivity and selectivity for electroactive analytes. ECD can be used to detect endogenous electroactive substances from femto- to attomole levels in single cell analysis by using CE [36]. ECD must be isolated from the CE separation in order to adapt the ECD potentials (mV) and currents (nA–pA) with much larger CE voltage (kV) and current (μA) [37]. This can be accomplished by off-column and end-column protocols. End-column is the most popular and small bore capillaries, usually 25 μm ID, are commonly used.

The working electrode is very important in the development of CE–ECD. Many kinds of working electrodes have been used, such as a platinum electrode [38, 39], carbon fiber bundle-Au/Hg dual electrode [40], gold/mercury amalgam microelectrode [41], carbon disk electrode [42], and carbon fiber micro-disk bundle electrode (CFMBE) [43-45].

Ascorbic acid (known as vitamin C, AA), as electroactive substances in vitamins, usually produces an obvious current response at a carbon or glassy carbon electrode. The potential applied to the working electrode directly affects the sensitivity, detection limit and stability of this method. CE-ECD has been successfully used for the determination ascorbic acid in grapefruit juice [46]. A hydrodynamic voltammetry experiment was performed to obtain optimum detection. An applied potential of +0.95 V was selected for high signal and low background current.

Single-cell analysis is interesting to the biological, medical and pharmaceutical fields and it has represented a trend in analytical science. A homemade carbon fiber micro-disk bundle electrode has been described for the direct determination of AA in individual rat hepatocytes based on CE-ECD. Individual rat hepatocytes were injected into a fused-silica capillary and lysed by 0.1% sodium dodecylsulfate (SDS) as cell lysis solution. The detection potential was 0.80 V (vs. saturated calomel electrode (SCE)). The CFMBE was

more friendly to the environment because it was free from mercury, and it was simple in terms of fabrication procedure and convenient in detection [47].

CE with ECD at a CFMBE was developed by the same group to determine ascorbic acid in individual rat peritoneal mast cells (RPMCs) [48]. In this study, a self-made holder made of foam was used to keep the capillary from swing, which assured the stability of the baseline of the electropherogram. The single cell was lysed completely within 5 s using the 0.1% sodium dodecylsulfate (SDS) cell lysis solution together with a lysis voltage of 2 kV.

The detection limit of a common electrode is usually 1×10^{-6} mol L^{-1}. It is difficult to determine a small concentration of AA by CZE-ECD with this sensitivity. It is well known that chemically modified electrodes [49-51], based on the incorporation of a catalyst or a redox mediator, have improved the sensitivity and therefore extended the application of CZE-ECD in trace analysis.

A novel chemically modified electrode was developed by using polyethylene glycol (PEG) and Cu_2O as double modifiers into conventional carbon paste for AA analysis in beverage [52]. The EC detection response of AA increased by adding PEG into the Cu_2O carbon paste modified electrode compared with carbon paste electrode modified with only PEG or Cu_2O.

Carbon fiber microelectrodes (MEs) or modified MEs have typically been used in single-cell analysis. The development of improved micro-biosensor fabrication techniques and various modified materials will enhance sensitivity and selectivity when these novel micro-biosensors are used as the detectors of CE in single-cell analysis.

Metal nanoparticles have emerged as a novel type of compound in chemistry, physics, biology, medicine, and material sciences due to their unique optical, electrical, magnetic and catalytic properties [53]. Platinum nanoparticles were electrodeposited onto the carbon fiber micro-disk electrode (PtNPsME) as electrochemical detector in CE for the analysis of ascorbic acid. The life-time of the modified electrode was more than one week since the

PtNPs on its surface were immobile and were not easily polluted [54].

Electrochemiluminescence (ECL) is another attractive detection method for CE. The electrogenerated tris(2,2'-bipyridine) ruthenium(II) ($Ru(bpy)_3^{2+}$) chemiluminescent reagent was used to enhance the ECL detection sensitivity. A novel method to detect AA was developed by combining CE with ECL detection based on $Ru(bpy)_3^{2+}$ in individual rat hepatocyte cells [55].

Luminol is also a widely used CL reagent. A method based on MCE with CL detection was developed for the determination of AA in single cells [56]. In this study, pre-column derivatization of sample is not required. Cell injection, loading, lysing, electrophoretic separation and CL detection were integrated onto a simple cross microfluidic chip. A single cell was loaded in the cross intersection by electrophoretic means through applying a set of potentials at the reservoirs. The docked cell was lysed rapidly under a direct electric field. The intracellular contents were separated within 130 s by MCE. CL detection was based on the enhancing effect of AA on the CL reaction of luminol with $K_3[Fe(CN)_6]$. Maximal CL signal was obtained with luminol at 2.5 mM in the running buffer and $K_3Fe(CN)_6$ at 0.8 mM in the reaction buffer at pH 12.5. The addition of NaBr to the running buffer increased the CL intensity. However, the reason for this was not clear.

3.3 Mass spectrometry

Mass spectrometry (MS) as a general detector is able to detect analytes without chromophore or fluorophore, on condition that the analytes can be ionized. The coupling of CE with MS provides a powerful tool due to its sensitivity and selectivity. The most frequently implemented MS ionization modes include electrospray ionization (ESI), matrix-assisted desorption/ionization and inductively coupled plasma ionization. The sheath liquid interface is the most robust set up while the dilution effect of the analytes has to be taken into account. There is no dilution effect when interfacing CE with a sheathless interface. Compared to the sheath liquid based interface, the low flow sheathless interface significantly improves ionization efficiencies as

well as reduces ion suppression of co-migrating analytes. Tandem mass spectrometry (MS/MS, with triple quadrupole, QqQ) is much more reliable since each analyte can be determined by two MS signals i.e. qualifier and quantifier. An on-line CE-ESI-MS/MS method was developed for the analysis of nine vitamins B in pharmaceuticals and dietary supplements [57]. The multiple reaction monitoring (MRM) mode was used for the simultaneous determination of nine vitamins B through the precursor-product ion transitions with cetirizine dihydrochloride as internal standard. The fragmentor voltage and collision energy were optimized to reach high MS intensity of the precursor and product ions, respectively. A coaxial sheath liquid with composition of 0.1% formic acid in 50% methanol was used at the interface of the CE-MS. The sheath liquid was co-injected with a syringe pump at a flow rate of 8 μL/min. The pressure of the nebulizing gas (N_2) flowing through the outer capillary channel was set to 10 psi. The drying gas temperature was 300 ºC with a flow rate of 5 L/min. The capillary voltage in the MS detector was set to 5 kV. The optimal protruding length of the CE capillary from the interface was about 0.2-0.3 mm.

Inductively coupled plasma mass spectrometry (ICP-MS) provides the advantage of a low detection limit for metal containing compounds. Instead of the determination of total metal content by ICP-MS, the separation of metal-containing compounds with the use of CE could be interesting. A CE-ICP-MS method was developed for the analysis of cobalamin in nutritive supplement and foodstuff [58]. Cobalamin contains a transition metal in its structure, therefore the analysis could be achieved by ICP-MS through the determination of Cobalt at *m*/*z* 59. A commercial interface of CE and ICP-MS (CEI-100) was used for a stable electrical connection. The nebulizer worked in the self-aspiration mode at a flow rate of around 5.4 μL/min. A detailed description of the CEI-100 capillary electrophoresis interface (CETAC, Omaha, NE) was given in a previous paper [59].

4 Different separation modes used in the analysis of vitamins

4.1 Micellar electrokinetic chromatography

Micellar electrokinetic chromatography has been used to separate both ionic and neutral analytes which are difficult to separate by CZE. The presence of micelles of surfactants in the background electrolyte (BGE) acts as a “pseudostationary phase”, which leads to both hydrophobic and electrostatic interactions with solutes. Therefore, the MEKC technique is applicable to the separation of both water-soluble and fat-soluble vitamins.

UV detection is one of the most used detection techniques in MEKC. A MEKC method with UV detection was developed for the simultaneous determination of three fat-soluble vitamins including ergocalciferol (vitamin D_2), α-tocopherol (vitamin E) and retinol palmitate (vitamin A) in multivitamin tablets and vitamin E soft capsules. In this study, three on-line concentration methods namely sweeping, field-enhanced sample injection and pressure-assisted field-enhanced sample injection (PA-FESI) were used to improve the detection sensitivities of the analytes. PA-FESI applied a positive pressure to counterbalance the reverse EOF in the capillary column during sample injection, which provided a little advancement in the concentration efficiency for the vitamins compared with the other two methods [60].

The stacking mechanism applies only to charged analytes, while sweeping preconcentrates both charged and neutral molecules. A MEKC method with field-amplified sample stacking (FASS)–sweeping was developed for the analysis of five vitamins B in bacterial growth medium [61]. A longer injection time resulted in the decrease of separation efficiency. The strongest signal amplification was obtained with an injection time of 30 s while 10 s can give a good compromise between signal amplification and separation efficiency. Enhancement factors obtained in the study reached up to 40-fold in comparison to CZE using a standard hydrodynamic plug (2 s, 0.5 psi).

Karamanos developed a MEKC method for analysis of AA in tear fluid of a healthy subject [62]. Tears were collected with Schirmer strips, extracted with a

low-pH phosphate buffer, centrifuged through membrane filters and an antioxidant was added. They were stable at −70°C for 15 days.

Zhang developed a simple MEKC method for the determination of folic acid, nicotinic acid and riboflavin in protein containing matrices from monoclonal antibody manufacturing processes [63]. This MEKC method was compared to a CZE method using the same running buffer containing no SDS. In CZE conditions, riboflavin migrated at the same time as the large matrix peak although both CZE and MEKC modes provided good baseline separation for the standards. The UV spectrum of all the analytes of the mixture was taken in the range of 190–300 nm to determine the best wavelength for detection. The 210 nm wavelength was chosen for all the experiments based on the overall response data.

The determination of several water-soluble vitamins is challenging. A MEKC method was developed for the analysis of eight water-soluble vitamins in energy and sport drinks [64]. Different surfactants including SDS, sodium cholate and sodium deoxycholate were tested. Of all surfactants, the optimal separation was observed with SDS. Vitamins B_3 (nicotinamide) and B_{12} could be resolved in the MEKC mode instead of co-migrating with the EOF peak in CZE with a borate buffer at pH 8.5.

The separation and determination of ten water-soluble vitamins was achieved by MEKC in a single run by da Silva [65]. A 2^3 factorial design was used to optimize the determination of the water-soluble vitamins. The stability test was performed dissolving the vitamins in the BGE (2.0 % SDS, 10.0 % ethanol, and 20.0 mM sodium borate at pH 8.70) at ambient temperature and in the absence of light. It was found that vitamins B_1, B_3, B_5, PP, B_6, B_8, B_9 and B_{12} were stable in the experimental condition. However, vitamins B_2 and C suffered severe degradation; vitamin C was highly unstable with oxidation of 64.1 %, 96.7 %, and 99.5 % after 6, 24 and 48 h, respectively, whereas vitamin B_2 degradation was 8.6 %, 18.0 % and 30.6 % after 6, 24 and 96 h, respectively. The degradation of vitamins was significantly reduced by keeping

the vitamin solutions frozen overnight. The degradation percentages are half of those observed in the samples that were kept at room temperature. The results indicated that temperature control is necessary for the analysis of these water-soluble vitamins.

4.2 Microemulsion electrokinetic chromatography (MEEKC)

Separation of fat-soluble analytes is difficult in the MEKC mode, because these compounds have an extreme affinity to the micelles, which results in long migration time and poor resolution [66]. In comparison with MEKC methods, MEEKC has enhanced selectivity capacity for non-polar compounds and can give better resolution for fat-soluble vitamins.

Microemulsions contain dispersed nanometer-sized droplets of oil-in-water or water-in-oil, stabilized by a surfactant and short-chain alcohol used as co-surfactant (1-butanol and 2-propanol). Most of these experiments were carried out in a common microemulsion of 0.81% (w/w) heptane, 6.61% (w/w) 1-butanol, 3.31% (w/w) SDS and 89.27% (w/w) 10 mM sodium tetraborate buffer [67]. The analysis of highly hydrophobic analytes takes a long time because these compounds have an extreme partition into the microemulsion droplet (about an hour). A higher concentration of SDS led to a longer migration time. Unlike the common SDS microemulsion used in MEEKC, a rapid and selective MEEKC method for simultaneous determination of 10 water-soluble and 3 fat-soluble vitamins within 30 min was developed [68] (see Figure 2). In this study, a novel microemulsion system was elaborated by using lower SDS concentration (1.2%) with the addition of 21% co-surfactant (1-butanol) to stabilize the microemulsion. Various types and concentrations of lipophilic solvents including n-hexane, n-octane and ethyl acetate were tested. The result indicated no significant differences for the analysis of the vitamins, which is consistent with the previous results stating that the internal lipophilic phase had a minor impact on the separation efficiency in MEEKC [69, 70]. Different organic solvents including methanol/ethanol and acetonitrile were tested. A stable baseline and good resolution were found with acetonitrile. A

higher proportion of acetonitrile resulted in increased resolution, but longer migration time and an unstable baseline.

4.3 Capillary electrochromatography

Capillary electrochromatography (CEC) is a separation technique combining HPLC with CE, and these two modes act simultaneously in the separation of analytes. In CEC, water-soluble vitamins can be separated mainly in CE mode, while fat-soluble ones can be separated mainly by their interaction with a stationary phase.

Vitamin E exists mainly as four forms of tocopherols (TOHs), namely α-, β-, γ- and δ-isomers. Due to the structural similarities, the separation of β- and γ-isomers is not easy on C8 or C18 particle-packed columns and could only be achieved by C30 stationary phase using HPLC. A CEC monolithic method with UV detection was developed for the separation of TOHs [71]. The monolith was prepared from pentaerythritol diacrylate monostearate-ethylene dimethacrylate (PEDAS-EDMA) according to Okanda and El Rassi [72]. The PEDAS-EDMA monolithic column was shown to have a better selectivity for β- and γ-TOHs compared to reverse phase C18 and compatible with the C30 stationary phase. Under the optimal condition, resolution (R_s = 1.2) of the β- and γ-isomers was achieved.

A method for simultaneous separation of water- and fat-soluble vitamins was achieved using pressure-assisted CEC with a methacrylate-based capillary monolithic column [73]. A mixture of six water-soluble and four fat-soluble vitamins was separated simultaneously within 20 min using isocratic elution. The sensitivity of the developed method was not sufficient for the analysis of all the vitamins in a commercial multivitamin tablet. A volatile buffer was used in the optimized mobile phase making it compatible with MS, for sensitive detection in the future.

5 Conclusions

The challenge of vitamins analysis by CE is the sensitivity on the one hand,

and multi-vitamins determination on the other hand.Sample preparation by SPE or LLE could be helpful for the extraction of low amounts of vitamins. Light protection and low temperature were used to ensure the stability of vitamins during sample pretreatment. High sensitivity of vitamins analysis could be achieved by fluorescence detection with sub nM detection limits, where the analysis was mainly applied to vitamin B_2 showing native fluorescence or on vitamins B_9 and B_3 through derivatization. The electrochemical detection was attractive for an electroactive substance like vitamin C. Chemically modified electrodes in CE-ECD have great potential in single cell analysis.

Most CE methods related to multi-vitamin determination have been performed using CE-UV. Borate buffers at different concentrations (10-100 mM) and at pH values comprised between 8.0 and 10.0 were applied. UV detection was performed at 214 nm for vitamins B and at 265 nm for vitamin C. The wavelength for fat-soluble vitamins was mostly selected at 280 and 290 nm. The separation mode of MEKC was mainly used in the determination of several water-soluble vitamins. Different on-line concentration techniques combined with MEKC were explored to improve the detection sensitivity of the analytes. MEEKC or CEC are more selective for fat-soluble vitamins and could be used in the simultaneous determination of water-soluble and fat-soluble vitamins.

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Figure legends

Figure 1. Electropherogram obtained from a standard containing 25 µg/mL of each tocopherol prepared in BGE (A) fluorescence detection (B) UV detection. Figure was adapted from [35] and reproduced with permission.

Figure 2. Electropherogram of mixed standard solution (a) and commercial vitamin tablet (b). Figure was adapted from [68] and reproduced with permission.

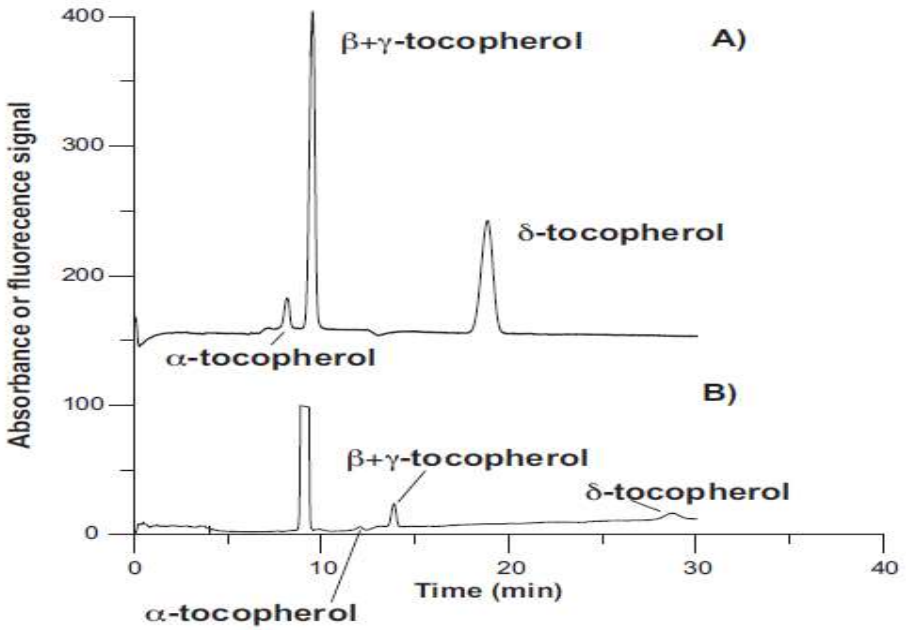

Figure 1

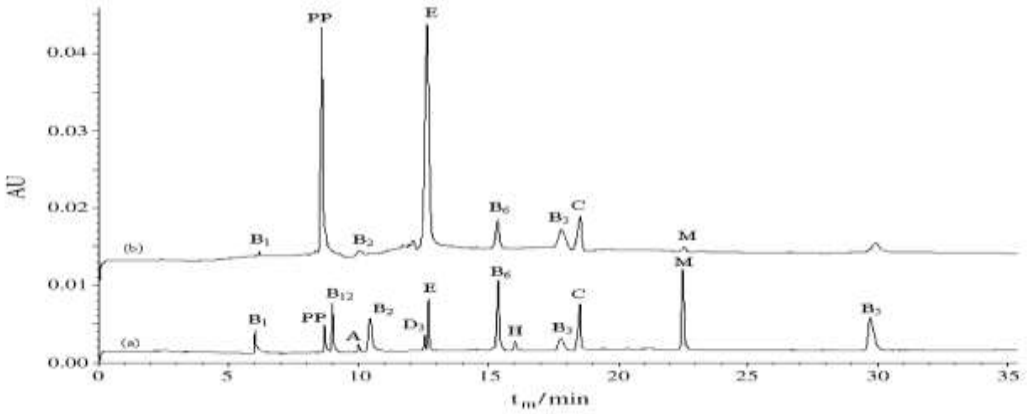

Figure 2

Table 1 Summary of vitamin analysis by CE

Sample	Vitamin	Sample pretreatment	Separation mode	Buffer	Detection	LOD	Ref
Urine & beverage	B_2	Dilution with water & Centrifugation & filtration	CZE	10 mM Borate pH 9.6, 10 % ACN	LIF EX: 474 nm EM: 515 nm	3 nM	31
Health drinks	B_9, B_3	Dilution & derivatization	CZE	25 mM Borate, pH 9.85	LIF EX: 488 nm EM: 520 nm	1-1.5 nM	32
Functional drink	B_3	Dilution & derivatization	MCE	100 mM Borate, pH 9.88	LIF EX: 635 nm	0.2 nM	34
Oil	E	SPE	NACE	12 mM Borate, 60 mM sodium cholate, 12 mM NaOH in MeOH	Xe–Hg lamp EX: 297 nm EM: 250 nm	0.18 - 0.56 µg/ml	35
Juice	C	Sonication with ethanol and Centrifugation	CZE	60 mM Borate at pH 9.0	EC	1 µg/ml	46
Hepatocytes	C	Sonication and Centrifugation	CZE	18.3 mM Na_2HPO_4–17 mM NaH_2PO_4 (pH 7.8)	EC	1.7 µM	47
Rat peritoneal mast cell (RPMC)	C	Sonication and Centrifugation	CZE	18.3 mM Na_2HPO_4–17 mM NaH_2PO_4 (pH 7.8)	EC	1.7 µM	48
Tea beverage	C	Dilution	CZE	30 mM NaOH, pH 12.5	EC	0.49 µM	52
Single cell	C	Sonication and Centrifugation	CZE	25 mM phosphate, pH 7.4	EC	0.5 µM	54
Hepatocytes	C	Trypsin digestion & Centrifugation &	CZE	73 mM Na_2HPO4–12 mM	ECL	10 nM	55

		sonication		NaH_2PO4 (pH 8.0)			
Hepatocytes	C	Trypsin digestion & Centrifugation	MCE	20 mM Na_2HPO_4 (pH 10.0), 2.5 mM luminol, 40 mM NaBr	ECL	1.3 µM	56
Pharmaceuticals and dietary supplements	B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}	Dilution for injection & sonication for tablets	CZE	50 mM formic acid with pH 2.05	MS/MS	Sub µg/mL	57
Nutritive supplements	B_{12}	Microwave-assisted extraction	MEKC	25 mM Tris buffer (pH 9.0) , 15 mM SDS	ICP/MS	0.3 ng/ml	58
Tablets and capsules	A, D, E	Sonication with MeOH	MEKC	80 mM borate-60 mM SDC-20 mM AOT-2 mM Brij 35 (pH 8.5)	UV: 290 nm	0.028 - 0.17 µg/ml	60
Bacterial growth media	B_1, B_2, B_3, B_6, B_9	Filtration	MEKC	10 mM NaH_2PO_4, 80 mM SDS, (pH 7.25)	UV: 200 nm	0.1 - 0.3 µg/ml	61
Tear	C	0.1 M phosphate buffer, pH 3.0, 1 mM EDTA at 4℃for 30 min	MEKC	25 mM borate at pH 10.0, 100 mM SDS	UV: 266 nm	5 µM	62
Cell culture media	B_9, B_3, B_2	10 kDa molecular mass cutoff filter	MEKC	40 mM SDS, 20 mM sodium phosphate, and 20 mM sodium borate at pH 9.0	UV: 210 nm	0.3 – 1 µg/ml	63
Energy drink	B_1, B_2, B_3, B_5, B_6, B_{12}, C	NA	MEKC	40 mM borate/ 40 mM SDS, pH 8.5, 5% MeOH	UV B: 214 nm C: 265 nm	0.1 - 1.2 µg/ml	64
Food supplement	B_1, B_3, B_5,	Sonication with BGE	MEKC	10.0 % ethanol, 2.0 %	UV: 214 nm	0.3 - 2.5	65

	PP, B_6, B_8, B_9, B_{12}, C			SDS, 20 mM borate (pH 8.70)		µg/ml	
Dietary supplements	C, B_1, B_2, B_3	0.1N HCl 90℃ 5 h	MEKC	Boric acid / SDS at pH 8.2	UV	5 ppm	26
Tablets	A, E, D_3, B_1, B_2, B_6, B_{12}, B_3, B_5, B_9, B_7, C	Ultrasonic with microemulsion for 15 min	MEEKC	1.2% SDS, 21% 1-butanol, 18 % ACN 0.8 % n-hexane, 20 mM borax (pH 8.7)	UV: 205 nm	0.2 - 12 µg/ml	70
Urine	C	SPE	MEKC	30 mM SDS, 100 mM boric acid and 5 mM sodium tetraborate, pH 7.75	UV: 280 nm	0.15 µg/ml	27
Serum	A, E	LLE	MEEKC	propan-2-ol (16.2 g), butan-1-ol (6.6 g), n-octane (0.8 g), SDS (2.883 g) and 10 mM sodium tetraborate pH 9.81	UV: 280 nm	0.15 - 0.25 µg/ml	27
Feed	A, D, E, K	LLE	MEEKC	10 mM phosphate buffer (pH 2.5), 2-propanol (16.2 g), 1-butanol (6.6 g), *n*-octane (0.8 g) and SDS (2.883 g)	UV: 280 nm	7 - 25 mg/kg	28
Capsule	E	Dilution with methanol	CEC	100 mM Tris buffer (pH 9.3)-MeOH-ACN (3:10:87,	UV: 200 nm	0.4 - 0.7 µg/ml	71

				v/v/v)		
Tablets	B_1, B_2, B_5, B_6, C, D, E	Sonication with ethanol	CEC	1 M formic acid (pH 1.9)-acetonitrile (30:70, v/v) , 10 mM ammonium formate	UV	73