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INCREASINGLY GREEN APPROACHES TO THE DETERMINATION OF SELECTED TRACE ORGANICS IN COMPLEX MATRICES. SHORT CHAIN CARBOXYLIC ACIDS

OZNACZANIE ŚLADOWYCH ILOŚCI ZWIĄZKÓW ORGANICZNYCH W PRÓBKACH O ZŁOŻONYCH MATRYCACH ZGODNIE Z REGUŁAMI „ZIELONEJ CHEMII ANALITYCZNEJ". KRÓTKOŁAŃCUCHOWE KWASY KARBOKSYLOWE

Abstract: Nowadays the great stress is put on environment protection and the activities to minimize the effect of chemical analyses on the environment are discussed. When environmentally friendly methodologies of analytes determination are applied the term "green analytical chemistry" is used. In general, the determination of organic compounds in complex matrices, which is a frequent analytical task, requires separation of sample components and often analytes enrichment. The latter has often been regarded as responsible for environment damaging effects to a higher degree, and this aspect of analysis was emphasized and characteristics of particular techniques reviewed. Methodologies to determine low molecular organic acids in a variety of sample compositions are comprehensively described and compared with respect to environmental impact and results quality. The trends to make the sample treatment as well as separation increasingly green have been inferred and supported by personal experience and literature evidence.

Keywords: green analytical chemistry (GAC), trace organics, sample preparation, analytical separation, organic acids

Introduction

Nowadays the environmental consciousness of mankind is relatively high and it is still increasing. Care to protect the environment from degradation has resulted in formulation of a set of rules, known as the 12 Principles of Green Chemistry [1, 2]. The very term "green chemistry" was coined by Anastas in 1991 [3]. Environmentally friendly approach is based on, among others, improving process economy, waste reduction, and elimination of risks and hazards in large-scale industrial processes [4], resulting in the environment of better quality, which, certainly should be monitored. One of important elements of quality monitoring of a given environmental compartment is determination of a variety of organic

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compounds. In many samples, the concentration of polluting organics can differ by several orders of magnitude. Some must be determined with high precision and accuracy even if present at very low concentration. The analytical tasks of that kind can be a challenge for many analytical laboratories and need the analytical instrumentation of high separation power, sensitivity and selectivity. Determination of trace organic components in samples of complex matrices must generally be preceded by sample pretreatment consisting in selective analyte isolation and enrichment from a large sample. If this is the case, the monitoring process itself can be a significant source of pollution, unless environmentally friendly or green analytical procedures are employed. For many years attempts have been made to reduce possible impact of analytical activities on the environment. In the process *Green Analytical Chemistry* (GAC) emerged, whose methods were first described in 1995 [5-7]. GAC incorporates the activities to develop new analytical technologies and to modify old analytical procedures so as to make chemical analysis less harmful to the environment [8, 9]. Implementation of the principles of green chemistry to analytical chemistry was comprehensively discussed at the Second International Symposium on Green/Sustainable Chemistry and the discussion is continued [10]. Quite a few excellent reviews on different aspects of GAC have been published quite recently [11-16]. Armenta et al [11] discuss the fundamentals of GAC, paying special attention to the strategies and tools to make analytical procedures greener. The current state of green analytical chemistry emphasizing sample preparation methods and progress in miniaturization is summarized in a tutorial review [12]. Reviews on determination of organic pollutants in aquatic environment and preparation of samples of different origin for trace organic analysis were published in 2010 [13] and 2009 [14], respectively. Separate reviews were devoted to greening analytical chromatography [15] and methodologies combining liquid phase microextraction with capillary electrophoresis [16].

Each step of chemical analysis should be taken into consideration in the process of making a given analytical procedure green or clean or environmentally friendly. The great effort has been made to reduce amounts of solvents, reagents, waste, energy and costs as well as to miniaturize analytical equipment. Miniaturization results in drastic reduction of the aforementioned elements. In determination of trace organics in the presence of many other sample components, especially those present at much higher concentrations special attention should be paid to the two basic steps, ie, sample preparation and the analysis proper using efficient separation.

In this paper the discussion of making the chemical analysis greener will be focused on *short chain carboxylic acids* (SCCA). These organic analytes are of great significance in the case of municipal and dairy farm wastewater, municipal solid waste leachate, animal farming, some food products, etc. SCCAs include volatile monocarboxylic acids or *volatile fatty acids* (VFA) containing up to 7-8 carbon atoms in a molecule, dicarboxylic acids, hydroxyacids, ketoacids, etc. The green approach to sample preparation for gas chromatographic determination of VFAs in wastewater was dealt with elsewhere [17]. This paper is focused rather on a separation step of a wider spectrum of organic acids.

Sample preparation

Different aspects of sample preparation for chromatographic analysis were reviewed in many papers [18]. An excellent book dealing with different aspects of sample preparation

was published in 2010 [19]. The greenest approach to sample preparation for the analysis proper would be excluding this step from the analytical procedure. However, this is a rare case in trace organics determination in real-life samples. The matrices of air samples (indoor, outdoor air, etc) are relatively simple and the organic pollutants present at the highest concentrations can sometimes be determined by injecting an air sample directly into a gas chromatographic column. In most cases organic compounds must be first isolated from an air sample by trapping on a solid sorbent or in liquids coated on a solid support and then extracted with, eg an organic solvent; such approach is unfriendly to the environment. A typical way of making this procedure greener is thermal desorption of organic analytes from the sorbent; in which process an enrichment factor can be considerably higher [20]. This approach has become dominant in air analysis for the contents of trace organics. At higher concentrations some organics can be extracted from the air into a stationary phase coated on a thin fused silica fiber and liberated by thermal desorption in a GC injector. The technique, which is simpler, is *solid phase microextraction* (SPME) [19, 21-23]; it is also greener and widely used nowadays, to extract organics from aqueous samples. It was used in determination of VFAs, in the air in the vicinity of wastewater treatment plants and municipal solid waste landfills [24].

In analysis of aqueous samples, the direct injection of sample into a chromatograph without any preparation is possible but in limited number of media, eg drinking water [25]. Many aqueous samples can be introduced into a chromatographic column using direct aqueous injection after removal of particulate matter by means of either filtration or centrifuging. This green approach has been applied quite often to determine SCCAs in many different samples by means of different separation techniques, eg VFAs in wastewater and solid waste landfill leachates by gas chromatography [26].

Research activity in the field of sample preparation is very dynamic and is aimed not only at making it greener but also at increasing selectivity and enrichment factors. The most noticeable inventions, which can be considered green at the present state-of-the-art are quite numerous. Extraction using organic solvents has been regarded as very unfriendly to the environment and attempts have been continually made to replace it by so called solventless techniques, in which organic and hazardous inorganic solvents are not used. However, *ionic liquids* (ILs) have emerged as a new type of green solvents whose properties can be designed to satisfy assumed requirements [27]. ILs can introduce new dimension in sample preparation techniques. They have been used, for example, in *dispersive liquid microextraction* (DLME) and *supported liquid membrane extraction* (SLME) but the spectrum of applications including new methods can considerably increase. Analytes which are volatile and poorly soluble in water can be effectively isolated and enriched using gas extraction with inert gases which certainly are green extractants. The most important and most often used varieties of gas extraction are *static headspace* (HS) and *purge and trap* (PT). In HS analytes undergo partition between an aqueous sample and the gas phase above it (head space) so no additional extractant gas is needed. The example can be determination of VFAs in animal farm waste water by means of GC with a capillary column equipped with an automatic HS sampler [28]. An exemplary chromatogram is presented in Figure 1. The detection limits are on the level of sub mg/dm^3 for VFAs with 2-6 carbon atoms in a molecule.

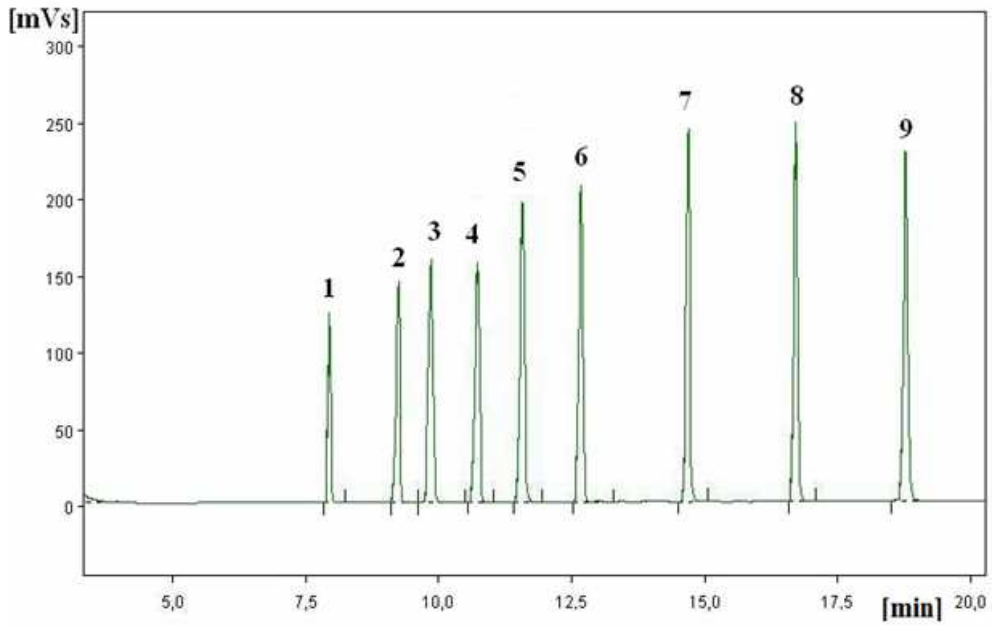

Fig. 1. DAI-GC-FID chromatogram of a standard aqueous sample containing VFAs at a concentration of 250 mg dm^{-3} each. Separation system: SUPEROX FA II (Bio-Rad) /polyethylene glycol/ (10 m × 0.53 mm × 1.20 µm), deactivated capillary (2 m × 0.53 mm), Restek Rtx-1 /dimethyl polysiloxane/ (30 m × 0.53 mm × 5 µm) and guard column (0.5 m × 0.32 mm). Volume injected: 2 (100 µm)^3. Temperature program: 80ºC (60 s) - 7ºC/min to 200ºC (3 min). *Analytes: 1 - ethanoic acid, 2 - propanoic acid, 3 - 2-methylpropanoic acid, 4 - butanoic acid, 5 - 3-methylbutanoic acid, 6 - pentanoic acid, 7 - hexanoic acid, 8 - heptanoic acid, 9 - octanoic acid* [26]

At present, the most widely recognized techniques are those which do not use solvents at all or in drastically reduced amounts or have been adopted to use solvents of minimal environmental harmfulness. Such solvents are mainly water under special conditions of temperature and pressure and supercritical carbon dioxide. The widely used sample preparation techniques are: *solid phase microextraction* (SPME) [29-39], *stir bar sorptive extraction* (SBSE) [40]; *solid phase extraction* (SPE) [41]; *microextraction in a packed syringe* (MEPS) [42]; *liquid phase microextraction* (LPME) [43]; *different modes of membrane extraction* (ME) [44]; *supercritical fluid extraction* (SFE) [45]; *subcritical water extraction* (SWE) [46]; *dispersive liquid microextraction* (DLME) [47]; *pressurized liquid extraction* (PLE) [48]; *needle trap extraction* (NTE) [49] and combination of the techniques, as for example HS-SPME. Some have already been applied in determination of VFAs [29-39, 41, 49].

Separation and detection - the analysis proper

Most of analytical tasks concerning SCCAs are their simultaneous determinations in samples of complex matrices. Therefore, efficient separation techniques must be applied, generally after pretreatment based on isolation and enrichment of analytes of interest. As in

the case of most organic analytes, the separation methods generally taken into account are chromatography (gas, liquid, supercritical fluid) and electrophoresis.

Gas chromatography

If a given group of analytes can be analyzed by means of gas chromatography (GC), this technique should be a method of choice, due to a number of reasons. In GC inert gas is a mobile phase called a carrier gas. Its flow rates range from the order of a single cm^3/min in *capillary gas chromatography* (CGC) to tens of cm^3/min when packed columns are used. Being inert, carrier gas should not make any harm to the environment and can be regarded as a green mobile phase. Due to much higher separation power and better thermal stability capillary commercial columns have been used for most analytical separations of organics. Inert gases used in GC can be considered completely green as such. However, they must be very clean, especially when used in trace analysis, and their production can directly and indirectly (manufacturing installations for gas production) can add to the total environmental impact. The capillary column generally contains less stationary phase than the packed and has longer lifetime. As a result CGC can be regarded as greener. With packed or with capillary columns, GC instruments are relatively large, especially when coupled with mass spectrometers and their production as well as disposal should be taken into account as well. CGC has been very widely used to determine many volatile and semivolatile organic compounds. Determination of SCCAs in a number of matrices has been comprehensively reviewed by Peldszus [50].

If concentration of VFAs is sufficiently high, then the greenest approach would be the direct injection of an original sample into GC for analysis provided that the chromatographic column is resistant to water and produces peaks of good quality and well separated. Though not easy, good separation of a aqueous solution of 9 VFAs was achieved using an in series connection of separation columns and pieces of empty capillaries in order (starting from the injector): a polar capillary column SUPEROX II FA (10 m × 0.53 mm × 1.20 μm), deactivated capillary (2 m × 0.53 μm), non-polar capillary column Rtx-1 (30 m × 0.53 mm × 5.0 μm), and a short capillary (0.5 m × 0.32 mm) [26]. The chromatogram of standard aqueous sample of volatile fatty acids is presented in Figure 2.

This approach could also be used for the "dirty" samples cleaned up of suspended particular matter, some inorganic compounds and high molecular organics. This has been achieved by sample refluxing in a special apparatus containing a small volume chamber collecting the rectificate. In the process the analytes are separated from column deteriorating sample components. Considering only organic solvent impact, such sample cleanup is green but, if other factors (eg time of analysis, energy consumption) will be taken into consideration it seems not so obvious.

Short chain non-volatile carboxylic acids (oxalic, malonic, etc.) can also be determined by means of GC, but only after analytes are converted to volatile derivatives. In most cases the process is not green, since some extra reagents are used and as such should not be recommended for the routine laboratories performing a lot of analyses.

The great progress in making analytical methods greener can be achieved by miniaturization. The lab-on-a-chip can give analytical instruments which are cheaper, faster and more flexible. The miniature devices consisting of a column, injector and valves can

separate a mixture of organics within 10 seconds [51]. Nowadays, due to the progress in wafer technology, miniaturized GC-MS coupled systems have been constructed. In such systems, the vacuum requirements are relaxed and carrier gas consumption reduced due to the small size of the unit [52, 53]. This is an inevitable trend which should result in a leap similar to that brought by introduction of PCs.

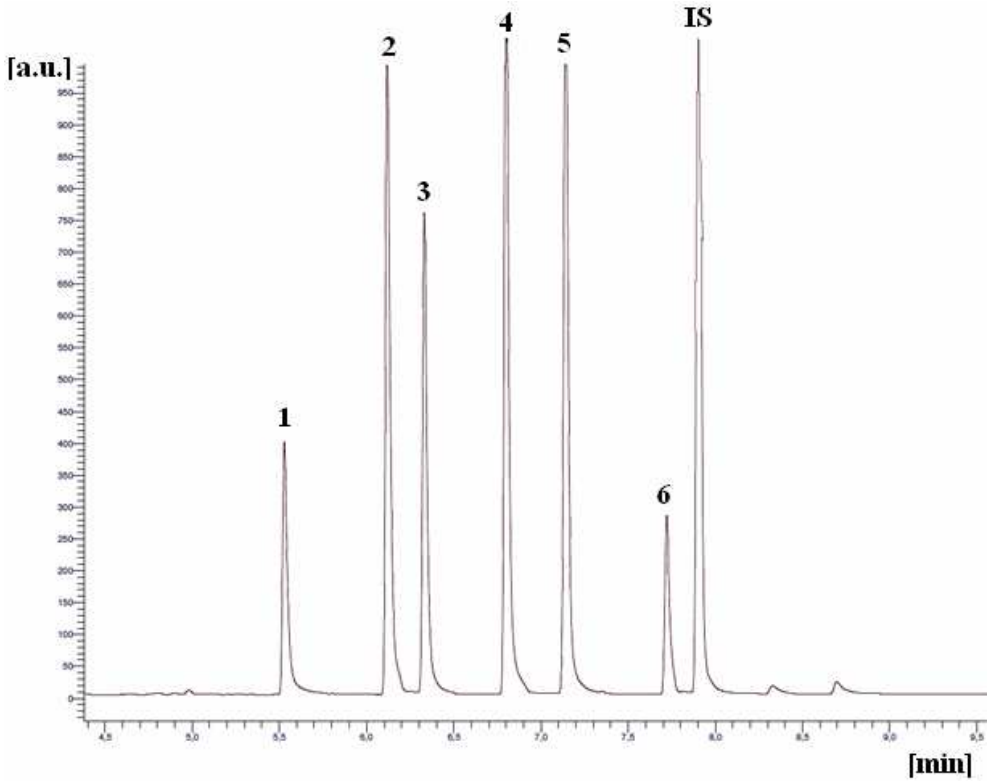

Fig. 2. HS-GC-FID chromatogram of a cattle farm waste water sample. Chromatographic column: Stabilwax DA (30 m × 0.25 mm × 0.25 µm). Temperature program: 70°C (1 min), 25°C/min to 160°C (1 min), 10°C/min to 230°C (5 min). Designations as in Figure 1 (*Internal Standard* (*IS*) - *2-ethylbutanoic acid*) [28]

Liquid chromatography

The spectrum of applications of high performance liquid chromatography to determine SCCA is wider than GC, since not only VFAs but also non-volatile acids can be determined. For many years now RP HPLC with columns of 4.6 mm in inner diameter and 25 cm long, packed with stationary phase particles of 5 µm in diameter has been usually used [54]. For such columns the optimal mobile phase flow rate is of the order of 1÷1.5 cm^3/min. Using a Kromasil RP-C18 (250 × 4.6 mm, 5 µm particle) oxalic, tartaric, acetic, succinic, butene dicarboxylic, and glutaric acids [55] were well separated in less than 8 min. A mobile phase was 25 $mmol/dm^3$ aqueous solution of dihydrogenphosphate buffered at pH 2.10 with

orthophosphoric acid and modified with methanol (11%). At the total mobile phase flow rate of 1.3 cm^3/min, methanol consumption was 0.14 cm^3/min. This does not seem to do big harm to the environment and the separation procedure was successfully employed to determine the above acids in Bayer liquors. With a UV-Vis detector at $\lambda = 215$ nm detection limits ranged from 0.0023 to 10.36 mg/dm^3.

However, at round-the-clock operation of many instruments, despite of relatively low flow rate, methanol can make some harm if care is not taken. Generally, if organic solvent is more hazardous as, for example, acetonitrile and its fraction in the mobile phase is high, the system can not be regarded as green by the present day standards. Solvent recycling, one of the ways to make technology greener has not got much attention in analytical separations [56, 57].

One way of decreasing solvent consumption is to use smaller particles which increase separation efficiency and hence analysis can be faster due to possibility of using shorter columns [58]. Quite recently, Stavova et al [59] have applied LC coupled with high resolution MS and tandem MS/MS to determine C1-C18 monocarboxylic acids and C2-C14 dicarboxylic acids. The reverse phase column of 100 mm length and 2.1 mm inner diameter was packed with 3 µm particles. The flow rate was only 200 mm^3/min. The optimal separation was obtained using gradient elution of water modified with formic acid (10 mmol/dm^3) and acetonitrile, whose content in a mobile phase increased from 0 to 90%. Acetonitrile is not a green solvent but its consumption was low (on average 90 mm^3/min) and, in our opinion, the procedure is greener than, eg GC based procedures which would require derivatization of the above acids. The disadvantages of the LC based procedure is that formic acid can not be determined and some correction for the matrix is necessary in the case of real-world samples.

Another way to make LC based analytical procedures greener is application of *ultra high performance liquid chromatography* (UHPLC), whose popularity has increased significantly in recent years [58, 60, 61]. In the technique, the use of smaller stationary phase particles is accompanied by increase in pressure which results in higher speed of separation. According to Welch [62], over 92% drop in solvent consumption can be achieved in this way when a 250 × 4.6 mm column packed with 5 µm particles is replaced by a 100 × 2.1 mm column packed with 1.7 mm particles. Quite recently UHPLC with a C-18 reverse phase column (100 × 2.1 mm, 1.7 µm) coupled with an electrospray ionization MS/MS was successfully applied to determine topramezone in soil, corn, wheat, and water [63]. Gradient elution was performed with a mobile phase composed of methanol and a 0.01% aqueous ammonium hydroxide solution. The average methanol content in the mobile phase was 50%. At the applied flow rate of 300 mm^3/min the consumption of methanol was 150 mm^3/min. Since this is accompanied by short separation time, the procedure may be regarded as being green to some degree. Accordingly, the application of UHPLC is a step towards greening analytical chemistry.

Another strategy to make LC separations greener is replacing classical mobile phases with less harmful alternatives. With respect to separation, acetonitrile was found to be the excellent organic modifier in reverse phase LC due to its advantageous properties. Replacement of acetonitrile with methanol is a step towards greener separation. Recently, the studies have been conducted to introduce even greener solvent, ie, ethanol [64]. In general, it is inferior to acetonitrile as a component of mobile phase but some researches

have shown that mobile phase modifying properties of ethanol can be improved by increasing temperature [62].

The next step in greening LC separation is the use of water as a mobile phase. Water under conditions of temperature and pressure approaching the supercritical state has quite different solubilizing properties than cold water. At ambient conditions NaCl solubility in water is 37% by weight while it drops to about 100 mg/dm^3 at supercritical conditions. The inverse relationship is observed for organic compounds many of which are miscible in supercritical water. For example, benzene is miscible in supercritical water but has a solubility of only 1700 mg/dm^3 at ambient temperature and pressure. Critical temperature and pressure of water are very high and it would be technically difficult to apply supercritical water as a mobile phase. However, *superheated water* (SHW) or pressurized water as an LC mobile phase received a lot of interest and the corresponding research is conducted. SHW is environmentally friendly solvent in the case of analytical chromatography and has real advantages for some analytical tasks. Background of SHWLC has been discussed and applications described in a comprehensive review published recently [65].

Carbon dioxide has the critical temperature and pressure which can be easily accessible and subcritical and supercritical states can be easily maintained. CO_2 appears quite a good solvent for organic compounds in a liquid or supercritical state and its solubilizing properties can easily be programmed in a wide range. Due to some specific properties of supercritical fluids *supercritical fluid chromatography* (SFC) have been developed as a separate chromatographic technique. It has been commercially available since 1982. SFC backgrounds and applications have been discussed in a number of papers and books [66]. Supercritical carbon dioxide is environmentally friendly and has many advantages over gas and liquid chromatography and SFC is the preferred chromatographic technique for many an analytical task, eg chiral separations. Amounts of CO_2 used in analytical SFC in comparison with its content in the atmosphere and the emission rate in combustion processes are so small that it can be neglected. CO_2 can be regarded as completely green solvent. However, it must be very pure for chromatographic usage and its production can have some environmental impact. Moreover, for some separations, its elution power must be changed by addition of some organic modifiers as methanol or acetonitrile so complete greenness of CO_2 as a mobile phase is questionable. The problem how green a solvent CO_2 is, has recently been raised [67, 68] and different aspects of SFC with a CO_2 mobile phase were thoroughly discussed [62].

Ion chromatography

Carboxylic acids can undergo dissociation and if pH is sufficiently high they are mainly in ionized form and can be separated as carboxylate anions by *anion exchange chromatography* (AIC). At low pH they can separated by *ion exclusion chromatography* (IEC).

In majority of applications of AEC to separate carboxylic acids (monocarboxylic, dicarboxylic, hydroxy- and ketoacids) as carboxylic anions a mobile phase is an aqueous solution containing such anions as of hydroxides, carbonates, bicarbonates, borates, etc. Aqueous samples usually do not require any sample pretreatment other than possible filtration prior to injection. To protect the analytical column from degradation and to

prolong its life time, guard columns are generally applied in-line prior to the analytical column. Generally, detection is based on conductivity so the mobile phase conductivity is suppressed before the eluate enters the detector. Nowadays the hydrogen cations suppressing the conductivity are generated by electrolysis and determination of carboxylic acids by AEC is of green nature. Of the 21 AEC applications to determine organic acids reviewed by Peldszus [50], only twice methanol and once acetonitrile were used as mobile phase modifiers. Raman and Hopke [69] for example, used AEC to determine water soluble short chain carboxylic acids (acetic, formic, propionic, glutaric, adipic, oxalic, succinic, malic, malonic, maleic) in ambient aerosols. The method was very simple and it allowed for simultaneous detection and quantitation of organic acids and inorganic acids. The mobile phase was aqueous KOH solution at a flow rate of 0.8 cm^3/min so it was environmentally friendly. The basic limitation of AEC is lower separation capability than modern LC systems.

The mechanisms of retention are very complex in IEC [69, 70]. They include Donnan exclusion, size exclusion, adsorption, polar interactions, hydrogen bonding, etc. Separation of organic acids in IEC is achieved in cation exchange columns. Eluents applied in IEC are aqueous solutions of mineral acids or organic acids. Organic solvents such acetonitrile and various alcohols are used as mobile phase modifiers to reduce tailing and retention times of more hydrophobic analytes. This can indicate that IEC must be considerably less friendly to the environment than AEC. Generally, conductivity detection is used in IEC but more powerful analytical detection and identification machines are produced by coupling IEC with different types of mass spectrometry [71, 72]. IEC is usually applied to analyse more complex samples and often the analysis proper is preceded by sample pretreatment. The technique was widely used in determination of SCCAs in a variety of samples [50]. In some recent applications, the authors evaluated their procedures with respect to impact on the environment. Dias et al [73] developed a method to determine acetic, propionic and butyric acids in dietary fiber extracts without any derivatization. The separation was shorter than 10 min, the mobile phase flow rate 0.6 cm^3/min and no organic solvent modifiers were used. So the environmental impact of the method should be low and the method green.

Applying IEC with a typical column (250 × 7.8 mm, 10 µm particles), solutions of perchloric acid ($HClO_4$), heptafluorobutyric acid ($CF_3(CF_2)_2COOH$) and sulphuric acid at different concentrations as mobile phases at a flow rate of 0.5 cm^3/min and conductivity detection after inverse suppression, 11 saturated and unsaturated low molecular mass organic acids were separated in 22 min [74]. To aid quantification of maleic and oxalic acid, anion exchange chromatography was used. Separation was performed using an anion exchange column (250 × 4 mm, 13 µm particles) and an aqueous solution of Na_2CO_3 and $NaHCO_3$ as a mobile phase at 0.5 cm^3/min.

In *ion chromatography* (IC), the waste is water containing small amounts of an electrolyte, generally not harmful to the environment, and sometimes also small amounts of organic solvents used as modifiers of mobile phases. The changes of the technique towards increasing green character depend on improvements in separation and suppression systems.

Capillary electrophoresis

Due to low consumption of reagents and solvents and small samples, *capillary electrophoresis* (CE) can be regarded as a green analytical technique. The sample injected

ranges from single pL (10^{-15} m^3) to nL (10^{-12} m^3) and buffer consumption is on the level of μL (10^{-9} m^3) per electrophoretic run [16].

A typical detection system used in CE is a UV-Vis absorption spectrometer, which is of rather low sensitivity especially that the sample injected is small and light path short. SCCAs are characterized by low absorption coefficients and often indirect detection is applied. Such approach was applied to determine oxalic, citric, tartaric, malic, succinic, carbonic, acetic, lactic, aspartic, glutamic, ascorbic and gluconic acids [75]. These acids were well separated using a capillary 75 μm in inner diameter and 70 cm (63 cm to the detector) long. A background modifier was *trimellitic acid* (TMA). *Tetradecyltrimethylammonium bromide* (TTAB) was used as an EOF modifier. The method developed is rapid, sensitive and environmentally friendly (the consumption of reagents is small). It is characterized by the detection limits of the order of $2.0 \cdot 10^{-6}$ mol/dm^3 and can be applied to determine these acids in real food samples.

Capillary electrophoresis with indirect UV detection was also used to separate nine organic (formate, acetate, propionate, butyrate, oxalate, malonate, succinate, phthalate and maleate) and seven inorganic anions [76]. The detection limits were below 0.5 mg/dm^3 for all the analytes mentioned (except for phthalate 1.0 mg/dm^3) and the procedure could have been used to analyze waste water samples. The only treatment was filtration through 0.45 μm membrane filter; in some cases dilution was required due to excessive levels of carbonate. The capillary was 60 cm long and 50 μm in I.D. Again EOF modifier was TTAB while pyridine-2,6-dicarboxylic acid was used as electrolyte.

Capillary electrophoresis with both direct (at λ = 185 nm) and indirect (at λ = 254 nm) UV detection was employed to determine carboxylic acids (formic, acetic, propionic, butyric, malonic, lactic) that are relevant for the evaluation and specification of silage quality [77]. It was found that direct UV detection was more suitable for the determination of the above analytes in complex matrices such as silage. The acids were extracted from silage with water and the extract was filtered through a 0.45 μm disposable filter cartridge. Separations were performed using fused-silica capillaries with effective lengths between 50 and 65 cm, an inner diameter of 75 μm and a detection window 8 cm from the capillary end. Aqueous and non-aqueous buffer systems were used. The latter offered unique selectivity and the information obtained with them can be regarded as complementary to that obtained with aqueous electrolytes.

Co-electroosmotic high performance capillary electrophoresis was successfully applied to determine saturated and unsaturated mono- and dicarboxylic acids which are intermediates and reaction products in the conversion of citric and itaconic acid in hot, compressed liquid water and supercritical water [78]. Direct and indirect UV detection at λ = 185 nm, with, respectively, borate-phosphate electrolyte and phthalate as background electrolyte were employed. Fused silica capillaries of 50 mm I.D. and effective length 24.5 cm were used. The CE determination lasting 2-3 min is much faster than when HPLC is used, which could take about 35 min.

A capillary electrophoresis based method was developed to determine short chain organic acids such as oxalic, formic, fumaric, aconic, succinic, malic, glutaric, citric, glycollic, propionic, and quinic in serum of natural latex [79]. The acids were separated using uncoated fused silica capillary (57 cm × 50 mm) and spectrometrically detected at λ = 200 nm. LODs ranged from 2 μmol/dm^3 for aconitic acid to 1612 μmol/dm^3 for

formic acid in standards, while in samples from 3 for fumaric acid to 1600 μmol/dm^3 for propionic acid. The sample pretreatment was limited to coagulation.

The simple filtration was used to prepare samples of swine manure for determination of volatile fatty acids such as propanoic, butyric, valeric and caproic acids [80]. The analytical conditions were as follows: fused silica capillary of an effective length of 40 cm, 50 mm I.D., 20 mmol/Tris and 10 mmol/dm^3 *p*-anisate buffer; 30 kV voltage and a temperature of 25ºC. VFAs were determined with good accuracy and precision at ppm level using samples as low as nanoliters. The separation complete within 10 min was time-saving as compared with chromatographic methods.

Wines are typically monitored for the content of SCCAs. CE with indirect spectrophotometric detection was used to analyze 23 Brazilian wines [81]. The separation was made using typical fused silica capillary (57 cm × 0.75 μm) and the electrolyte consisting of 10 mmol/dm^3 3,5-dinitrobenzoic acid (DNB) at pH 3.6 and cetyltrimethylammonium bromide as flow reverser. DNB has a good effective mobility similar to acids of interest, good buffering capacity and good chromophoric characteristics for indirect detection at λ = 254 nm. The procedure gives good quality results. The detection limits range from 0.64 to 1.55 mg/dm^3, sample preparation is simple (dilution and filtration) and separation fast (5.5 min to separate six acids, ie tartaric, malic, lactic, succinic, acetic, citric) and analysis cost is low. So it has a feature of green method.

The limits of detection of SCCAs can be lowered by converting them to derivatives for which the detector is more sensitive and selective. Often it is the conversion of the acids mentioned above to the fluorescent products [82]. Eleven organic acids which can occur in such beverages as wine, beer, vegetable and fruit juices were well separated and detected as their acid hydrazides by means of capillary zone electrophoresis with photodiode array detector [83]. Separation of the acids was achieved within 12 min and detection limits ranged from 2 to 10.0 mg/dm^3 at 42 nL (100 μm)^3 sample injections. A typical fused silica capillary 40 cm long and 75 μm I.D. was used. The acid hydrazides were detected at λ = 230 nm. The corresponding procedures are less green since additional reagents and processes are applied. However, analytical procedures can still be regarded as green as compared with those based on gas or liquid chromatography since generally samples need only tiny pretreatment and the amounts of samples and reagents used are really very small.

CE can be made even greener by miniaturization. CE microchips are characterized by high degree of integrity, portability, minimal solvent and reagent consumption, high performance and high speed. Applications of microchip CE with electrochemical detection for determination of environmental pollutants including some acidic organics were reviewed by Chen et al [84].

Conclusion

Green analytical chemistry is a very important trend in different areas of analytical chemistry. As far as trace organic determination in complex matrices is concerned the great stress should be put on sample preparation and a lot has been done to make this step of analysis greener. However, to challenge the analytical tasks of that kind, powerful separation systems must be often employed. Nowadays the separation methods are modified to decrease organic solvent consumption and also to replace them with solvents which are less harmful, eg ethanol or totally harmless (water in *Reverse Phase Liquid*

Chromatography) for the environment. The other approach is developing analytical procedures based on inherently greener methods, for example, replacing HPLC with SFC or with electrophoresis. The great leap in reduction of consumption of solvents and reagents is development of miniaturized systems. It seems to be the future of analytical chemistry. The above-mentioned activities are observed in determination of traces of short chain carboxylic acids in complex matrices. Many analytical procedures proposed recently are based on capillary electrophoresis. Volatile fatty acids can be separated by gas chromatography which is regarded as a method of choice since it is greener than other separation techniques and is simple and easy to apply. Some GC systems allow for the analysis of aqueous samples and direct aqueous injection is preferred whenever the samples are sufficiently clean. Such approach has been used to determine VFAs in some environmental samples. When analytes must be isolated and enriched prior to GC analysis solventless techniques are frequently applied. All this makes monitoring of short chain carboxylic acids increasingly green.

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OZNACZANIE ŚLADOWYCH ILOŚCI ZWIĄZKÓW ORGANICZNYCH W PRÓBKACH O ZŁOŻONYCH MATRYCACH ZGODNIE Z REGUŁAMI „ZIELONEJ CHEMII ANALITYCZNEJ". KRÓTKOŁAŃCUCHOWE KWASY KARBOKSYLOWE

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Abstrakt: Obecnie społeczeństwa kładą duży nacisk na ochronę środowiska. Działania w tym kierunku są prowadzone także w zakresie chemii analitycznej, co określa się terminem „zielona chemia analityczna (GAC)". W artykule przeanalizowano wprowadzanie nowych metod i technik analitycznych oraz modyfikację już opracowanych metodyk oznaczania w kierunku bardziej przyjaznych środowisku. Konkretne procedury oznaczania krótkołańcuchowych kwasów karboksylowych zostały ocenione pod kątem ich ewentualnego wpływu na środowisko.

Słowa kluczowe: „zielona chemia analityczna", śladowe ilości związków organicznych, przygotowanie próbek, techniki rozdzielania, kwasy organiczne