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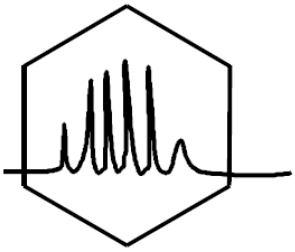

THE XXXIInd SYMPOSIUM

'CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS'

JUNE 3rd – 5th, 2009
KATOWICE – SZCZYRK

Oral presentations

PERFORMANCE OF HPLC COLUMNS PACKED WITH VERY FINE PARTICLES. CONSEQUENCES OF THE HEAT EFFECT

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The demand from the pharmaceutical industry to increase speed, throughput, and resolution of HPLC analyses has pushed the manufacturers of packing materials to prepare new brands of fine silica particles. Several types of columns packed with sub-2 μm particles are now commercially available. The permeability of these columns is much lower than that of conventional columns and their optimum velocity larger. So, in order to exploit their full potential, they must be operated at inlet pressures as high as 1 kbar. However, high linear velocities of the mobile phase combined with steep pressure drops along the column generate an important amount of heat, due to the friction of the mobile phase against the bed through which it percolates. This heat escapes through axial convection, radial and axial conduction. As a consequence, columns packed with very fine particles cannot be isothermal. Depending on the external environment (adiabatic column, moderately insulated column, or temperature-controlled column wall), significant longitudinal and/or radial temperature gradients are formed inside the column. This new type of column heterogeneity causes most serious problems that need to be investigated from both theoretical and experimental viewpoints.We measured and calculated the temperature distributions along and across 2.1 x 50, 100, and 150 mm columns packed with 1.7 μm bridged ethylsiloxane - silica hybrid particles. We determined the relationship between their apparent efficiency and the mobile phase velocity. Emphasis will be given to the worst set of experimental conditions, in which the column wall is kept at a constant temperature, resulting in an important radial temperature gradient, hence in a large radial gradient of mobile phase velocity across the column. This gradient warps the solute bands and deleteriously affects the elution profiles of their elution peaks, hence the apparent column efficiency. We show the results of measurements of the temperature profiles along the wall of a column and across its exit section and illustrate the experimental dependence on the flow rate of the HETP of naphtha[2,3-a]pyrene. We compare calculated and measured elution peak profiles and calculated and experimental HETP curves for this compound. Finally, advice will be presented regarding the most appropriate ways to use this type of columns and conclusions drawn sketching the probable frontier of future applications of columns packed with ultra-fine particles.

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NEW CHIRAL STATIONARY PHASES FOR LIQUID-PHASE ENANTIOSEPARATION TECHNIQUES

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In this presentation the results of our recent studies on development of novel chiral stationary phases (CSP) for enantioseparations using high-performance liquid chromatography (HPLC), capillary liquid chromatography (CLC) and capillary electrochromatography (CEC) are summarized. In the first part of the presentation the emphasis will be made on novel phenylcarbamate derivatives of cellulose and amylose as useful CSPs for analytical and preparative scale enantioseparations. These novel materials are applicable for HPLC enantioseparations in combination with normal phase-, polar organic mobile phase and reversed-phase eluents, as well as for SFC enantioseparations at higher pressure. In the second part of the presentation our attempt will be described in order to combine high enantiomer resolving ability of polysaccharide derivatives with favourable dynamic properties of monolithic silica materials. Modification of silica monoliths in common diameter columns as well as in capillary columns by *in situ* coating or covalent immobilization of a chiral selector onto the surface will be described. The common-size columns obtained by above mentioned techniques were applied for enantioseparations under normal- and reversed phase conditions in HPLC and capillary columns in CLC and CEC [1-3]. It was possible to obtain high efficiency enantioseparations with short analysis time in all 3 techniques. Comparative characteristics of chiral stationary phases based on monolithic and particulate silica materials will also be presented.

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On the Role of Thin-Layer Chromatography in Studying Reaction Mechanism: Selected Examples of Condensation

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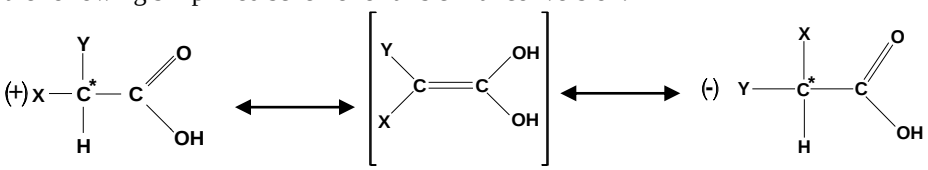
In previous studies, we reported the spontaneous oscillatory chiral conversion of α-substituted propionic acid derivatives in abiotic aqueous media (e.g., [1-5]). We proposed the following simplified scheme for this chiral conversion:



enantiomer (+) enol enantiomer (-)

where X = -NH_2, -OH, -Ar, etc., and Y = -C_nH_{2n+1}.

In papers [4,6], we introduced a model chemical oscillator (two linked Templators), that simply yet adequately describes the above process.

In subsequent studies [7], we discovered the oscillatory nature of α-substituted carboxylic acids not derived from propionic acid and another phenomenon, rapid peptide formation of *R,S*-(±)-phenylglycine, which we confirmed by several analytical techniques (including chromatography, ^{1}H NMR and Raman spectroscopy, and the biuret test).

In this study, we discuss spontaneous peptide formation in a wider selection of α–amino acids, which we suggest is triggered by the oscillatory chiral conversion of these compounds. We have modified our model of two linked Templators to incorporate two simultaneous phenomena, the oscillatory chiral conversion of α-amino acids and their polycondensation to form peptides coupled through peptide (NH-C=O) bonds.

Moreover, polycondensation can also occur with α-hydroxy acids and profens. We have demonstrated experimentally, for example, the transformation of *L*-lactic acid to poly(lactic acid), coupled through O-C=O bonds. A modified model of two linked Templators can describe this type of polycondensation as well.

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The work of two of the authors (M.G. and D.K.) was partially supported by PhD scholarships granted to them in 2008 within the framework of the 'University as a Partner of the Economy Based on Science' (UPGOW) project, subsidized by the European Social Fund (EFS) of the European Union.

SELECTIVITY AS THE DRIVING FORCE FOR THE POWER OF LIQUID CHROMATOGRAPHY

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Selectivity is the most driving parameter in any type of separation technology whether it is in gas-phase of liquid phase (GC, SFC, LC, CE, CEC). However, what drives selectivity? Phenomenologically speaking and simplifying the events, it is based on intra- and inter-molecular interactions of molecules and surfaces and this in turn is a result of the functional groups and structural increments of the molecules being involved in the interaction event and of the liquid medium (solvents) in the case of liquid phase separation. With other words, selectivity needs to be discussed on a molecular level and we call the molecular descriptor of the stationary phase, the ligand, as selector (SO) and the analyte it interacts with as selectand (SA). Both species can be non-polar, polar, chiral, ionizable, etc. whereby these properties which are a consequence of the functional groups of the SO-SA can be switched on or off in a wide window in the course of intermolecular interactions by the influence of the solvents (mobile phase). Stereochemical aspects need special attention.

Thermodynamically these events can be described by the Gibbs binding energy ΔG and the $\Delta\Delta G$ values are directly correlated to the observed selectivity values. On top of these considerations one may also consider in liquid chromatography selectivity principles derived from different partition coefficients of the SAs in two different "non-mixable" liquid layers of which one is the stationary and the other one is the mobile phase. For all these events one needed to consider also de-solvation and re-solvation aspects and the dynamics of conformational changes of the SO and SA molecules as factor of the local environment and of the temperature.

In reality the observed selectivities in separation technologies are often based on mixed mode/modal interaction models and it is not so trivial to de-convolute the increments actually responsible for selectivity changes, etc. However, for the practitioner the reproducible triggering of selectivity parameters counts most but one should be aware of the sensitivity separation systems may respond on small changes of the ligand (stationary phase) structure and on mobile phase compositions.

As the subject of this lecture is very broad special focus will be given on recent developments of new stationary phases and separation systems thus including also HILIC and enantioselective LC-methodologies dealing with polar analytes.

MICROCOLUMN TECHNOLOGIES FOR INNOVATIVE SEPARATION SCIENCE

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Abstract: Three micro scale liquid chromatographic techniques will be dicussed: capillary action liquid chromatography (caLC), electrochromatography (CEC) and electrically assisted liquid chromatography (eLC).

Driven by capillary action flow, caLC has been developed as a new mode of column liquid chromatography. Using a centrifugally dry packing method, capillary columns packed with a great variety of HPLC grade particulate stationary phases were manufactured in a high throughput and low cost way. Using a simple instrumental setup with a digital microscope, caLC separations of dye mixtures were monitored by real time imaging. Performance in normal phase caLC on Hybrid 2.6 μm silica, with a plate height of 8.8 μm for a weakly retained analyte, approached that of HPLC.

A new single particle fritting technology was developed for CEC column fabrication. By contrast to the conventional sinter fritted capillary columns, the new columns present improved separation efficiency and excellent stability irrespective to the operation conditions (thermostatting and pressurization).

The combination of chromatography and electrophoresis, eLC, has been investigated using silica monolithic columns. Due to the high permeability of the column material, high performance LC separations were achieved at the low pressures available on a CE instrument. When electrical field was combined with a low hydraulic pressure (0-100 psi), high efficiency and high selectivity separations of charged compounds were obtained. Chromatography and electrophoresis have also been combined in a sequential way, coupled LC-CZE, using a duplex column configuration. Initial experiments with an oligopeptide mixture have shown the potential of this coupled microcolumn separation technique for the analysis of complex mixtures.

Optimization of chromatographic systems for analysis of basic analytes and determination their lipophilicity parameters

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In case of chromatographic analysis of compounds having character of weak bases with the use of aqueous mobile phases, in which these compounds dissociate, number of analytical problems appear. Asymmetric and wide peaks on chromatograms appear, efficiency of systems is low and separation selectivity of mixtures of basic compounds insufficient. It is caused by various retention mechanisms of both forms of these substances. Neutral form interacts with stationary phase ligands by hydrophobic interactions typical for reversed phases, whereas ion form undergoes ion exchange process on residual silanols of silica matrix.

Disadvantageous interactions of basic cations of analytes with silanol groups can be limited by the use of low pH mobile phases to suppress of free silanols' ionization and the use of high pH mobile phases when dissociation of basic analytes is suppressed. Various mobile phase additives can be also applied: the use of ion pair reagents to creation of unionized associates or the use of reagents playing the role of silanol blockers. Moreover the use of special ligands of stationary phase causing additional blocking of free surface silanols was examined.

In order to selection of optimal conditions for qualitative and quantitative analysis of basic compounds the effect of mobile phases pH, kind and concentration of anionic reagents, kind and concentration of silanol blockers were examined.

The second problem is the selection of systems for determination of lipophilicity. The presence of both, neutral and ionic forms in chromatographic systems causes difficulties in evaluation of lipophilicity parameters. Retention coefficient for basic analytes in system non-polar ligands/water (log k_w) can be determined from retention – modifier concentration dependencies. Log k_w values for basic analytes determined in various chromatographic systems were correlated each other and with log P values calculated by ChemIDplus software. Very poor correlations between log P and log k_w values were obtained in case when log k_w values were extrapolated for log k = f (c) dependencies in organic modifier – water or organic modifer - buffer of low pH. Also the addition of silanol blockers does not give significant improvement of correlation. The most optimal for determination of lipophilicity were systems with buffered mobile phases of high pH and especially with the addition of ion-pairing reagents.

Bioanalytical possibilities in food-drug interaction research

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The sensitivity and selectivity of LC-MS and/or LC-MS/MS applications are high of importance in the pharmaceutical food-drug interaction research. Bioanalytical methods play an important role in the original and generic drug development. In the recent years it has come to the centre of attention that food intake exerts a complex influence on the biological availability of certain drugs. A number of *in vivo* studies have been published, however, only a few *in vitro* data are known. Fed and fasted conditions can be simulated *in vitro* with dissolution tests using dissolution media with appropriate food components according to the Ph. Eur. 5.

Our results gained on model drug molecules (fluoroquinolone type ciprofloxacin and anxiolytic drug deramciclane) in food-drug interaction studies indicate the importance of further development of sample preparation and bioanalytical methods. A potential problem with the increasing use of quinolone type antibiotics for systemic illness is the chelation and inactivation of these compounds by multivalent cations, therefore dairy products present a risk in their ability to be absorbed. The amount of the free, non-complexed ciprofloxacin can be quantified with LC-MS method after the SPE sample preparation of the milky samples.
Further studies gave evidence that also in case of deramciclane the presence of food has a significant impact on the bioavailability of the drug. *In vivo* data were obtained by the determination of the absorbed deramciclane amount from different plasma origin with newly developed LC-MS/MS method. Comparing the *in vivo* LC-MS/MS and *in vitro* data derived from GC-NPD a good correlation can be observed (IVIVC).

Chemometric analysis of gel electropherograms

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Two-dimensional (2D) gel electrophoresis is a core analytical technique applied in proteomics studies. In the so-called comparative proteomics, the main goal is identification of proteins that differentiate the studied classes of objects (e.g., the control samples and the diseased or treated samples). This identification is very important for medical diagnostics, drug design and/or our understanding of biological processes. In this type of studies, the comparison of samples is performed based on the resulting digital images of the 2D gel electropherograms. Thus an overall success of comparative proteomics critically depends on accuracy and reliability of the analysis of gel images. This is a multi-steps process, and all individual steps heavily influence final results, i.e., the obtained set of proteins differentiating the studied classes.

For instance, gel images require enhancement, transformation, normalization, warping, spots detection, spots quantification, etc. At each step of data analysis, different software makes use of different approaches and the choice thereof, and also of the input parameters, is up to the user. However, different methods of data pre-processing or different input parameters to the given algorithm can lead to a significant amount of the software induced variance (e.g., [1]).

Another serious drawback of the available approaches is caused by the fact, that analysis is performed based on the spots volumes, i.e., spot detection and quantification is required (based on images segmentation or the model-based quantitation). As reported in [2], this can lead to many missing elements in the spots table. Problem with missing elements in the spots table can be omitted, if instead of spots the analysis is performed at the pixels level [3,4].

The available software allows a comparison of an abundance of the corresponding proteins in the univariate manner. In many cases this approach can be successful, but it can also happen that there are no individual proteins significantly differentiating the studied classes. It does not mean that there are no differences in the entire protein profiles, but these can be revealed and used for medical diagnostics, based on the multivariate approach only.

To eliminate these drawbacks of the available software, we propose the start-to-end approach [3], in which the spot detection step is eliminated, image enhancement is optimized, feature selection is multivariate and cross-model validated. Performance of the proposed approach is demonstrated on the real data sets.

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HERBAL FINGERPRINTS: DEVELOPMENT AND EXTRACTION OF INFORMATION

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Worldwide, herbs are used for preventive and therapeutic goals. Therefore, identification and quality control of these products of natural origin is required. Determination of some of the active compounds does not always allow assessing their total intrinsic quality. Since 1991 the World Health Organization accepts fingerprint chromatography as identification and quality evaluation technique for medicinal herbs. In fingerprint development, the first step is to create general conditions to maximize the peak capacity within an acceptable analysis time. To evaluate the global quality of a fingerprint chromatogram, different criteria such as the sum of Resolutions (ΣRs), the Hierarchical Chromatographic Response Function (HCRF), the Chromatographic Response Function (CRF) and the Chromatographic Optimization Function (COF) potentially can be used.

A fingerprint can be developed for a number of reasons: identification, classification or calibration purposes. Identification is to confirm that a sample is originating from the herb expected and to exclude that it is another, i.e. to attain a better quality control of the herbs. Classification can be performed to classify samples according to, for instance, their origin. This can be either a geographic origin or to distinguish between natural and synthetic compounds, e.g. vanillin from herbal, synthetic or microbiologic origin. Such evaluation is most often done by a principal component analysis, occasionally by a cluster analysis. A multivariate calibration can be performed when the herb or its extract also can be characterized by an activity, e.g. an antioxidant or a cytotoxic activity. The activity then can be modelled as a function of the complete chromatogram. The most commonly used modelling techniques are stepwise multivariate regression, principal component regression and partial least squares. The goal of the modelling can be either to built models that are able to predict the activity for future samples based on the chromatogram (e.g. the antioxidant activity from green tea) or to identify the main compounds/peaks responsible for a given activity.

THE NOISE PROBLEM IN THIN LAYER CHROMATOGRAPHY – THEORY AND METHODS OF ITS SUPPRESSING

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The chromatograms made by HPLC technique are often processed chemometrically as unique signals (fingerprints of samples). Although TLC plates can be also scanned densitometrically or videodensitometrically to obtain and process similar signal-like data, there are almost no such approaches in worldwide literature.

Before further processing, such as baseline removal, warping and final multivariate analysis, the chromatograms often must be properly denoised. The noise nature and denoising proposals are well studied in HPLC, CE or other techniques, but there is a lack of such investigations regarding TLC.

The noise nature was studied in three cases: densitometry (where the noise resulted to be significantly pink and heteroscedastic) and videoscanning (1D and 2D), where CCD noise was almost white and homoscedastic.

The representative densitogram and univariate videoscan were then denoised by various approaches: Savitzky-Golay filters, ADPF Filter, Whittaker smoother, Butterworth and Chebyshev IIR filters, Fourier Denoising and wavelet shrinkage with different parameters. The results were compared to reference signals, denoised by multiple scanning and averaging. The best results were obtained using Savitzky-Golay filters and wavelet shrinkage with soft thresholding, high decomposition level and Haar mother wavelet.

ADVANTAGES AND COSTS OF TWO-DIMENSIONAL HPLC

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We need two-dimensional chromatography because we have more and more highly complex samples to analyze. We cannot prepare the uni-dimensional HPLC columns that could separate them and, if we could, the analysis times would be too long. So, we need two-dimensional chromatography because it takes far less time to generate twice in a row a peak capacity n_c than to generate once a peak capacity n_c^2.

In two-dimensional chromatography, the sample is successively separated on two columns packed with different adsorbents that operate after different retention mechanisms (e.g., HPLC and IXC, HILIC and SEC). Small fractions of the eluent of the first column are injected into a second column, the chromatograms are recorded and combined by software. The requirements for success are (1) the sample components must be eluted over a wide range of elution times on each column; (2) the two retention mechanisms must be independent or nearly so and there is little correlation between the retention patterns on the two columns; (4) the separation performed by the first column must not be wasted during the transfer of fractions of the first column eluent to the second column. The reasons for these conditions and their consequences will be explained. There are three different ways to combine the chromatographic analyses performed on two columns. These are on-line, stop-and-go, off-line comprehensive two-dimensional chromatography. We review, discuss and compare the potential performance of these combinations, their advantages, drawbacks, problems, perspectives, and results.

CAPILLARY ELECTROPHORESIS AS AN ALTERNATIVE OR COMPLEMENTARY TECHNIQUE TO HPLC FOR THE QUALITY CONTROL OF PHARMACEUTICALS

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Capillary electrophoresis has proven to be a powerful and versatile technique in many areas for the analysis of small inorganic ions and organic compounds. It is used in food, beverage, pharmaceutical and biotechnology industries for research and/or quality control purposes. This presentation focusses on CE applications in the quality control of drugs in the pharmaceutical industry. Major applications areas of CE such as assay of the principal component, impurity confirmation and determination, determination of stoichiometry and chiral analysis will be illustrated with examples of CE methods developed in our laboratory for pharmaceutical companies. The results obtained by both CE and HPLC will be compared and it will be shown how CE can be an attractive alternative or complementary technique to HPLC, which is widely used for the quality control of chemical drugs. Some examples of the quality control of biotechnological products using CE pharmacopoeia methods will be also given. Although the range of applications of CE and HPLC are similar, it will be shown that CE should be considered first for the resolution of charged polar analytes; it excels in the rapid analysis of small ions and chiral separations, and it is well suited to the analysis of basic compounds and analytes with no chromophore. CE offers the advantages of high resolution, fast method development and analysis, and low operating costs. However, it suffers some limitations, principally with regard to sensitivity of detection and precision of injection (an internal standard is needed) which are, in general lower than in HPLC.

QUALITY OF PHARMACEUTICALS

Lois Ann Beaver

LAB Enterprises, Rockville, MD, USA

Changes in approaches to the way that pharmaceuticals are manufactured and regulated are currently being implemented. The background of these changes, international harmonization activities and industrial response will be addressed. A deeper understanding of the concepts involved and a sense of the impact of changes in the pharmaceutical quality system will be presented.

ON THE DIFFERENTIAL LIPOLYTIC CAPABILITIES OF DIVERSE TISSUES FROM YOUNG ADULT GUINEA PIG. A CHROMATOGRAPHIC STUDY

F. M. Helmy(USA)

We have recently reported the preferential deacylation of cardiolipin (CL) in a variety of mammalian myocardia and pectoral muscle of some birds by endogenous phospholipases (PLA1 and/or PLA2) during *in vitro* incubation at pH 7.4 and 38°C for 60 minutes of whole tissue homogenate (as source of phospholipids and phospholipases) with production of monolysocardiolipin (MLCL) and concurrent reduction of CL. In contrast, whole tissue homogenate of rat spleen treated similarly selectively deacylated ethanolamine plasmalogen (PE) producing lyso alkenyl PE.

In the present study, we conducted *in vitro* incubation of whole tissue homogenate of guinea pig heart, kidney, testis and spleen at pH 7.4 and 38ºC for 60 minutes. Subsequent TLC (thin layer chromatography) analysis revealed: a) a noticeable high level of MLCL and a very low level of CL in control testis, b) guinea pig heart and kidney revealed a high level of CL and no MLCL was detected. MLCL was only produced (in both heart and kidney) subsequent to *in vitro* incubation, c) very low level of CL was shown in control sample of guinea pig spleen and no MLCL was produced, d) guinea pig heart has both ethanolamine (PE) and choline plasmalogens (PC). PE plasmalogen is the only alkenyl species present in guinea pig kidney, testes and spleen.

These data raised some questions pertaining to the lipolytic capabilities of diverse tissues and will be discussed in details.

CHARACTERISTICS OF CITRUS FRUITS: RESULTS OF THE STUDIES *IN VITRO* BY CHROMATOGRAPHY, AND *IN VIVO* ON PATIENTS SUFFERING FROM ATHEROSCLEROSIS

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The health protective effect of fruits, including the citrus fruits, is mostly related to their antioxidants: phenolic compounds and to a lesser extend – to dietary fiber. As a consequence, consumption of these natural products is inversely related to coronary atherosclerosis. However, the differences between several citrus fruits were less investigated, therefore oranges, red and blond grapefruits, and sweeties (a hybrid of pummelo and blond grapefruit) as a supplement to atherosclerosis preventing diets were studied. The FTIR spectra and fruorimetry of polyphenols were used to characterize the citrus fruits. It was found that the total and free polyphenols, flavonoids, flavanols, tannins, anthocyanins and the antioxidant activity determined by five radical scavenging assays (CUPRAC, DPPH, ABTS, FRAP and β-carotene-linoleic acid) differ in investigated samples. Phenolic acids (free, esters and glycosides), hesperidin, naringin, quercetin, kaempferol, apigenin and ascorbic acid were determined by HPLC analysis. It was found that the contents of bioactive compounds, especially phenolics, flavonoids and the antioxidant capacity of the red grapefruits are higher than in blond cultivar. Diets supplemented with red grapefruits significantly hindered the rise in the plasma lipid levels in cholesterol fed rats. Diets supplemented with red grapefruits significantly decreased the plasma lipid levels especially triglycerides in patients suffering from coronary atherosclerosis and related hypertriglyceridemia**:** a) total cholesterol (TC) by 15.5% and 7.6%; b) low density lipid cholesterol (LDL-C) by 20.3 % and 10.7%; c) total triglycerides (TG) by 27.2%, and 5.6 %, for two experimental groups EG1 (red grapefruit) and EG2 (blond grapefruit), respectively. In conclusion, the combined results of the *in vitro*, *in vivo* and on humans investigations are a solid basis for recommendation to include red grapefruit in atherosclerosis preventive diet, especially in patients suffering from hypertriglyceridemia.

CHROMATOGRAPHIC APPLICATIONS OF RECURRENT RELATIONS

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One of the most interesting features of trends in various physicochemical constants of organic compounds in any homologous series is their identity. Most of homologous property (A) changes vs. number of carbon atoms in the molecule (n_C) can be described with high precision by the simple linear (first order) recurrent relations [1]:

$$A(n_C+1) = aA(n_C) + b$$

Besides that the same equation describes monotonous variations in different properties (x) of chemical systems depending of temperature ($x = T$), pressure ($x = P$), or concentration of constituents ($x = C$):

$$A(x+\Delta x) = aA(x) + b\,, \quad \Delta x = \text{const}$$

In chromatography the application of recurrences permits us to optimize the solution of the following principal problems:

- The approximation of homologous dependencies of retention parameters (both in GC and HPLC)	$t_R(n_C+1) = at_R(n_C) + b$
Partial case of the same problem: evaluation of hold-up time of chromatographic systems (t_0)	$t_R(n_C-1) = at_R(n_C) + b$* [n-fold application of this equation until $(n_C-1) = 0$]
- The approximation of temperature dependencies of retention parameters in GC	$t_R(T+\Delta T) = at_R(T) + b$
- The approximation of concentration dependencies of retention parameters in HPLC (C – is the concentration of organic solvent in an eluent)	$t_R(C+\Delta C) = at_R(C) + b$

Instead of n-fold application of recurrent relation (*), the evaluation of hold-up time of chromatographic systems can be based on the simple calculation of the corresponding limiting value:

$$t_0 = \lim[t_R(n_C)]\,\big|_{nC=0} = b / (1-a)$$

The possibility to apply the single equation in describing the different dependencies is caused by unique mathematical properties of recurrences.

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Quality Assurance of *Boswellia* Resins by TLC and GC-MS

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Olibanum, also called **Frankincense,** is an aromatic resin obtained from trees of the genus *Boswellia*, particularly *Boswellia carterii* (syn. *B. sacra*, *B. thurifera*) (Burseraceae). Olibanum is tapped from the very scraggly but hardy *Boswellia* tree by scraping the bark and allowing the exuded resins to bleed out and harden. The terpenes of the resins or oils are located in excretion or resin channels of the bark or wood of stems or roots.

Olibanum (*B. carterii*) has been traded on the Arabian Peninsula and in North Africa for more than 5000 years. It is used in incense as well as in perfumes. **Olibanum** was lavishly used in religious rites as an ingredient for incense.Gold, olibanum and myrrh were among the gifts to Jesus by the three Magi. The resin of *B. frereana* is known for its sweet smelling qualities. It is used as a deodorant, as a flavouring for food, drink and toothpaste.

In Ayurvedic medicine **Indian frankincense** (*B. serrata*) has been used for hundreds of years for treating arthritis. In Traditional Chinese Medicine (TCM), *B. carterii* and *Pistacia lentiscus* (Mastix) have been used as "Ru Xiang" (**olibanum**).

Nowadays, standardized preparations of Indian frankincense from *B. serrata* are being investigated in scientific studies as a treatment for chronic inflammatory diseases such as Crohn's disease, ulcerative colitis and osteoarthritis. The main active compounds of Indian incense are viewed as being boswellic acids and tirucallic acids. These acids are present in the other species as well and that is why they can be used alternatively as leukotriene inhibitors.

The most successful technique used in the separation of volatile terpenes is their direct resolution by GC-MS. For non volatile terpenes (>diterpenes) silylation has to precede the GC separation or other chromatographic techniques are needed. For a rapid quality assurance TLC separation and derivatization with anisaldehyde reagent will be the method of choice. TLC reveals fingerprints of the essential oils and resin extracts which are strikingly different and assign to the species or products in particular. But some specific volatile terpenes lack functional groups and need to be analyzed by GC-MS:

Octyl acetate with its pungent smell, e.g., is a remarkable feature of olibanum (*B. carterii)* resins. It can only be detected by GC.

Verticilla-4(20), 7,11-triene is very specific for *B. carterii*. It can be detected after derivatization with anisaldehyde reagent, but the Rf values of the bright violet bands does not differ very much from the various cembrenes of the other species. On the other hand the very specific diterpenes of *B. carterii*, incensol and incensol acetate, are clearly visible.

B. serrata is characterized by another diterpene, serratol (cembrenol), whereas *B. frereana* reveals the bluish kessane , violet dimers of α-phellandrene and the violet lupeol. Again, TLC is recommended for detection of decomposed substances. During processing of the resin - e.g. to obtain the volatile (essential) oil, the acidic fraction or a medicine according the TCM procedures - esters are hydrolyzed or hydroxyl groups are oxidized and dehydrated. Two-dimensional TLC procedures are very suitable to verify this course of events. This will be demonstrated by an example, e.g., the conversion of 11-hydroxy-β-boswellic acid to 9,11-dehydro-β-boswellic acid. The representative videoscan was also denoised (as whole image) by various image denoising techniques: averaging, circular averaging, Gaussian averaging, 2D Savitzky-Golay filter, 2D FIR filters, adaptive (Wiener) filter, median filter and wavelet shrinkage. The results, compared with reference denoised image (obtained by grabbing multiple frames) showed that median filter is the winner of this competition. Wavelet shrinkage with Haar mother wavelet and high decomposition level is also very sufficient way to videoscan denoising.

Application of Pressurized Planar Electrochromatography for chiral separations

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Pressurized planar electrochromatography (PPEC) has been known for five years since Nurok et al. [1] published the first paper on this mode in 2004. Principle of the mode is based on application of electroosmotic effect to drive into movement a solution of the mobile phase relative to an adsorbent layer of the chromatographic plate. PPEC process is performed in a closed system – the adsorbent layer of the chromatographic plate is covered with special foil under pressure. In that way vapor phase is eliminated from the separating system of PPEC, contrary to the nomal thin-layer chromatography mode. It should be underlined that the PPEC process can be performed under equilibrium conditions similar as it is in the column liquid chromatography. So the chromatographic conditions of PPEC system are similar to those of HPLC and capillary electrochromatography (CEC).

Important advantages of PPEC mode such as high performance (similar to HPLC) and short time of separation process (even 24 times shorter separation with PPEC than with TLC) have been reported in publications[1-4]. Another advantage concerns the change of separation selectivity relative to liquid chromatography modes. It is especially valid, when ionized compounds are separated.

PPEC mode has been applied by our group for the enantiomer separations. In the first paper on this subject [5], it was demonstrated that this new mode is very attractive for enantiomer separations by the Ligand-Exchange Chromatography mechanism. Enantiomer resolution obtained with PPEC mode is considerably enhanced in comparison with TLC. The time of the separation process can also be significantly shortened in comparison with TLC. It was observed that variables such as polarization voltage, modifier concentration, pH and buffer concentration of the mobile phase influence migration and separation selectivity of the enantiomers. Influence of these variables on separation of enantiomers in PPEC systems will be discussed in this presentation.

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ONE-DIMENSIONAL CHROMATOGRAPHY VERSUS SPECIAL MODES OF DEVELOPMENT FOR CONSTRUCTING MEDICINAL PLANT FINGERPRINTS

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In recent years, the need for quality assurance tools to ensure the identity, purity, and quality of botanical materials has risen dramatically. Among a variety of quality control methods, chromatographic fingerprinting has gained more attention recently. HPTLC with digital scanning is becoming more and more popular in chemical fingerprint construction, mainly due to its ability to produce picture-like images, that can be stored as documentation, and easily compared with other images. However in case of very complex samples, fingerprints constructed by a single chromatogram, are usually inadequate. One of the solutions of the problem is the application of multidimensional and/or multimodal thin-layer chromatography methods for fingerprints' development. The use of special modes of development enables the resolution of substances being structural analogues or spanning a wide polarity range.

In the analysis of botanicals and herbal preparations the following modes of multidimensional planar chromatography have been commonly applied: comprehensive 2D planar chromatography realized on mono- and bilayers, coupled-layer chromatography, combination of MD-PC (multidimensional planar chromatography) techniques and hyphenated methods.

The presentation compares the traditional and multidimensional techniques for fingerprint construction, advantages and disadvantages of both methods are outlined, as well. The use of special modes of development for fingerprint construction of the following herbs is presented: *Heracleum* spp, , *Peucedanum* spp., morphologically similar plants from the family *Apiaceae*, *Salvia* spp. and *Eleutherococcus* spp. The examples of secondary plant metabolites, that are well resolved with the use of special modes of development, are described, too. Information are also given in what cases, the special modes of development should not be used, as may lead to decomposition or chemisorption of the analyzed compounds.

MOLECULAR MASS ANALYSIS OF STAR-SHAPED POLYMERS

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Keywords: luminescence, carbazolyl group, star-shaped polyethers

Star-shaped polyethers (Fig 1.) were obtained by the anionic polymerization of various oxirane monomers in the presence of cyclic oligo(potassium glicydoxide) as the macroinitiator. Polymerization processes were controlled with IR spectroscopy and Size Exclusion Chromatography. The end-products obtained were analyzed with GPC and MALDI-TOF systems.

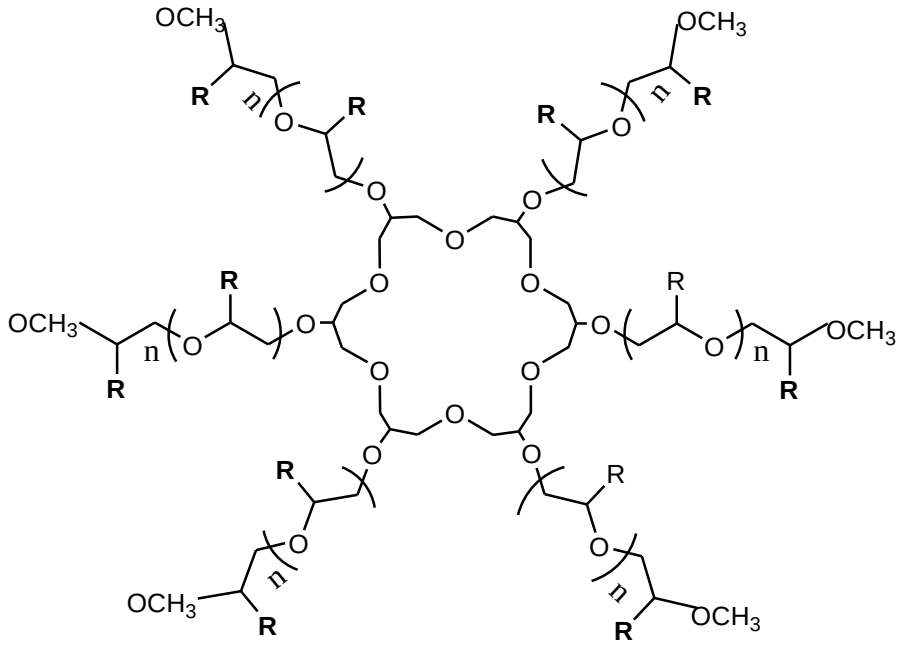

Figure 1. The structure of star-shaped polyether

It was found that the SEC technique allows to control the anionic polymerization of oxirane monomers and the MALDI-TOF technique gives information about the structure as well as molecular masses and dispersity of the polymers.

POSTER PRESENTATIONS

HSP FOR PHARMACEUTICAL ACTIVE SUBSTANCES BY MEANS OF IGC

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In a pharmaceutical design and preparation of different formulations very important is knowledge of properties of their components (an active material, excipient). Determination of some physicochemical parameters can facilitate a description of behavior of different materials in real systems, including such phenomena as: miscibility, adhesion and wetting. Proposed by Hansen three dimensional solubility parameters ($\delta_d, \delta_p, \delta_h$) or Hansen solubility parameters (HSP) consists of three components δ_d, δ_p i δ_h representing dispersive, polar and hydrogen bonding contribution, respectively.

Determination of the HSP for solid materials, in comparison to liquid materials, is relatively difficult. HSP values for Ibuprofen have been estimated by using an indirect technique, gas-solid chromatography method. It was based on the model of adsorption described by Snyder and Karger and requires the knowledge of value of adsorption energy for the respective test solutes.

$$-\Delta E^A = V_i\left(\delta_d^i\delta_d^j + \delta_p^i\delta_p^j + \delta_h^i\delta_h^j\right). \tag{1}$$

When *N* test solutes are used in IGC experiments one obtains a system of *N* equations corresponding to eq. (1) shown below in matrix form:

$$\begin{pmatrix} -\Delta E_1 \\ \cdots \\ -\Delta E_n \\ \cdots \\ -\Delta E_N \end{pmatrix} = \begin{pmatrix} V_1\delta_{1d} & V_1\delta_{1p} & V_1\delta_{1h} \\ \cdots & \cdots & \cdots \\ V_n\delta_{nd} & V_n\delta_{np} & V_n\delta_{nh} \\ \cdots & \cdots & \cdots \\ V_N\delta_{Nd} & V_N\delta_{Np} & V_N\delta_{Nh} \end{pmatrix} * \begin{pmatrix} \beta_1 \\ \cdots \\ \beta_n \\ \cdots \\ \beta_N \end{pmatrix} + \begin{pmatrix} \varepsilon_1 \\ \cdots \\ \varepsilon_n \\ \cdots \\ \varepsilon_N \end{pmatrix} \tag{2}$$

$$Y = X\beta + \varepsilon \tag{3}$$

where: *Y* is the column vector containing the *N* values of experimental measurements of the energy of adsorption ($-E_n$) of *N* solutes, X is the experimental matrix, formed of elements (X_{nk}), where $X_{nk} = V_n\delta_{nk}$, V_n is the molar volume of the n^{th} solute and δ_{nk} is one of the Hansen solubility parameters of type *k* (*k* = *d*, *p*, or *h*) of the respective solute. The β vector contains the real values of HSP of the adsorbent, i.e. δ_{jd}, δ_{jp}, δ_{jh}.

The experimental procedure will be presented with discussion of the possible sources of inconveniences and/or errors.

QUANTITATIVE STRUCTURE-RETENTION RELATIONSHIPS OF SOME SCHIFF BASE LIGANDS AND THEIR COMPLEXES BY THIN LAYER CHROMATOGRAPHY ON SILICA GEL

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The aim of this work was development of a model which defines the effects of molecular structure of some Schiff base ligands and their copper(II) complexes on the separation mechanisms and prediction of chromatographic behaviour of the structurally similar compounds. Copper(II) complexes contain ligands obtained by condensation ethane-1,2-diamine or propane-1,2-diamine as the amine part and pentane-2,4-dione and/or 1-phenylbutane-1,3-dione; pentane-2,4-dione and/or 1,1,1-trifluoropentane-2,4-dione; 1,1,1-trifluoropentane-2,4-dione and/or 1-phenylbutane-1,3-dione as a β-diketone.

In order to obtain predictive and interpretative models, we performed chromatographic separations by thin layer chromatography on silica gel plates, using a Camag horizontal HPTLC development chamber in the tank configuration with five mono- and nine two-component mobile phases. The obtained R_F values were converted to R_M before performing computations. All structures were optimizated with the HyperChem 7.0 program. In order to obtain structural electronic descriptors, the geometry optimization of molecules was performed by the molecular mechanics MM+ force field method, followed single-point calculation with the semi-empirical quantum chemical method ZINDO/1. Chemical descriptors: volume, surface area, energy of the highest occupied molecular orbital, energy of the lowest unoccupied molecular orbital, dipole moment, refractivity, hydrophilic-lipophilic balance, ClogP and polarizability were calculated from the structure and related to their retention parameters by multiple linear regression analysis. The best model was selected based on the multiple squared correlation coefficients (r^2), the mean square error (MSE) and the value of Fischer significance, *F*-value (a statistic for assessing the overall significance). The predictive power of the QSRR models was validated by a leave-one-out cross validated analysis. These correlations provide insights into the mechanisms of chromatographic retention on a molecular level.

A VERY FAST AND SIMPLE METHOD FOR THE ANALYSIS OF SULFONAMIDES IN SOIL SAMPLES BY LC-MS/MS IN THE MRM MODE

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One of the oldest groups of veterinary chemotherapeutic agents, sulfonamides have been widely used for more than fifty years, thanks to their low cost and their broad spectrum of activity in preventing or treating bacterial infections. Nowadays, sulfonamides and other antibiotics are regularly detected in a wide variety of environmental samples, including natural waters, sediments and soils. Since the environmental concentrations of sulfonamides are usually very low and their occurrence multicomponental, their determination in these matrices still pose significant analytical problems. The present paper describes the optimization of ESI-MS/MS parameters and the chromatographic separation of twelve sulfonamides commonly used in veterinary medicine. The methodology developed in this study, unlike many others, satisfied the criteria of EU Commission Decision 2002/657/EC, which defines the criteria for both screening and confirmatory methods with respect to drug residues on the basis of identification points. Each MRM transition was tested not only for the qualitative but also for quantitative analysis of sulfonamides. The method was validated for its analytical performance parameters and applied to the determination of sulfonamides in soil samples.

THERMODYNAMICS OF INTERACTIONS OF OLEFINS WITH STATIONARY PHASES CONTAINING CO(II) AND NI(II) SALTS CHEMICALLY BONDED TO SILICA

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Coordinative compounds of transient elements included in a stationary phase for CGC are capable of selective interactions with electron-donor adsorbates, thus creating reverse complexes of different stability. Taking into account the fact that creation of complexes is a very selective process which depends not only on structures of complexing compounds, but also on a temperature, using such complexes in chromatography research makes it possible to separate compounds of similar chemical structure and boiling temperatures, e.g., different types of isomers, isotopes included. Large number of factors showing an influence to the stability of the created compounds let control the retention to obtain required separations. Moreover, the feasibility of wide control of the above parameters makes the packings interesting not only from the analytical, but also from physical and chemical points of view. These packings allow for investigation of interactions among sorbates and complexes of transition metals.

This poster presents some results of studies of packings containing Co(II) and Ni(II) salts chemically bonded to silica via β-diketonate groups. We concentrated our research on the packings containing such metal salts as chloride (Cl), acetylacetonate (acac), and hexafluoroacetylacetonate (hfac). Special attention was paid to fluorinated acetylacetonate, because a replacement of hydrogen atoms of methyl groups, present in the acetylacetonate ligand, by fluorine atoms results in a decrease of values of σ-donor and π-donor properties of the ligands. At the same time the capability of bonding additional ligands to the metal increases.

To characterize the packings investigated, the specific retention volume (V_g) was measured and used to calculate the following thermodynamic parameters: free energy of adsorption (ΔG_a), heat of adsorption (ΔH_a), and entropy of adsorption (ΔS_a). These parameters enable a characterization of specific interactions among adsorbate molecules and metal complexes chemically bonded to a silica surface. The study was performed using the above-mentioned adsorbates for the analysis of aliphatic linear and branched hydrocarbons.

INTERACTIONS AMONG ELECTRON-DONOR COMPOUNDS AND PACKINGS MODIFIED WITH CYCLAM-TRANSITION METAL COMPLEXES

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Nowadays there is continuous need for selective gas chromatography stationary phases, to be applied not only to analytical chemistry, but also to the pharmacy and other similar application domains. Development of such phases can help to achieve better resolution parameters in many difficult chromatographic separations. Recently, significant attention has been drawn to macrocycles. Macrocycles are useful in the determination of large group of analytes, mainly due to their ability to form specific interactions with many other molecules. For this reason macrocyclic stationary phases are highly specific towards many organic and inorganic molecules.

This work is devoted to the newly synthesized packings with cyclam (1,4,8,11-tetraazacyclotetradecane) and it's complexes with copper and cobalt, chemically bonded to silica surface. The packings under study were investigated by means of elemental analysis, DSC, EPR, and UV-VIS techniques. The work depicts interactions among the synthesized stationary phases and a given groups of electron-donor compounds, to be applied for gas chromatography.

The work is concentrated on a description of the synthesis of above-mentioned stationary phases, as well as an evaluation of their application for gas chromatography. The research aims to emphasize the specific interactions among the complexes formed at the silica surface, and simple compounds, such as ethers, aromatic compounds and other electron-donor molecules. A discussion follows regarding the structure-related factors in turn determining separation processes on macrocyclic stationary phases.

EVALUATION OF APPLICABILITY OF SPME/HPLC IN TRACE ANALYSIS OF EXPLOSIVES

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Most of the explosive compounds are thermally unstable, thus the method of choice in theirs analysis is HPLC. In case of solid samples (e.g. material taken from the place of explosion) the analytes must be transferred to the proper solvent, miscible with the HPLC mobile phase. The method which is used most often is solvent extraction. Important drawback of this method is lack of selectivity - besides analytes, numerous interfering compounds are co-extracted from the sample making difficult or sometimes even impossible to detect/determine the analysed compounds. Some authors propose to solve this problem by introducing the additional step to the procedure of samples preparation, i.e. application of SPME for selective adsorption of analytes, previously extracted from the samples with organic solvent. SPME/HPLC interface is commercially available (e.g. Supelco). The aim of presented research was to verify if SPME can be used to selectively concentrate explosives before HPLC analysis.

A standard mixture of 8 explosives was used in the research containing 1 mg/cm^3 of following compounds: RDX; HMX; TNT; nitrobenzene; 1,3-dinitronitrobenzene; 2,4-dinitronitrobenzene; 1,3,5-trinitrobenzene and 2-amino-4,6 dinitrotoluene. PDMS/DVB, PA, PDMS and CW/TPR fibers were tested. The preliminary experiments conducted according to the conditions taken from published papers (Furton K. et al. J Forensic Sci 2000, 45(4), 857-864), enabled to rank the fibers in order of increasing efficiency, as follows: PDMS, CW/TPR, PA and PDMS/DWB. Optimization research were carried out for PDMS/DVB and the optimal conditions were established: concentration of NaCl water solution: 25%, ratio of volume of acetonitrile 'extract' and NaCl solution: 1:99; adsorption time - 60 min with maximum stirring rate, followed by 20 min of passive desorption with acetonitryle. The most important observation was that the recoveries were different for different compounds, and generally very low (few percent). Additionally the resolution was very poor. These problems were not caused by chromatographic system itself or SPME/HPLC interface as it was regularly checked by injecting the standard mixture directly to the desorption chamber. Results of preliminary experiments conducted to check efficiency of SPME/HPLC did not prove it to be as useful tool in trace analysis of explosives as one could think on the basis of the results presented in papers.

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DEVELOPMENT AND EVALUATION OF HPLC METHOD FOR DETERMINATION LAMOTRIGINE IN SERUM

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Lamotrigine is a phenyltriazine anticonvulsant drug which is chemically and pharmacologically unrelated to currently used antiepileptic medications. It acts by inhibiting pre-synaptic voltage-sensitive sodium channels and excitatory neurotransmitter release (principally glutamate), and inhibits repetitive firing for action potentials characteristic of epileptic foci. It has been effective against refractory partial seizures, as well as generalised tonic-clonic seizures and other generalised seizures.

A simple HPLC method was developed and validated for quantitation of lamotrigine in human serum. The samples were alkalinised with sodium hydroxide solution (1M), and then lamotrigine together with internal standard (phenacetin) were extracted from serum samples using dichloromethane. The HPLC separation was performed on C18 LiChrospher column (125mm x 4 mm). The mobile phase was a binary mixture of acetonitrile-water (25:75, v/v) modified with ortophosphoric acid to adjust pH to 2.7. Chromatograph was operated at flow rate 1.8 ml/min and the UV detector was set on 207 nm. Under those circumstances the retention times for lamotrigine and phenacetin were 3.9 min and 3.0 min respectively. Additionally, under operation conditions chosen not any interferences with endogenous substances were observed. Therefore the elaborated method can be classified as specific.

The method was statistically validated for specificity, linearity, precision, accuracy, quantitation limit and the percent recoveries of lamotrigine. Such a statistical evaluation is usually required by the major drug registration agencies. Concluding, the method was found to be simple, specific, precise, accurate, and reproducible. Therefore it might be stated, that the described method is suitable for pharmacokinetic investigations as well as in program of therapeutic drug monitoring.

The elaborated method was applied to pharmacokinetic and also bioequivalence studies. Bioequivalence means comparison of two pharmacokinetics which characterise studied and reference drugs. Mention above studies were performed on 24 healthy volunteers.

APPLICATION OF DIFFERENT HPLC DETECTORS FOR ESTIMATION OF DRUG SUBSTANCES CONCENTRATION IN HUMAN BLOOD

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Pharmacokinetics and or bioequivalence studies required precise and reliable determination of drug substances in blood on low concentration levels, ranged from μg to pg per mL, depending on the drug dose and individual response. Up till now the HPLC method is widely accepted for drug substance concentration measurements. Hence, the detector sensitivity is very important factor. The most popular in such a system is UV detector, although in many applications it is not enough selective and sensitive for these purposes. Therefore various other detectors were applied, i.e. refractometric, light dispersion, fluorescence, electrochemical and so on. Currently, the HPLC-MS/MS systems are subject to a lot of advertisements, simply because they are sometimes provide opportunity to simultaneous qualitative and quantitative analysis of drug substances in biological fluid. Nevertheless it is not a general rule. Hence the previous instrumental methods of detection are still used.

The work which will be presented concerns determination of three drug substances, i.e. carbamazepine, glibenclamide and propafenone in human serum. Taking into account different physicochemical properties of these substances various detectors were applied.. Carbamazepine was successfully determined using UV detector, glibenclamide requires fluorescence detector and propafenone amperometric detection.

Elaborated methods were fully validated in accordance to the world widely registrations agencies requirements. Chromatograms, results of methods validation will be presented. Additionally some pharmacokinetic data will be discussed in connection with analytical results achieved.

CHROMATOGRAPHIC SEPARATION AND ISOLATION OF ARYLSTILBAZOLIUM CIS/TRANS ISOMERS

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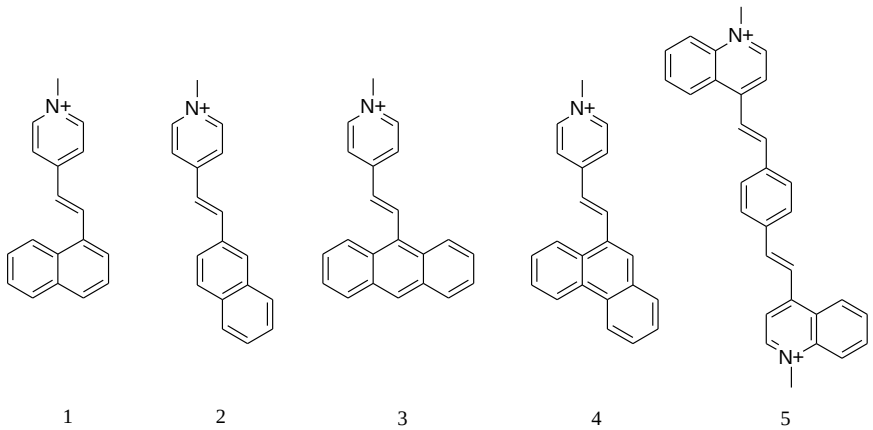
The subject of the study is separation and isolation of *cis* and *trans* isomers of arylstilbazolium ligands. The isomeric forms of arylstilbazolium derivatives (Figure 1) can be separated by HPLC technique on the analytical scale, as we reported previously [1]. In this study, we report the preparative separation of *cis-trans* forms of the compounds 1 – 5, in order to perform DNA binding studies for particular isomers.



1 2 3 4 5

Figure 1. Molecular structure of *trans* isomers of arylstilbazolium ligands.

It is known, that *cis*/*trans* isomerization might influence ligand/DNA complex formation [1]. Using an equilibrium dialysis experiment we observed binding between arylstilbazolium *trans* isomers and DNA [2]. It has appeared that arylstilbazolium derivatives can stabilize different structures of nucleic acids. What is very interesting, ligands **1** and **4** show the highest binding preference for the quadruplex formed by C-MYC oncogene sequence. It is worth verifying if *cis* isomers bind DNA stronger than *trans* and complete binding equilibrium studies are required.

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SALTING-OUT THIN-LAYER CHROMATOGRAPHY OF SOME PYRIDINIUM SALTS

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Several unsubstituted and substituted pyridinium salts bearing a 4-oxothiazolidinyl moiety were studied by salting-out thin-layer chromatography (SOTLC). As it is known, pyridinium salts are interesting compounds as they show antimicrobial activity. Also, molecules containing the 4-oxothiazolidinone ring are known to possess a wide range of biological properties. Therefore, these compounds, as congeneric group of substances, could be an ideal choice to prove suitability of SOTLC parameters in QSAR analysis.

Chromatographic investigations were performed on cellulose and silica gel plates. Aqueous ammonium sulfate solutions of different concentration were used as solvents. A linear relationship of the corresponding R_M values and ammonium sulphate content in the solvent was observed. Regression data of the plots obtained were used to determine the lipophilicity parameters R_M^0 and C_0. Lipophilicity determined in this way was correlated with calculated logP values. Satisfactory correlation between the slope m and the intercept R_M^0 is indicative of the suitability of SOTLC for lipophilicity estimation.

THE COMPARATIVE ANALYSIS OF TWO-DIMENSIONAL CHROMATOGRAPHIC SIGNALS WITHOUT PRIOR SIGNALS ALIGNMENT

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Hyphenated chromatographic techniques, e.g., chromatographic techniques coupled with mass spectrometry, are frequently used to obtain fingerprints of complex samples, for instance, biofluids (e.g. urine and serum samples), environmental samples, peptides and food samples. As a result of an analysis, two-dimensional analytical signals are obtained [1]. They are represented by a two-way data table with the abundance values observed for a given retention time and mass to charge ratio (*m*/*z*) as its elements.

Unfortunately, instrumental instabilities that are often encountered during an experiment cause peak shifts in signals [1]. It is a serious problem when the samples are to be compared by means of chemometric method. Therefore, prior to their comparative analysis of samples alignment of the time and *m*/*z* dimensions in all samples is a must.

Usually, three strategies for handling peak alignment are considered. These are: (1) identification of peaks and matching them across all signals [2], (2) correction of peak shifts using different alignment techniques [3] and (3) comparing a set of two-dimensional signals without any prior alignment using approach developed by Danielsson et al. [4].

Here, yet another concept of comparing individual two-dimensional signals that falls into the third category of the discussed approaches is discussed [5]. It is based on a new chromatographic, the so-called Gram matrices, $\mathbf{X}\mathbf{X}^{\mathrm{T}}$ (*m*/*z*×*m*/*z*). In this approach, we assume no peak shifts in the *m*/*z* direction. To compare individual Gram tables (now describing individual samples) the Rv coefficients [6] are used.

In order to illustrate performance of this strategy and methods developed by Danielsson et al. [4] real data was a subject of comparative analysis using different chemometric methods. The major advantages of the new method for comparing the two-dimensional chromatographic signals are: simplicity, fast performance, no input parameters, good visualization properties and possibility to construct unsupervised and supervised models for samples.

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CHEMOMETRICS IN COMPARATIVE ANALYSIS OF CHROMATOGRAPHIC FINGERPRINTS OF THE EXTRACTS FROM DIFFERENT SAGE (*SALVIA* L.) SPECIES

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Recently, we have witnessed to a considerable growth of interest in characterization of chemical samples by their analytical signals obtained from the fingerprinting techniques [1]. Among different analytical techniques well-suited for the fingerprinting purposes, chromatographic methods have their special place, since they offer a possibility to separate and quantify components of the analyzed mixtures. With very complex samples (e.g., those of natural origin), their chromatographic profiles (fingerprints) are often used for characterization and comparison. The comparative analysis of samples on the basis of their fingerprints is very challenging, since the chromatographic data have large dimensionality and individual chromatographic signals often require specific pre-processing. The comparative analysis of the samples can largely be facilitated using chemometric methods. In chemometric arsenal, many approaches can be found developed to improve the quality of individual chromatographic signals (methods for signal denoising, background removal and peak alignment [1]) and to visualize similarities among the samples (clustering techniques [2] and projection methods [3]).

The goal of this study is to present the chemometric strategy for the analysis of complex samples of herbal extracts from the different sage (*Salvia* L.) species. Its performance is illustrated on two experimental data sets of chromatographic fingerprints obtained for twenty different sage species, known to possess pharmacological activity. In our study, extracts from the different sage species were characterized using the two types of chromatographic fingerprints, i.e., those obtained from HPLC [4] and the head-space GC/MS [5]. Further, the sage fingerprints were pre-processed and compared by means of different chemometric approaches.

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PUTRESCINE, A PRECURSOR FOR THE FORMATION OF *N*-NITROSAMINES DURING MEAT PROCESSING?

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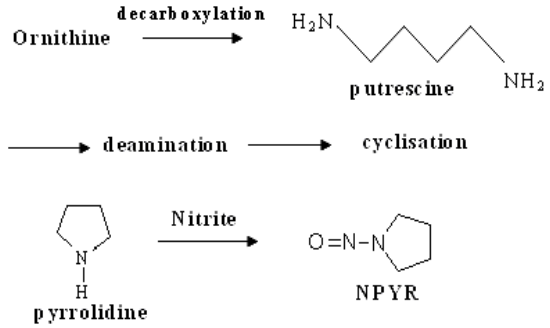
During the processing of cured meat products, *N*-nitrosamines (NAs) may be formed out of the reaction between nitrite, a protective agent against *Clostridium Botulinum* and colour and flavour stabilisator, and N-containing substances being naturally present in meat, such as amino acids and secondary amines. As demonstrated below, *N*-nitrosopyrrolidine (NPYR) might be formed out of pyrrolidine, the deamination and cyclisation degradation product of putrescine, the biogenic amine:



The aim of this study was to determine the role of putrescine in the formation of *N*-nitrosamines in cured meat products. Experimental evidence was produced using gas chromatography in combination with Thermal Energy Analyzer (GC-TEA). The obtained analytical results were statistically evaluated by means of the Univariate Analysis of Variance ANOVA approach. The meat samples (model-type) were prepared with different nitrite concentrations (0, 120, 480 mg/kg meat resp.), variable temperatures (85 °C, 120 °C, 160 °C, 220 °C resp.) and different concentrations of putrescine (0, 10, 100, 1000 mg/kg meat).

Although in literature putrescine has been described as a precursor of NPYR, the presence of NPYR in the tested meat products could not be confirmed, except for extreme "not-realistic" conditions (1000 mg/kg meat) in which 0,77 – 1,01 µg/kg NPYR were detected. As a consequence, it could be concluded that putrescine is no N-source for the formation of NAs. Nevertheless, the higher the temperature of the meat processing heating procedure and the higher the amount of sodium nitrite added, the higher the concentration of another formed NA, i.e. *N*-nitrosodimethylamine

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METHODS OF DETERMINATION SELECTED PRIORITY SUBSTANCES IN SAMPLES CHARACTERISING WATER ENVIRONMENT: IN FLOWING WATERS, SEDIMENTS AND ALLUVIUM.

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The reason for undertaking this research was the Water Framework Directive, which defines framework of cooperation in the field of water policy. The directive gives parameters of water chemical state assessment made by indicating substances with proven or highly probable, espiecially harmful effect on ecosystems and water of so called priority substances.
The aim of this work was to devise methods of determination all priority substances characterised as priority hazardous substances: hexachlorobenzene, hexachlorobutadiene, hexachlorocyclohexane, pentachlorobenzene, chloroalkanes C10-13, pentabromodiphenyl ether, nonylphenols, trybutyltin hydride and benzo(a)pirene in samples of water, alluvium and bottom sediments.
For determination of analised priority substances, researchers used a gas chromatograph compound with mass spectrometer GCMS-QP2010S produced by Shimadzu. The following three methods of data acquisition were developped: SCAN method that monitors mass numbers in whole scope of measurement, SIM method that minitors a selected number of ions and EX-SIM method, which also monitors selected ions, but in a considerably narrower scope than SIM method.
The following two methods of samples of sediment and alluvium extraction were compared: the ultrasonic extraction and accelerated solvent extraction in conditons of raised temperature and pressure (ASE). Each technique was tested in a system of two extraction mixtures: hexane/acetone and hexane/dichloromethane/acetone. A technique of extraction to stationary phase (SPE) was choosen for water samples, comparing the two kinds of sorbents, C18 phase and copolymer Amberlite XAD-4.
The usefulness of the devised methods was estimated by comparing obtained limits of quantification of examined substances with limit values according to ordinance of the Ministry of Environment 20 August 2008 regarding the way of classification of surface water state (DZ. U. Nr 162, poz. 1008)
The devised methods were tested on naturaral environmental samples collected near the point of idustrial wastewater discharge to river.

DETERMINATION OF LIPIDS FROM *DENDROLIMUS PINI* EXUVIAE OBTAINED FROM LARVAL - LARVAL MOULT BY HPLC-LLSD AND GLC-MS

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The cuticular surface of insects is covered by a thin layer of lipids, the function of which is to restrict water evaporation and provide chemical communication. Lipids also reduce the penetration of chemicals, provide an interface between insects and its environment. This layer contains mainly free lipids consisting aliphatic polar and non-polar compounds. *D. pini* effectively resists many insecticides, but it can be controlled by the use of bioinsecticides such as entomopathogenic fungi. For the use of microbial agents for biocontrol of *D. pini*, it is of importance to identify cuticular lipids of this pest, if we are to understand the factors responsible for preferential adhesion or selective repulsion of entomopathogenic fungi that are potentially useful for biocontrol.

Lipids from *D. pini* exuviae were extracted by petroleum ether and then a second time in dichloromethane. The exuviae extracts were separated into several classes of compounds using high-performance liquid chromatography (HPLC) in the normal phase. Laser light scattering detection (LLSD) was used as the detection system. HPLC with LLSD detector is infrequently use for determination of cuticular insects lipids, though it gives very good results in the separation of lipids [1]. GLC–MS analysis was performed with a SSQ 710 equipped with an HP 6890 GC. The lipids in *D. pini* exuviae were found to consist of hydrocarbons, triacylglycerols, and free fatty acids. They were identified on the basis of the characteristic ions e. g. fatty acids were identified on the basis of the characteristic ions of silyl derivatives (m/z=73, m/z=117, m/z=129, m/z=132, m/z=145, $M^{+\cdot}$ and $(M-15)^{+}$)

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VALIDATION METHOD FOR DETERMINATION PERFLUORINATED CARBOXYLIC ACID BY GAS LIQUID CHROMATOGRAPHY

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Perfluorinated carboxylic acids (PFCAs) and the salts of thereof have been recognized as an important class contaminants in the global environment. Perfluororinated carboxylic acid are the subject of the big number of examinations. It is necessary to have the suitable methods of the quantitative and quality analysis.

A rapid method for the derivatization, extraction and determination of the perfluorinated carboxylic acid has been developed. The analytical procedure for the synthesis of PFCAs derivative was based to one described earlier [1]. The modifications were aimed at decreasing time of the analysis and improving efficiency of the reaction of the synthesis.

The perfluorinated acids were identified on the basis of characteristic anilide derivative ions. GLC-MS analyses and then GLC of the same samples were used for identification of each signals PFCAs in GLC chromatograms.

The GLC method was validated for linearity, sensitivity, specificity, inter-day and intra-day precision and accuracy. Overall, the method developed in this study for the measurement perfluorinated acids are robust; they are capable of measuring the target compounds. The method can be applied in the analysis of water, biological and technological matrices, so that we can better understand the fate of perfluorinated compounds in the environment.

The analyses were carried out on a Clarus 500 (Perkin Elmer) gas chromatograph. The RTX 5 column was used. Confirmation analysis of perfluorinated carboxylic acid derivatives was carried out by GLC/MS in the selected ion monitoring (SIM) mode and total ion current (TIC). Mass spectra (70 eV) were recorded on a SSQ 710 mass spectrometer (Finnigan). The samples were introduced through a Hewlett-Packard 5890 gas chromatograph.

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QUALITATIVE HPLC ANALYSIS OF PHENOLICS IN MOLDAVIAN DRAGONHEAD (*DRACOCEPHALUM MOLDAVICA* L.) IN DIFFERENT PLANT ORGANS.

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The Dragonhead genus (*Dracocephalum* L.), which belongs to the *Lamiaceae* (*Labiatae*) family, consists of about 40 -70 species. They are annual, biennial or perennial – mostly herbaceous plants that appear in all northern hemisphere.

Moldavian Dragonhead (*Dracocephalum moldavica* L.) is the best known species of this genus, widely used as aromatic, medicinal and melliferous plant. In the past, it was used to adulterate *Melissae folium* (lemon balm leaf).

There are numerous papers concerning chemical composition of herb and seeds of Moldavian Dragonhead. The presence of volatile oil, phenolic acids (mainly rosmarinic acid), flavonoids, anthocyanins, tannins and coumarins can be found in the herb; in seeds there is fat oil with a high content of unsaturated fatty acids.

In our experiments we have focused on the HPLC qualitative analysis of phenolic acids and flavonoids in various plant parts (organs) of Moldavian Dragonhead collected in various ontogenetic phases.

The experiments were carried out on plant materials from *Dracocephalum moldavica* L. (roots, stems, leaves, inflorescences and fruits) collected in the Pharmacognostic Garden of Medical University of Lublin. Plant materials were collected in three phases of ontogenesis: 1. vegetative phase, 2. generative: in-bloom phase, 3. generative: fructification phase. Each plant material (after drying) was milled, sieved and exhaustively extracted in the Soxhlet apparatus using chloroform – to remove nonpolar ballast substances. Afterwards, the extraction with methanol allowed to obtain a polar fraction. Such obtained extracts were purified using SPE method (RP-18 cartidges, eluted with mixtures of methanol and water), and finally analysed using HPLC method in the following conditions: RP-18 / gradient of acetonitrile in water + 1% of acetic acid. Next, qualitative analysis of phenolic acids and flavonoids was performed. The presence of such compounds in Moldavian Dragonhead as: rosmarinic acid, caffeic acid, p-coumaric acid, protocatechuic acid, chlorogenic acid, wanillic acid, syringic acid, m-OH-benzoic acid, apigenin, 7-Glu-apigenin as well as other compounds was confirmed.

As the result of the performed experiments, qualitative and quantitative differences in the chemical composition of the examined plant organs have been determined.

APPLICATION OF GAS CHROMATOGRAPHY IN EXPERIMENTS OF HYDROGEN PRODUCTION IN STEAM-GASIFICATION OF CARBONACEOUS MATERIALS

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Hydrogen is widely recognized as a new, price competitive and environment friendly energy carrier. It can be produced among the others in the process of fossil fuels such or biomass gasification. Biomass advantage in these terms over for example coal stems from the fact that it may be converted into high quality fuels and electricity in gasification process with the amounts of CO_2 produced equaling the level of CO_2 fixed from the atmosphere by plants during their growth. In this way the process is considered to be not contributing to the increase of CO_2 concentration in the atmosphere.

The study was focused on comparative tests of hydrogen oriented steam gasification of coal and biomass with CaO added for hydrogen-rich gas production. The experiments were conducted in a laboratory scale fixed bed reactor at the temperature range of 675-900^{o}C in three series. The composition of the outlet gas mixture was analyzed online via a gas chromatograph and the amount of gas produced in the gasification process was measured with a flow meter.

In the first series the coal/biomass were gasified without addition of CaO whereas in the second and third series they were gasified with addition of CaO layered on a coal/biomass sample and mixed with a coal/biomass sample, respectively. The CaO increased both the hydrogen yield and content in gaseous products mixture in comparison with series I. Moreover, mixing of CaO with coal/biomass sample improved the effects in terms of hydrogen yield and concentration in outlet gas when compared to the experiments with CaO layered on a coal/biomass sample. An effective CO_2 absorption was observed in tests with CaO mixed with a coal sample and at relatively low temperatures. At higher temperatures CO_2 concentration increase in the product gas mixture was observed.

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY GAS CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION

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Policyclic aromatic hydrocarbons (PAHs) are the most widespread organic pollutant and are a group of compounds considered to be a potential health hazard, because of their mutagenic and carcinogenic properties. One of the source of PAH is combustion or pyrolysis of fossil fuels.

Measurement of pollutants concentration on real objects are known as reliable assessment measure of the quantity of the harmful substances inserted into the environment. Growing requirements of the environmental protection require continuous PAH monitoring in the environment.

Methodology of determining was worked out in ICHPW and relay onto two stages extraction. Next, extract is analysed by use of GC-MS. PAHs is identified by their retention times and confirmed by comparing their mass spectra with the mass spectra of the NIST library. Quantitation of sixteen US EPA specified PAHs and their methyl derivatives is done on the GC-MS in the selected ion monitoring (SIM) mode using the internal standard method.

ANALYSIS OF NICKEL COMPLEXES WITH SCHIFF BASE LIGANDS USING HPLC ASSAY WITH UV AND ELECTROCHEMICAL DETECTORS

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Schiff complexes are widely investigated from the end of nineteen centaury. They are important in the coordination chemistry as well as in metabolism processes in living organisms. From the chemical point of view they are imines RCH=NR` with the characteristic >C=N– group. They are able to coordinate transient metals creating many different structures (many different donors) and stabilize they different oxidation steps.

In the presentation separation of five nickel (II) complexes with Schiff base ligands has been investigated using RP-HPLC assay with UV and amperometric detectors. It turned out that good separation of all complexes was obtained at low (5°C) temperature. From the other side, at this temperature very wide (broaded) peak of the complex with salpn ligand was obtained. Probably it was caused by kinetic effects.

It was found that UV (DAD) detector can be used to monitor separation. Because central ion, Ni(II), as well as ligands can be easily oxidized/reduced we have also tested the amperometric detector. However, it turned out that non-sensitive, irreversible results were obtained. Therefore, the electrochemical behavior of separated complexes has been investigated in strong and weak donating solvents by cyclic voltammetry. Nickel (II) species can be reduced to nickel (I) in several solvents. Characteristic feature has been observed in weak donating solvents, nickel (II) complexes with Schiff bases ligands create conducting polymeric films at the electrode surface. The properties of nickel (II) complexes was compared in different solvents.

COMPARISON OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND CAPILLARY ELECTROPHORESIS IN QUANTITATIVE ANALYSIS OF FEXOFENADINE HYDROCHLORIDE IN HUMAN PLASMA

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The aim of this work was the development, validation and comparison of RP-HPLC and CE and method for determination of fexofenadine hydrochloride.

Fexofenadine is a second generation antihistaminic drug and is clinically effective in the treatment of seasonal allergic rhinitis and chronic idiopatic urticaria through the antagonism of histamine receptors H_1.

The determination of drugs in biological fluids in many clinical laboratories are generally performed by HPLC method. However, CE method has shown advantages over HPLC in terms of simplicity, shorter analysis time and less organic solvent consumption, which is great economical and ecologically benefit. Moreover, the major strength of CE is that the basic separation principles are different from those of HPLC and other chromatographic techniques.

Two reported analytical methods were developed and validated by analyzing a series of plasma samples containing fexofenadine hydrochloride in different concentrations. The extraction procedures are simple and no complicated purification steps in both techniques. The calibration curves for fexofenadine were linear over a range 20-600 ng/ml for HPLC and CE. The detection limits were 20 ng/ml and 40 ng/ml for fexofenadine with HPLC and CE (UV detector was applied for analysed substances in both cases), respectively. It provides approximately two-fold lower detection limit in case of HPLC, but total time of single separation was two times shorter in CE than HPLC. The separation efficiency are good for both techniques. CE as well as HPLC method is selective, robust and specific allowing reliable quantification of fexofenadine and can be useful tool for clinical and biomedical investigations.

Both proposed techniques may be applied as routine methods in bioavailability or bioequivalence studies and drug monitoring after administration of a fexofenadine therapeutic dose. These methods are sensitive enough for pharmacokinetic study at all intervals after dosing of drug and can be routinely applied to drug monitoring in adult subjects.

HPLC/UV VALIDATED METHOD FOR SIMULTANEOUS DETERMINATION OF TESTOSTERONE AND EPITESTOSTERONE IN HUMAN URINE

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The analysis of steroid hormones in biological samples in urine is routinely used in clinical diagnoses being an essential source of information concerning metabolic pathways, disorders of metabolism, occurrence of cancer on endocrine basis as well as the abuse of anabolic substances. Testosterone (T) is one of the endogenous anabolic androgen steroids and epitestosterone (ET) is the inactive 17α-epimer of testosterone. The ratio T/ET is used as a marker for T doping. According to the International Olympic Committee regulations, an athlete with a T/ET ratio greater than 4 is considered as potential user of T.

The aim of this study was to develop a quick method for determination of simultaneous determination of T, ET and T/ET ratio in human urine. The investigations were performed on a group of 112 healthy volunteers both sex.

A sensitive and simple high-performance liquid chromatographic (HPLC) method for simultaneous determination of T and ET in human urine was described. The urine samples containing conjugated steroids after hydrolysis at temperature 90 °C were transferred to solid phase columns (Merck, LiChrolut RP-18, 500 mg). Next the compounds were diluted using dichloromethane. Finally quantification of steroids in human urine was performed by RP HPLC method with UV detection at wavelength of 240 nm. Chromatographic separation was achieved on an analytical column C18 Nucleosil-100 (125 x 4 mm, 5μm). The mobile phase was acetonitrile and water (48:52 v/v). Under these conditions the retention times of T, ET and methyltestosterone (internal standard) were 4.9, 5.6 and 6.3 respectively. The method was validated for its linearity, accuracy, precision and selectivity. Because of its high sensitivity and reproducibility, this RP HPLC method with UV detection will be suitable for routine analysis of endogenous T and ET in urine samples. Moreover, the short time of determinations allows it to be applied for routine control of T doping in athletes.

COMPARISON OF DERIVATIZATION AGENTS FOR DETERMINATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS BY GC METHODS

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During the last decade pharmaceutically active compounds (PhACs) have been considered to constitute a health risk for humans and aquatic ecosystems. A variety of pharmaceuticals have been detected in many environmental samples worldwide. Due to their pharmacological activity their monitoring is necessary. Pharmaceuticals are present in environment at very low levels, therefore analysis of residuals of these compounds requires selective and very sensitive methods of detection. Non-steroidal anti-inflammatory drugs (NSAID's) are commonly used in human health care and in veterinary medicine applications. They are among the group of pharmaceutical compounds most often prescribed and also hundred of tons of these drugs are sold over-the-counter. NSAID trace residues have been detected in the effluent of water treatment plants, rivers and groundwater, which witnesses the massive use of these drugs in developed countries. The aim of this study was to compare three different derivatization agents used for the determination of non-steroidal anti-inflammatory drugs by gas chromatography in order to select the most proper one. The tested reagents were *N*-methyl-*N*-[tert-butyldimethyl-silyl]trifluoroacetimide (MTBSTFA), commercialy available mixture of 99% *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), 1% trimethylchlorosilane (TMCS) and trimethylsilyldiazomethane (TMSD). Trimethylsilyldiazomethane (TMSD) as a derivatization reagent was used for this class of compounds at the first time. The study was performed using ibuprofen, ketoprofen and naproxen as model compounds. GC analyses of native compounds were also performed. Influence of several parameters in the efficiency of derivatization step was investigated. The parameters identified as influential were the volume of derivatization reagents, time of the reaction and temperature conditions.

VALIDATION OF HEAT AND MASS TRANSFER MODEL FOR CHROMATOGRAPHIC COLUMN WORKING UNDER VERY HIGH PRESSURE DROP.

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The current trend in the liquid chromatography is toward the use of still finer particles in order to achieve faster analyses and higher throughputs. The requirement of the shortest possible time of analysis enforces the application of high mobile phase velocity, which causes a large value of head pressure to overcome the viscous forces. Operating columns in the optimum range of mobile phase velocities require pressure between 100 and 400 bar for High Pressure Liquid Chromatography (HPLC) and up to 1000 bar for Very High Pressure Liquid Chromatography (VHPLC), depending on the column length and the mobile phase viscosity.

The high back pressure causes heat generation inside column and as a consequence the formation of both a radial and an axial temperature, density, viscosity and velocity gradient.

All above gradients, finally cause a loss of column efficiency. It should be expected that the loss of column efficiency would depend on the method on temperature control of column wall (adiabatic column, thermostated column, column working in natural convection conditions).

To understand the phenomenon described above and the influence of column working conditions on the column efficiency, we have proposed the detailed model of heat and mass balance coupled with the model of mobile phase velocity distribution.

The aim of the work is to present that model and validation of heat balance model by comparing calculated and measured chromatography column wall temperature. The experiments were performed in HPLC as well as in VHPLC on column length from 3 to 25 cm filled with 1.7 and 5μm particles. The pressure drop was from several dozen to 1000bar. The agreement between calculation and experiment was good.

We will present also the theoretical results regarding the temperature and mobile velocity distribution distributions inside column working in different column wall control temperature conditions. The validation of full heat and mass balance model will be discussed in our second presentation [1].

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OPTIMALIZATION OF THE CHROMATOGRAPHIC CONDITIONS FOR THE TRACE ANALYSIS OF ENROFLOXACIN BY HPLC WITH UV-VIS DETECTION

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The biologically active substances referred to as pharmaceuticals are not used exclusively to protect human health: large amounts of these are also produced for the needs of husbandry and veterinary medicine. Hence, there are many ways in which these medicines can get to the environment. Their main sources are the following: pharmaceutical industry, farming and veterinary medicine, health service centers and household. It is estimated that in the majority of environmental matrixes, almost 50% of medicines occur in their basic form, while the rest take the shape of variously modified metabolites. In order to adequately asses the threats posed by the presence of pharmaceuticals in the environment, as well as to foresee their future behavior and to investigate the risks of toxicological exposure, it is crucial to analyze and identify both the metabolites and the products of these compounds' degradation. It usually takes place in natural conditions, as well as during wastewater and water treatment. Special attention is given to monitoring the mostly used medicines, as well as those that are especially durable. Enrofloxacin belongs to the group of fluoroquinolone antibiotics, which are antimicrobials marketed in human and veterinary medicine for treating a variety of bacterial diseases. It is a lipophilic and amphoteric antibacterial which is used exclusively in veterinary medicine. The aim of this work was to develop and validate a simple and sensitive analytical method for determining enrofloxacin in environmental samples by HPLC-UV-VIS method. The present work has been aimed at selecting the best possible parameters for the chromatography analysis of the compound in question, which includes the choice of: analytical wavelength, the composition and flow rate of mobile phase, the type of column, the program of gradient elution and the time of analysis. The optimal conditions for chromatography analysis of the compound were as follows: the analytical wavelength was 280 nm, a 5μm particle size, 250 mm × 4.6mm C_{18} reversed-phase column (Phenomenex, USA) was used, the mobile phase flow rate was 0.7 ml/min; it was the mixture of acetonitrile and trifluoroacetic acid (pH 3,4). The validation of the method has been carried out. Attempts at extraction and condensation of enrofloxacin from artificial seawater samples fortified by known amount of the analit have also been made by solid phase extraction (SPE) procedure.

APPLICATION OF CHROMATOGRAPHIC METHODS FOR DETERMINATION OF THE HETEROGENEITY STRUCTURE OF SALMONELLA TELAVIV O-POLYSACCHARIDE

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Non-typhoidal *Salmonella* species are a major cause of human gastroenteritis in many parts of the world. Most of these infections are zoonotic and are transmitted from healthy carrier animals to humans through contaminated food. Unlike other bacterial genera, *Salmonella* organisms are differentiated by serotyping analysis based on different specific types of somatic (O), flagella (H) and surface (Vi) antigens. The serological O-specificity is defined by the structure of the O-polysaccharide (OPS), which is a part of the lipopolysaccharide (LPS), one of the major component of the outer surface of smooth-type Gram-negative bacteria. More than 2500 serotypes (serovars) of *Salmonella* have been described. The *Salmonella* Telaviv bacterium belongs to the O:28 serogroup and its somatic O-antigen expresses the factors $O28_1$ and $O28_3$. This work describes application of chromatographic methods for determination of *S.* Telaviv OPS heterogeneity related to the non-stechiometric distribution of glucosyl residue and digalactose branching chain along the main OPS chain of LPS. This structural heterogeneity cannot be demonstrated by separate chromatographic techniques. Only the combination of chromatographic method of separation using gel permeation chromatography (GPC), gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS) can yield the relevant results. The determination of the heterogeneity structure of *S*. Telaviv O-polysaccharide allowed to determine the resonances in the anomeric region of the proton and carbon NMR spectra of *S*. Telaviv OPS. It should be mentioned, that structural heterogeneities of *S*. Telaviv LPS can influence animal/plant–microorganism interactions.

INFLUENCE OF MUTUAL SOLUBILITY OF ORGANIC SOLVENTS ON SELECTIVITY OF CHROMATO-PARTITION METHOD

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So-called chromato-partition method is based on the distribution of analytes between two layers of heterophaseous systems of solvents. The application of this method makes the duration of analyses longer, but it allows us to characterize the analytes by not only retention indices (RI), but the partition coefficients (K_p), also. The main factor preventing wide application of this method in routine analytical practice is the absence of detailed databases on K_p values for individual compounds. Fortunately, joint application of RI and K_p values permits us to attribute unknown analytes to the corresponding homologous series. In accordance with this concept, parameter *j*, which is the linear combination of RI and lgK_p, was suggested [1]:

$$j = aRI - lgK_p$$

Parameter *a* characterizes heterophaseous system, namely its selectivity in relation to compounds of different homologous series. It should be constant for all homologous series. The whole procedure of data processing includes: i. the determination of K_p and RI values for homologues, ii. the calculation of coefficient *a* for every series, iii. the averaging of *a*-values for all series under consideration, iv. the calculation of *j*-values for every homologue, v. the averaging of *j*-values as the invariants of different homologous series.

Six heterophaseous systems were characterized: hexane/acetonitrile, hexane/nitrometane, decane/acetonitrile, hexane/2,2,2-trifluoroethanol, perfluorodecalin/chloroform, and perfluorodecalin/octane. It implies the determination of mutual solubility of their constituents and the calculation of the average value of coefficient *a* using (RI, K_p) data for compounds of different homologous series. The comparison of data obtained indicates that selectivity of various solvent systems depends strongly on their mutual solubility in reciprocally proportional manner. The lower mutual solubility corresponds to the higher selectivity.

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DEALING WITH CENSORED DATA IN THE CHEMOMETRIC ANALYSIS OF CHROMATOGRAPHIC DATA

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Experimental measurements which have values below or above a given threshold are commonly called censored. Left censored data often appear in chromatographic experiments when the levels measured for some chemical components in the samples are found to be below the limit of detection. These values are then reported either as 'less-thans' or 'non-detects'. The lack of knowledge about the exact concentrations for some chemical components is problematic for the following chemometric analysis of the collected data.

Principal component analysis, PCA, is usually the method of choice when the aim is to see the similarities among samples and to investigate the correlation among variables. However, the classic PCA method cannot handle censored data directly. Therefore, the most widely used approach for dealing with such data is to substitute them with values corresponding to one-half of the detection limit. Substitution leads to an inappropriate estimation of the data mean and standard deviation and destroys the true correlation structure of the data. In this work, we present and compare the performance of two approaches to PCA (expectation-maximization PCA [1,2] and maximum likelihood PCA [3,4]) that can be considered to explore chromatographic data containing values below the detection limit.

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APPLICATION OF ION CHROMATOGRAPHY WITH CONDUCTOMETRIC DETECTION FOR SEPARATION AND ANALYSIS OF IONIC LIQUID MIXTURES WITH SIMPLE INORGANIC IONS INORGANIC AND IONIC LIQUID CATIONS

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Ionic liquids are an interesting group of compounds, existing in liquid state over wide range of temperature and characterized by negligible vapor pressure, solvent solubility in extraordinary polarity range, non-flammability, high electric conductivity and high thermal stability. They typically consist of nitrogen-containing organic cations together with an inorganic or organic anion commonly containing fluorine. Current research indicates that replacing conventional molecular solvents with these media can bring about remarkable improvements in well-known processes such as organic synthesis and catalysis, in the separation sciences, as electrolytes in batteries and solar cells, and as alternative lubricants.

In this study ion chromatography was tested to verify its applicability in analysis of ionic liquid cations such as alkyl entities of imidazolium, phosphonium, pyrrolidinium, pyridinium, quinolinium, morpholinium and ammonium together with typical inorganic cations such as Li^+, Na^+, K^+, Ca^{2+} and Mg^{2+} usually found in various matrices. During optimization process reliable and sensitive method was proposed to separate mixture of these ions in one run. All experiments were conducted using Metrosep C4 cation exchange silica-based column. The acetonitrile content in the mobile phase had the strong influence on selectivity of separation of imidazoliums and divalent inorganic cations. The influence of nitric acid content in the mobile phase was also observed, indicating usefulness of low acid concentrations if univalent cations shall be selectively separated. Low content of acid and organic modifier however resulted however in a very poor peak shapes. The method for simultaneous determination of ionic liquid and inorganic cation was validated with acceptable analytical performance parameters.

WEALTH OF NUTRITIENTS IN THE ABDOMEN'S MUSCLE IN BALTIC SHRIMP CRANGON CRANGON DETECTED BY HPLC-LLSD, MALDI-TOF AND GLC-MS TECHNIQUES

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Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) create primary source of fatty acids in shrimps. Consumption of the seafood is growing, they constitute the wealth of nutritients and the source of long-chain, unsaturated omega-3 fatty acids [1]. These acids can improve the effect of the immune system [2], influence cardiovascular and anti-inflammatory [1], whereas their low consumption and providing the organism are supporting such disease as cerebrovascular disease, unipolar major depression, neurodegenerative disease, Alzheimer's disease, allergy, asthma, diabetes and obesity [2].

The main aim of this work was the determination of lipid classes and composition of fatty acids in triacylglicerols and phospholipids in baltic shrimp *Crangon crangon*. High performance liquid chromatography with laser light scattering detector (HPLC-LLSD) was used for separation of the groups of lipids and further, matrix assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF) to verify recognized classes, as also for identification of particular lipid compounds. Additionally the composition fraction of free fatty acids was determined using gas-liquid chromatography-mass spectrometry (GLC-MS).

The following lipid groups were separated using HPLC-LLSD: triacylglycerols, fatty acids, sterols and phospholipids. HPLC-LLSD analysis showed the dominance of sterols (lack in MALDI-TOF mass spectra). TAGs were the next equally numerous group and remaining FFA and phospholipids (in small amount). MALDI-TOF method was also used to determine a composition of lipids. Composition of lipid classes was repeated, only sterols weren't detected by this method. Phospholipids occurred to be predominated group containing the following compounds: PC, PE, PS, PI, SM. TAGs were the second common group, but DAG and FFA were also present in spectra.

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NON COVALENT CAPILLARY COATING WITH HIGH MOLECULAR WEIGHT POLYELECTROLYTES FOR PEPTIDE AND PROTEIN ANALYSIS

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Capillary electrophoresis (CE) is now accepted as a high performance separation technique in the field of protein and peptide analysis. However CE separation, mainly for proteins, can be strongly affected by their adsorption onto the bare silica capillary wall, yielding poor repeatability and performance deterioration. To prevent or reduce this effect, a dynamic coating of the capillary wall by polyelectrolytes in single or Successive Multiple Polymer Layer (SMIL) can be performed. However, optimal coating conditions have not been defined so far. In this study, a systematic investigation of coating conditions has been carried out for PDADMAC (polydiallyldimethylammonium chloride) and PSS (polysodium 4-styrene sulfonate) coatings. Optimal coating conditions, coating stability (under acidic, alkaline and organic solvent conditions) and separation performance for peptides and proteins are presented.

DETERMINATION OF CORTISOL IN HUMAN URINE SAMPLES BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Cortisol is the major glucocorticoid produced by the adrenal glands in humans and regulated a myriad of biological function and processes. Moreover is a biological biomarker of stress, anxiety and depression. Urinary free cortisol (UFC) reflects the fraction of nonprotein bound plasma cortisol. Measurement of UFC is important in the diagnosis and management of adrenal disorders. Capillary electrophoresis, especially in micellar elektrokinetic chromatography (MEKC) mode, is becoming an important tool for the analysis of wide range of neutral and lipophilic molecules. This publication presents the evaluation and optimization of procedure for the determination of cortisol (hydrocortisone) with micellar electrokinetic chromatography.

Analysis was done using a Beckman P/ACE 2100 (Beckman Instruments, Fullerton, CA, USA) equipped with selectable fixed-wavelength UV detector. The unmodified silica capillary cartridge contained a 75 μm i.d., 57cm total length. The voltage was maintained at 20 kV. The resultant electropherograms were monitored at 254 nm. The background electrolyte for electrophoresis consisted of 50 mM SDS, 10 mM sodium tetraborate, pH 8,8.

Urine samples (15ml) were spiked with 500 ng/ml dexamethasone (Internal Standard) and various concentrations (5-400 ng/ml) of cortisol. Preconcentration of cortisol from urine samples was achieved by using extraction disc cartridges (Merck, LiChrolut RP-18).

Proposed method was validated for specificity, linearity, limits of detection and quantitation, precision and accurancy. The limit of detection was 3 ng/ml with 15 ml urine extraction, calculated by taking signal to noise ratio=3. Linearity ranged from 50 to 400 ng/ml; correlation coefficient was 0,9989. The mean recoveries were 103,7%. Intra-assay and inter-assay precision as RSD ranged from 2,7-9,6% and 2,9-12,1%, respectively. Specificity of both analytical methods was determined on the basis of blank and extracts samples.

This report describes the successful development and application of sensitive methodology for urinary cortisol detection. Cortisol measurement levels were in range 58-339 ng/ml. CE offers several potential advantages: high separation efficiencies, extremely small injection volumes (in the nanolitre range), short analysis time, rapid method development and low reagent costs.

NEW SPME FIBER FOR QUALITATIVE ANALYSIS OF VOLATILE COMPOUNDS IN PRINTING INKS

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Solid-Phase Microextraction (SPME) is a modern sample preparation method for the sample preparation for chromatographic analysis. SPME consists in a partition of analyzed compounds between the matrix and stationary phase, in turn present on a thin glass or quartz fiber. SPME is widely used in chemical analysis, due to the fact that this low-cost method is relatively simple and effective, with a possibility of combining with other analytical techniques, such as GC and HPLC. In addition, SPME allows to analyze trace amounts of volatile and non-volatile organic compounds in the food, water, soils, as well as plastic and floral samples. SPME permits to provide several analytical procedures simultaneously, to mention suitable sampling, and further a preconcentration and a determination of the chosen analyte by a suitable method.

There are many commercially available SPME stationary phases, characterized by different analytical possibilities. These phases are differentiated by the polarity, thermal stability, and sorption parameters.

This work is devoted to a preparation of SPME fibers covered with silica modified with ketoimine groups, as well as an optimization of the conditions for the qualitative analysis of some volatile compounds in printing inks.

The poster contains some results of qualitative determination of volatile compounds in printing inks using combined HS-SPME-GC technique.

APPLICATION OF THE SIMPLEX-HPLC METHOD FOR THE SEPARATION OF SELECTED IMIDAZOLIUM IONIC LIQUID CATIONS

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In recent years, room-temperature ionic liquids have increasingly attracted attention as the green, high-tech reaction media of the future. Typical ionic liquids consist of an organic cation with a delocalized charge and an organic or inorganic fluoro anion. At temperature below 100 °C, these compounds are ideal non-volatile solvents for a variety of industrial chemical syntheses. They are also applicable as biocatalytic media, phases and additives in separation processes, or alternative lubricants. Therefore, the development of new analytical methods for the rapid and reproducible separation and identification of ionic liquids is a prerequisite for further biological and environmental research of these substances. The variable-size simplex algorithm was applied to optimize the separation of a congeneric group of imidazolium ionic liquid cations and one alkylpyridinium cation. A chromatographic response function (CRF), which included the number of peaks, the resolution between adjacent peaks, a specified analysis time and the individual retention times relative to a minimum retention time, was calculated to evaluate the quality of the individual chromatograms. The mobile phase at the point corresponding to the optimum consisted of 10 % MeOH and 90 % 15 mM KH_2PO_4/H_3PO_4 with pH = 3.43. Using an optimized method, ten typical alkylimidazolium and alkylpyridinium ionic liquid cations were successfully separated in one chromatographic run.

SIMPLE THIN-LAYER CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF RANITIDINE AND ITS PHARMACEUTICAL FORMULATIONS: APPLICATION TO THE ANALYSIS OF PRODUCTS OF THEIR PHOTOLYTIC DEGRADATION

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The chromatographic properties of ranitidine hydrochloride and its degradation product has been examined on TLC plates: Kieselgel 60 WF_{254s} RP-18 F_{254s}, Aluminiumoxid 150 F_{254}, Cellulose F, NH_2 F_{254s} and HPTLC plates: Kieselgel 60 F_{254}, RP-18 WF_{254s}. Chromatographic experiments concerning ranitidine in pharmaceutical preparations (Ranic, Ranisan, Ranitin, Ranigast, Zantac tablets) and products of their photolytic degradation were performed on HPTLC Kieselgel 60 F_{254} plates. Chromatograms were developed by a series of following solvents: methanol-water, methanol-acetonitrile, methanol-dimethyl sulfoxide (DMSO), methanol-ammonia, methanol-ethyl acetate, acetonitrile-DMSO.

Comparing the data obtained when studying standard ranitidine and the product of ranitidine photolytic degradation on the slides covered with silica gel, octadecysilane, aluminum oxide, cellulose, and aminopropyl phase it was found out that the transport of both substances required higher concentrations of the methanol modifying additive, particularly acetonitrile on thin layer plates, Kieselgel 60, RP-18 and Aluminiumoxid. The non-aqueous mobile phase composition was investigated in order to determine whether elution behaviour of ranitidine hydrochloride and its degradation product is related to specific properties of water. Addition of DMSO to acetonitrile considerably decreased chromatographic retention of studied compounds, what can be attributed to the blockage of the silica active centers by this solvent. Admixture resulted in almost complete washing out of ranitidine, selected drugs and their degradation products. TLC method, using normal-phase and mobile phases including acetonitrile and methanol, proved an efficient separation method of several products of photolytic degradation of ranitidine generic drugs. Such chromatographic system may be recommended for further studies on photolytic degradation of ranitidine generic drugs. The most successful separation of ranitidine and a product of ranitidine photolytic degradation was obtained in plates RP-18W, in the wide range of methanol-water solutions concentrations.

USE OF POLAR AND NON-POLAR STATIONARY PHASES FOR DETERMINATION OF SELECTED FLAVONOIDS

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The retention behavior of two flavonoids (hesperetin and quercetin) has been examined on TLC plates: Kieselgel 60 WF_{254s}, RP-18 F_{254s}, Aluminiumoxid 150 F_{254}, Cellulose F, Polyamid 11 F_{254}, and HPTLC plates: Kieselgel 60 F_{254}, RP-18 WF_{254s}; wide range (from 0 to 100%, *v/v*) mixtures of n-alcohols with dimethyl sulfoxide (DMSO), hexamethyldisiloxane (HMDSO), acetonitrile and water were used as mobile phases.

A strong bonding of hesperetin and quercetin by aluminium oxide incapacities the use of this mobile phase for further analysis of the compounds above. Only water modifier used for alcohol mobile phase resulted in occurrence of hesperetin elution. It should be stressed that retention decreased with the increase in the alcohol carbon chain length. Silica gel, RP-18, cellulose and polyamide also adsorbed tested flavonoids to a different degrees, both in mobile water phases and non-aqueous phases with HMDSO modifier. Addiction of DMSO to alcohol mobile phases, by and large, resulted in the maximal flavonoids elution. Minimum retention, in the widest range of concentrations was observed for binary phase in methanol-DMSO. Carbon chain length of alcohol, in mixtures alcohol-DMSO type, significantly influenced the chromatographic retention of compounds under consideration. On silica gel and RP-18 plates with the increase of carbon chain length of alcohol, the range of concentrations for mobile phases where minimum retention occurred decreased considerably. On the contrary, a reverse dependence was observed in cellulose and polyamide. The widest range of concentrations, where quercetin and hesperetin parameters R_F reached maximal values, was observed on plates covered with cellulose. When analyzing results the absence of separation of quercetin and hesperetin in the reverse phases system was observed. On the other hand, a satisfactory distribution of examined flavonoids was obtained on silica gel, cellulose and polyamide when variable combination of binary mobile phases was applied.

HPLC TOTAL ANTIOXIDANT POTENTIAL ASSAYBASED ONHYDROXYL RADICALS REACTION WITH P-HYDROXYBENZOIC ACID

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Free radicals adversely modify biologically active molecules as well as whole cells and are implicated in various degenerative diseases and aging. Their mediated process have been implicated in the pathogenesis of several diseases. It is widely believed that these modifications are preventable by exogenous antioxidants. There is a need for a method to assess and compare strength of particular antioxidants in order to select these of the highest potential for further development as drugs. However, it turned out that frequently more information (e.g. synergetic effects) is obtained measuring total antioxidant potential (TAP) of biological samples than concentration of particular antioxidants separately.

In the literature we can find many methods describing the hydroxyl radicals analysis using HPLC. They are based on reaction of these radicals with the spin trap reagents. Products of these reaction can be monitored using electrochemical as well as fluorescence detection. Previously we have showed that these assays can be also applied to the TAP measurements. In this case hydroxyl radicals were generated in the Fenton reaction. They were detected using: (i) *p*-hydroxybenzoic acid (*p*-HBA) with the electrochemical detection or (ii) terephthalic acid (TFA) with the fluorescence detection. In the presentation TAP measurements using *p*-HBA with fluorescence detection will be discussed. The elaborated assay will be tested on alcoholic beverages as well as on pure compounds, such as indoles, flavones and triazines.

RAPID DETERMINATION OF LORATADINE IN HUMAN SERUM BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD WITH FLUORESCENCE DETECTION

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Loratadine is a rapidly effective and long-lasting tricyclic antihistamine with selective peripheral histamine H_1-receptor antagonist activity. This drug has been widely used because of its efficacy in treating seasonal and perennial allergic disorders and chronic idiopathic urticaria without significant central and autonomic nervous side effects such as sedation and aniticholinergic properties. Despite its widely use, only a few analytical technique have been developed for the determination of loratadine in human serum [1-3].

The aim of this work was the development and validation of HPLC method with fluorescence detection for the determination of loratadine in human serum. The presented method is simple, rapid and no complicated purification steps or derivatization is required. The solid-phase procedure extraction (SPE) has been used before HPLC analysis. Chromatographic separation was carried out on a C_{18} analytical column using a mixture of acetonitrile and water (70:30, v/v), adjusted to pH 2.7 with ortho-phosphoric acid, as mobile phase. The column was maintained at 28°C. Analyses were run at a flow-rate of 1 min mL^{-1}. Fluorescence detection was performed at an excitation wavelength of 265 nm and an emission of 454 nm. The whole of drug determination procedure was approach through validation specification regarding specify, linearity, sensitivity, accuracy, precision and stability. The absolute recovery of loratadine was above 93.0%. The limit of detection (LOD) and quantification (LOQ) were 0.07 and 0.2 ng mL^{-1}, respectively. Linearity was confirmed in the range of 0.2 – 30 ng mL^{-1} with a correlation coefficient of greater than 0.9998**.**

The presented HPLC method is selective, robust and specific and would be efficient in analyzing large number of serum samples supporting pharmacokinetic, bioavailability or bioequivalence studies after therapeutic doses of loratadine.

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HYDROPHILIC INTERACTION PLANAR CHROMATOGRAPHY OF SOME ANAESTHETICS

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Hydrophilic interaction liquid chromatography (HILIC) has recently been introduced as a highly efficient chromatographic technique for the separation of a wide range of polar solutes [1]. In the HILIC mode, an aqueous–organic mobile phase combined with a polar stationary phase was used to provide normal-phase retention behavior. HILIC is often considered as a normal-phase separation in a reversed-phase mode [2]. This technique is especially suitable for the separation of polar low-molecular-weight compounds such as hydrophilic amino acids, di- and tripeptides, and organic acids, which often not sufficiently retained in RPLC mode.

In scope of our research of retention behavior of various analytes, the chromatographic behavior of seven anaesthetics was investigated under both HILIC and RP conditions on thin layers of silica-gel and alumina, using simple mixtures of water and organic solvent (methanol or acetonitrile) as mobile phase. Considering the effect of nature of the mobile phase, it can be noticed that an increase in the amount of the water content in mobile phase, relatively to organic component, results in conversion of a separation mechanism. The results obtained shows that in a wide range of water content in a mobile phase, the chromatographic behavior of the investigated compounds is determined by hydrophilic interactions in chromatographic system.

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DETERMINATION OF TIOCTIC ACID IN DRUG FORMULATIONS AND IN DIETARY SUPPLEMENT PREPARATIONS

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Thioctic (alfa-lipoic) acid is a drug used for the treatment of diabetic polyneuropathy. Due to its antioxidant properties, thioctic acid is nowadays widely used both in dietary supplement preparation alone, and in combination with amino acids, L-carnitine and the other compounds.

There are not so many data available on quantitative determination of thioctic acid in dietary supplements.

Therefore the aim of these investigations was to develop and validate a TLC method for determination of thioctic acid after derivatization by the palladium(II) chloride reagent.

Separation of thioctic acid was performed on RPTLC plates (20 cm × 10 cm), using 2-propanol : methanol : acetone : water : acetic acid (6:4:2:8:0.2, v/v) as mixed mobile phase. The plates were immersed in the solution of palladium(II) chloride and yellow spots were scanned at 375 nm. The retention distances of thioctic acid and its reduced form were 45 and 32 mm, respectively.

Relationship between the peak areas and the amounts of the substance applied was evaluated with use of the linear (1 – 3 μg/spot) and the second degree polynomial regression function (0.5 – 5 μg/spot). For the proposed procedure, coefficient of linear correlation (r=0.999), limit of quantification (0.3 μg/spot), recovery (98.5 – 105.2%) and precision (0.9 – 2.9%) were found as satisfactory.

The developed method was applied to determination of thioctic acid in the drug dosage formulations and in dietary supplement preparation. The contents of thioctic acid were found as equal to 98.5 – 102.0% in the drug dosage formulations and 50.0 – 185.0% in some of the dietary supplement preparations.

ESSENTIAL OIL CONSTITUENTS OF SCHINUS EREBINTHIFOLIUS RADDI

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Schinus terebinthifolius Raddi (Anacardiaceae) is a plant growing native in South and Central America. This species is subdivided into four chemotypes[1]. As a shade and ornamental tree the plant was cultivated and spread rapidly around the equator. In traditional medicine it has been used for the treatment of inflammations[2] whereas the fruits are still used as spice[3]. So far, the essential oil of *S. terebinthifolius* has been subject of only few investigations[4]. In order to obtain additional data, leaves and fruits of *S. terebinthifolius* were collected in several botanical gardens in Germany. The essential oils were extracted by a Clevenger apparatus and analyzed by means of GC and GC/MS (GC-column: CP-Sil-5). Enantioselective GC (GC-column: 6-Me-2,3-Pe-ß-CD) was used to analyze the enantiomeres of mono- and sesquiterpenes[5]. Monoterpenes form the main fraction; α-pinene (89 - 72 %, (–) >95 %) and β-pinene (5 – 20 %) were identified as major components. One sample was markedly different from the others containing only 30 % α-pinene ((–) 0,2 %) yet 66,8 % limonene ((+) 97,8 %). The predominating compound in another of the samples was α-phellandrene (36,6 %, (+) 93 %) besides α-pinene (30 %, (–)40,6 %), β-phellandrene (14,7 %) and limonene (13,6 %).

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APPLICATION OF DIMETHYL SULPHOXIDE AND HEXAMETHYLDISILOXANE AS MODIFIERS OF MOBILE PHASES IN TLC

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The retention behavior of macrocyclic antibiotics (erythromycin, troleandomycin, tylosin, rifamycin B, rifampicin) has been examined on Kieselgel 60 F_{254} HPTLC, Kieselgel 60 WF_{254} TLC, LiChrospher Si 60 F_{254s} HPTLC, RP-18 F_{254} HPTLC and RP-18 WF_{254} TLC plates and porphyrins (uroporphyrin I, uroporphyrin III, coproporphyrin I, coproporphyrin III, protoporphyrin IX) on Kieselgel 60 F_{254} HPTLC as stationary phase; wide range (0 to 100 v/v) mixtures of alcohols, esters, ketones and xylenes with methyl sulfoxide (DMSO) and hexamethyldisiloxane (HMDSO) were used as mobile phases.

Use of HMDSO and DMSO in the mobile phases had very different effects on the migration of the macrocyclic antibiotics and porphyrins investigated. HMDSO strongly affected chromatographic retention of these antibiotics, causing it to increase, whereas DMSO led to a substantial decrease in the retention of the antibiotics and porphyrins. This can be explained by supposing that the ionic form of DMSO occupies adsorption centers on the silica surface not covered by the impregnating agent, resulting in reduced retention capacity. Mixtures of HMDSO and alcohols with the OH group on the 2 position had the greatest effect on retention of antibiotics, causing it to increase. Use of mixtures of DMSO and alcohols with OH group on the 2 position increased elution of these compounds. In practice the antibiotics can be used as a chiral stationary and mobile phase modifiers.

The mobility of the porphyrins depended on their molecular weight. Transport of these compounds required the presence of DMSO in the mobile phase. Mobile phases containing esters had the greatest effect on the retention and separation of coproporphyrins. Increasing the length of the carbon chain of homologous esters and ketones resulted in increased chromatographic retention of the porphyrins. The position of the methyl groups in the structures of esters affected the chromatographic behaviour of the porphyrins. The best separation of coproporphyrins was obtained by use of mixtures of DMSO and ketones.

ON SPONTANEOUS OSCILLATORY CHIRAL CONVERSION OF DIFFERENT CARBOXYLIC ACIDS IN AQUEOUS MEDIA

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In papers [1-3], we reported on the spontaneous oscillatory chiral conversion of 2-phenylpropionic acid and several optically pure profen drugs dissolved in abiotic aqueous media. All these compounds are structurally related as α-substituted propionic acid derivatives.

In papers [4,5], we demonstrated the ability of selected optically pure α-amino acids which can also be derived from propionic acid to undergo oscillatory chiral conversion when dissolved in an abiotic aqueous medium.

We have also investigated a selection of six optically pure α- and β-hydroxy acids (derived from acetic, propionic, and butyric acids), for their ability to undergo oscillatory chiral conversion in an abiotic aqueous medium (e.g., [6]). In each case, this ability was experimentally confirmed.

In our studies, we have employed a wide selection of analytical techniques, including thin-layer chromatography, ^{13}C NMR, and Raman spectroscopy. To elucidate the process of spontaneous oscillatory chiral conversion, we developed a simple model of a chemical oscillator (two linked Templators [7,8]). The cornerstone of this model is the assumption that cyclic homodimers of the respective carboxylic acids (coupled via a pair of hydrogen bonds) act as templates for short-lived achiral enol intermediates, which upon interacting with a given template take on the chiral characteristics of the template.

This presentation is meant as a review paper, aimed (i) to present the range of chiral carboxylic acids found with varying chemical structures that undergo chiral conversion; (ii) to extensively discuss the best analytical techniques for monitoring oscillatory chiral conversion; (iii) to furnish empirical evidence of the oscillations; and (iv) to discuss the qualitative agreement between the basic features of our experimental results and the simulations performed with the linked Templator model.

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EXPERIMENTAL EVIDENCE OF A SPONTANEOUS CONDENSATION OF PROFENS, AMINO ACIDS AND HYDROXY ACIDS IN AQUEOUS ETHANOL

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It is generally believed that condensation of amino acids and hydroxy acids (resulting in peptides and poly(hydroxy acids), respectively), and also of profens is rather difficult because energetically unfavourable, as it needs a considerable energetic input in order to split one water molecule from each pair of binding compounds [1]. This conviction affects many present-day presumptions regarding, e.g., prebiotic condensation of amino acids resulting in formation of peptides coupled through peptide bonds (NH-C=O). Hence, the experiments are still devised which involve ion irradiation of amino acid solutions to imitate the presumable prebiotic conditions of peptide formation [2]. Moreover, computational simulations are carried out to prove that energetically, polycondensation of amino acids (and hydroxy acids) would be more favourable, if carbon, oxygen, and/or nitrogen atoms in amino acid and hydroxy acid molecules were replaced by their respective analogues, i.e., silicon, sulphur, and phosphorus atoms [3].

In this study, an experimental evidence is provided to prove that condensation with certain low-molecular-weight profens, amino acids and hydroxy acids carried out at ambient temperature can be effortless, if it is carried out in aqueous ethanol (with predominant proportion of alcohol), or in the course of chromatographic procedure on microporous surface of silica gel, evidently due to dehydrating properties of both, ethanol and silica gel. Further it seems that energetically effortless condensation of profens, amino acids and hydroxy acids is inseparably linked with an ability of these acids to undergo a spontaneous oscillatory chiral conversion, first described in papers [4-6].

In our study, we provide an inventive experimental evidence on condensation of selected profens, amino acids and hydroxy acids obtained, e.g., by means of thin-layer chromatography, ^{13}C NMR spectroscopy, and biuret test. From our results it comes out that an indispensable precondition of a spontaneous self-condensation is that the two candidate molecules are the enantiomers of an opposite chirality. In the other words, the most rapid condensation takes place with amino acid or hydroxy acid racemate. If the starting material is, however, optically pure, first it has to undergo a spontaneous oscillatory chiral conversion to at least partially transform to its own antimer, and only then condensation can become analytically traceable.

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A COMPARISON OF THE EFFICIENCY OF RETRIEVING VOLATILE CONSTITUENTS FROM THE DIFFERENT SAGE SPECIES (SALVIA SPECIES L)

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Sage (*Salvia species L.*) is the plant which belongs to the family of *Lamiaceae*. In natural habitat, representatives of this family appear in tropical regions and in warmer areas of the moderate climatic zone. The family of *Lamiaceae* embraces ca. 1000 different plant species. The name *Salvia* is derived from the Latin noun *salus*, which means health. This name alone indicates that the plant has been recognized in natural medicine for ages now, due to its high curative potential. Presently, the sage species are used not only in pharmacy, but also in the cosmetic and food industry, due to the high contents of essential oils and an appreciated taste valour with many representatives of the family.

Carefully selected plant material used in this study originates from Botanical Garden of the Department of Pharmacognosy, Medical University of Lublin, Lublin, Poland. The aim of this study was to compare the efficiency of retrieving volatile constituents from five different sage species (i.e. from *Salvia lavandulifolia L.*, *Salvia triloba L.*, *Salvia nemorosa L.*, *Salvia staminea L.*, and *Salvia hians L.*), and to optimize these approaches in terms of the analysis time and the reagents consumption, using the following working techniques:

- thermal desorption in the head-space GC autosampler;
- vapour distillation in the Deryng apparatus; and
- accelerated solvent extraction (ASE).

In each scrutinized case, the analysis of the volatile constituents was carried out with use of the GC/MS system. A comparative evaluation was performed of the applied retrieving methods and thermal desorption in the head-space GC autosampler proved the best performed one.

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A COMPARISON OF THE PHENOLIC ACID AND FLAVONOID CONTENTS IN TWENTY DIFFERENT SAGE SPECIES (SALVIA SPECIES L.) BY MEANS OF TLC/DENSITOMETRY

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Sage is the herb grown in natural habitat in many places around the world. This plant has been known and utilized in natural medicine for many hundreds of years, due to its curative properties and good performance in combating various diseases. These advantages of sage can be ascribed to the richness and unique composition of organic compounds contained in the plant. In despite of its well recognized curative potential, chemical composition of sage is far from being sufficiently explored and it still needs an effort in the areas of phytochemistry and pharmacognosy to better scrutinize its constituents and the therapeutic properties thereof.

The aim of this study was to compare the phenolic acid and flavonoid composition in twenty different sage (*Salvia L.*) species, and namely in *S. amplexicaulis, S. atropatana, S. azurea, S. cadmica, S. canariensis, S. deserta, S. forskaohlei, S. glutinosa, S. hians, S. jurisicii, S. lavandulifolia, S. nemorosa, S. officinalis, S. pratensis ssp. Hematodes, S. sclarea, S. staminea, S. stepposa, S. tesquicola, S. triloba*, and *S. verticillata.*

In our experiment, we used extracts from dried leaves of the aforementioned twenty sage species, collected in the course of the vegetation period in 2007 and 2008. This plant material was certified and it originated from Botanical Garden of the Department of Pharmacognosy, Medical University of Lublin, Lublin, Poland.

Chromatograms were developed in the horizontal sandwich DS chambers (Chromdes, Lublin, Poland), then visualized with 2-aminoethyldiphenyl borate and PEG 4000, and finally scanned by means of the Desaga (Heidelberg, Germany) CD 60 densitometer. Visualization was also carried out by means of the UV lamp (Camag, Muttenz, Switzerland) at the wavelength λ = 366nm.

The obtained results point out to distinct differences in qualitative and quantitative phenolic acid and flavonoid composition, depending on the individual species considered.

The work of one of the author (Ł.W.) was partially supported by PhD scholarships granted to them in 2008 within the framework of the 'University as a Partner of the Economy Based on Science' (UPGOW) project, subsidized by the European Social Fund (EFS) of the European Union.

A COMPARISON OF THE PHENOLIC ACID AND FLAVONOID CONTENTS IN TWENTY DIFFERENT SAGE SPECIES (SALVIA SPECIES L.) BY MEANS OF HPLC/DAD

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Phenolic acids and flavonoids are among the most widespread groups of compounds, omnipresent in the plant kingdom and they generally exert positive effect on human health. Particularly important are their biological functions, i.e., antioxidative potential and radical-quenching action. Moreover, they demonstrate anti-inflammatory and anti-allergic properties, and also an ability to absorb the UVA and UVB light, qualities that have been utilized in cosmetology and in aesthetic dermatology.

Many phenolic acids and flavonoids are inherent of the sage species, and some of them exhibit anti-tumor properties. E.g., *p*-coumaric acid and chlorogenic acid hamper formation of carcinogenic elements, while elagic acid neutralizes such elements, which might cause degeneracy of the cell DNA.

The aim of this study was a qualitative and quantitative comparison of availability of phenolic acids and flavonoids in twenty sage (*Salvia L.*) species, and namely in *S. amplexicaulis, S. atropatana, S. azurea, S. cadmica, S. canariensis, S. deserta, S. forskaohlei, S. glutinosa, S. hians, S. jurisicii, S. lavandulifolia, S. nemorosa, S. officinalis, S. pratensis ssp. Hematodes, S. sclarea, S. staminea, S. stepposa, S. tesquicola, S. triloba*, and *S. verticillata*. Plant material utilized in this study originated from Botanical Garden of the Department of Pharmacognosy, Medical University of Lublin, Lublin, Poland.

The investigations were carried out with aid of HPLC/DAD and the aim thereof was to compare individual plant species from one and the same family (*Salvia L.*), additionally taking into the account the difference in the vegetation period, as the investigated plants have been collected in 2007 and 2008. Finally, an attempt was made on chemotaxonomic characterization of the individual sage species.

The obtained results point out to distinct differences in qualitative and quantitative phenolic acid and flavonoid composition, depending on the individual species considered.

The work of one of the author (Ł.W.) was partially supported by PhD scholarships granted to them in 2008 within the framework of the 'University as a Partner of the Economy Based on Science' (UPGOW) project, subsidized by the European Social Fund (EFS) of the European Union.

THE ANALYSIS OF ESSENTIAL OILS CONTAINED IN THE DIFFERENT SAGE (*SALVIA L.*) SPECIES BY MEANS OF TLC/DENSITOMETRY

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Essential oils have been recognized for ages as substances of plant origin that possess highly appreciated curative, cosmetic, and nutritional properties. In purely chemical terms, essential oils are the multi-component mixtures of mono-, di-, tri-, and sesquiterpenes. Structurally, these compounds can belong to the groups of hydrocarbons, alcohols, aldehydes, ketones, esters, and ethers. At ambient temperature, many of them appear as liquids and display an oily consistency.

Carefully selected sage material used in this study originates from Botanical Garden of the Department of Pharmacognosy, Medical University of Lublin, Lublin, Poland.

Presented research was carried out in two different aspects. Firstly, our work focused on targeting the most efficient method of isolating volatile compounds from plant material, which can be considered as a critical step in studying volatile constituents of the natural origin materials. Then we undertook an effort to select a thin-layer chromatographic system which might provide the best possible separation of essential oils contained in sage (*Salvia L.*). Our separation experiments were carried out by means of TLC/densitometry, the technique for obvious reasons not being a tool of primary choice in investigating volatile substances and this probably made the most challenging part of this research.

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IDENTIFICATION OF PRODUCTS OBTAINED BY FENTON'S METHOD DEGRADATION OF IONIC LIQUIDS USING GLC-MS

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Since ionic liquids are nonvolatile at ambient conditions, nonflammable and ionically conductive in wide temperature range, the use of ionic liquids immediately spread into various field in electrochemistry, synthetic and catalytic chemistry. They are regarded as environmentally friendly chemicals and hence are good alternative to toxic conventional volatile solvents. The mere reduction of gaseous emissions, however, does not automatically make a process environmentally friendly, and many other facts have to be taken into account before such a statement can be made. The few studies have been done so far to investigate the degradability of ionic liquids has shown these to be highly resistant to microbial degradation, in particular, the most common imidazolium-based compounds.

The removal of harmful organic pollutants from waters and wastewaters has been investigated by several of chemical processes. Among them the Fenton's reagent has been used successfully. The paper aim is to investigate the intermediates of 1-butyl-3-methylimidazolium ionic liquids degradation in the Fenton like system (Fe^{3+}/H_2O_2). Reaction mixtures were obtained by taking the appropriate aliquot of 1-alkil-3-methylimidazolium chloride stock solution, adding Fe^{3+} (1mM), and adjusting the pH with perchloric acid to a value of 3.0 or 3.5. The reaction was started by the addition of 100mM or 400mM of H_2O_2. The reaction mixtures were analyzed for the by-products by gas liquid chromatography-mass spectroscopy (GLC-MS). Identification was based on the comparison to library spectra. Mass spectra (70 eV) were recorded on a SSQ 710 mass spectrometer (Finnigan). The samples were introduced through a Hewlett-Packard 5890 gas chromatograph.

It was found that formed intermediates are different for oxidation process by dose of 100mM and 400mM H_2O_2. In vigorous Fenton –like reaction (400mM) the main by-products were compounds with aromatic ring, while in the oxidation by lower dose of H_2O_2 they were the intermediates only at the first stage of reaction. This fact suggested different mechanisms of 1-alkil-3-methylimidazolium chloride oxidation in the conditions applying with high and low dose of H_2O_2.

DETERMINATION OF IONIC LIQUIDS IN THE PRESENCE OF PERFLUOROCARBOXILIC ACIDS IN WATER SOLUTION BY HPLC

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Ionic liquids (ILs) are good solvents for various chemical and electrochemical reactions, they are nonvolatile and nonflammable. Therefore, ILs have been proposed as "green solvents" for chemical reactions and separation processes. However several authors have already mentioned ecotoxicological influences of ILs on some selected organisms. Perfluorinated acids and their salts have been widely used in industry as surfactants, lubricants & corrosion inhibitors; they are used as emulsifying agents in polymer synthesis and as surface treatment agents in photolithography. The widespread application, environmental persistence and bio-accumulative potential of perfluorinated compounds results in the global occurrence of these substances in air, sediment, water, animals and humans. These facts indicate that ionic liquids could appear in wastewater together with perfluoric compounds, and may form new ion pairs to have different physico-chemical and biological properties than the parent compounds.

Reversed-phase high-performance liquid chromatography (HPLC) methods were used for the determination of ionic liqids: 1-butylo-3-methyloimidazolium chloride and 1-butylo-4-methylopyridinium chloride in presence of three perflouoro acids: perfluorohexanoic, perfluorooctanoic, perfluorodecanoic. The analysis was performed using a 150 × 4.6 mm Gemini C_6-Phenyl column and UV detection at 218 nm. The mobile phase was acetonitrile-water-TFA (8 : 92 : 0,01, v/v/v) (isocratic elution), and flow-rate of 0.8 mL/min.

It was observed that the interactions between imidazolium ring and carboxilic group in perfluoro componds was strong. The retention time of $bmim^+$ in the presence of PFOAs changes sensitively over a wide range. It could be connected with the changes of the polarity of medium microenvironment, formed by this associated entity. The similar trend but in the smaller extend was observed for pyridynium salt.

RETENTION MECHANISM OF CARBOXYLIC ACIDS ON DIFFERENT HPLC PHASES

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Carboxylic acids plays important role as flavors (aliphatic) or preservatives (aromatic) components of food products, antioxidants, medicines and environmental contaminations. Therefore, their analysis is crucial for a lot of scientific disciplines. Frequently High Performance Liquid Chromatography (HPLC) is used for this purpose.

In the presentation the retention mechanism on different columns is discussed. In reversed phase HPLC the main retention mechanism is hydrophobic adsorption. It can be assumed that dissociated forms of carboxylic groups do not interact with the stationary phase. Increase of their retention is observed when ion-pairing reagent is added to the mobile phase. From the other side inclusion compounds, like cyclodextrines, decrease their retention.

In ion exclusion chromatography (IEC) the dissociated forms of acids are repulsed from the stationary phase, characterized by the same sign of the electric charge. Additional effects, like hydrophobic adsorption and screening effect increase or decrease retention, respectively. Aromatic acids are much stronger retained then aliphatic ones, because of their π-electron interaction with the resin skeleton.

Very strong π-electron interactions, and in consequence retention, are observed on the Porous Graphitized Carbon (PGC) column. Additionally, induced dipoles are responsible for interaction of the polar (or ionic) compounds with the PGC phase. It is so called PREG-effect (*Polar Retention Effect by Graphite*).

The described effects are discussed, and experimentally confirmed, for aliphatic and aromatic acids.

DETERMINING OF BIOMASS REACTIVITY USING GAS CHROMATOGRAPHY

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Increases in the world energy demand resulted in more restrictive regulations regarding greenhouse gases emission and made the research society search a new and environment friendly alternative to fossil fuels. Biomass used as an energy source does not contribute to the CO_2 emission increase. The Salix Viminalis, the Miscanthus X Giganteus (MXG) and the Andropogon Gerardi are considered to be very promising in terms of their application in production of synthesis gas and hydrogen in the gasification process. Biomass reactivity is an important parameter determining its ability to undergo thermochemical transformations in the particular process.

In the study the reactivities for 50% of carbon conversion and the maximum reactivity of the tested biomass samples in the temperature range of 650-900^{o}C were determined experimentally with a use of two-channels gas chromatograph Agillent 3000A for measurements of the compositions (H_2, CO, CO_2, CH_4) of dry and clean samples of synthesis gas. Gasification tests were conducted in a fix bed reactor with steam as a gasifying medium. The amount of the cooled, dried and cleaned gaseous product mixture was measured with the mass-flowmeter. The highest reactivity R_{50} was observed for the MXG in the whole temperature range. Hydrogen content in the synthesis gas was comparable for the MXG and the Andropogon Gerardi and lower for the Salix Viminalis, while the volumes of the synthesis gas and hydrogen were highest for the MXG at all temperatures.

CHEMOMETRIC APPROACH TO INVESTIGATE THE CORRELATIONS BETWEEN CHROMATOGRAPHIC AND ACTIVITY DATA OF SOME NEWLY SYNTHESIZED S-TRIAZINE DERIVATIVES

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1,3,5-Triazines (or s-triazines) are a class of compounds well known for a long time, and still continue to be the object of considerable interest, mainly due to their applications in agriculture as the basis for various herbicides. The concern over the use of synthetic herbicides in agriculture makes the need to discover new, potent s-triazines with no or low toxicity to plants, mammals and insects increasingly urgent. Furthermore, some 1,3,5-triazines display important biological activities, such as cytotoxic, anticancer or antibacterial what makes them attractive for various medical uses.

Seeing the activity of a compound as very complex process it cannot be considered to result only from the specific interactions between active compound and appropriate receptor. The ability to reach the intended site of action is equally important. This occurs via absorption on the site of the application, distribution within the body, metabolism and elimination (ADME) processes. Hence, absorption and distribution pathways are very important since they have a decisive influence on the overall activity.

It's been well known that many of the processes of drug action have much in common with the chromatographic separation because the same intermolecular interactions determine the behavior of chemical compounds in both biological and chromatographic environments. Finding a similarity between chromatographic data and activity of the compounds in preliminary stage of investigation of potentially active compounds can help in identification and elimination of candidate molecules that are unlikely to survive later stages of development. Using calculated activity data in combination with QSAR makes it possible to avoid time consuming and costly experiments. Chemometric exploratory and similarity methods, such as PCA, may be very helpful in achieving that goal.

QUALITY CONTROL OF GC IDENTIFICATION OF CYCLOHEXANE CHLORINATION PRODUCTS IN REACTION MIXTURES

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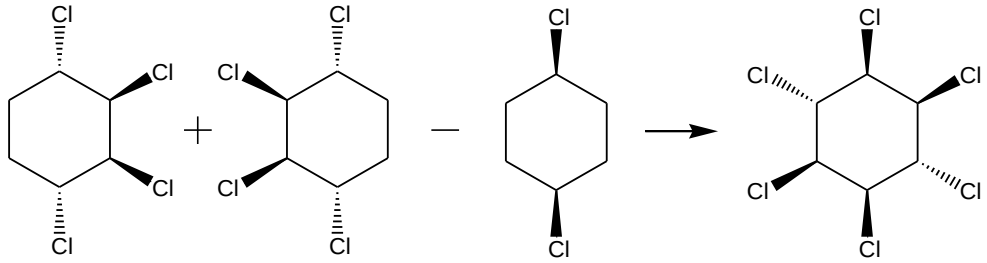
Free-radical chlorination of the cyclohexane (**I**) is the example of low stereo and regio selective reactions. Among its possible products, reference GC retention indices (RI) are known only for monochloro-, some of dichloro-, and hexachlorocyclohexanes. New additive scheme based on consequent "assembly" of higher polychlorocyclohexanes from the simpler precursors was recommended for identification of the products of **I** chlorination [1]. The assembly of structures with **n** chlorine atoms in a molecule seems to be correct only if precursors with (**n-1**) chlorines are selected.

In spite of advantages of this additive scheme, it should be subjected to a quality control. We have considered four modes of this control. #1 and #2 characterize the algorithm, while #3 and #4 characterize the objects (polychlorocyclohexanes).

1. RI evaluation for compounds of other homologous series (e.g., polymethylcyclohexanes);
2. Duplication of whole procedure of identification in different conditions of GC analysis;
3. Comparison of experimentally measured RIs of higher congeners with their precalculated RIs. One of chlorocyclohexanes characterized in details is the lindane (γ-hexachlorocyclohexane, 1r-*cis*-2-*trans*-3-*cis*-4-*cis*-5-*trans*-6-isomer). Precalculation of its RI can be performed with the following scheme:



Calculated RI: 1404±4
RI of the component in reaction mixture:1398±5

Exp. RI:1081±2

Calc. RI: 1715±7
Exp. RI :1721±10

The restriction of the total number of products by the special choice of substrates. Namely, the chlorination of 1,1-dichlorocyclohexane provides formation of only three from 12 trichlorocyclohexanes and 15 from 32 tetrachlorocyclohexanes.

This work was supported by RFBR (grant №08-03-00980).

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INVERSE GAS CHROMATOGRAPHY AN INSIGHT INTO PHYSICOCHEMICAL PROPERTIES OF NANOMATERIALS

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Inverse gas chromatography method is widely used for characterization of polymers and polymer blends, surfactants, biopolymers, solid food, petroleum pitches. In this method an investigated material is placed in a column and characterized by using volatile probes of known properties being carried by a mobile phase.

Surface properties of the particles are of high relevance in such applications as catalysis, crystallization, agglomeration, dispersion technology, nanotechnology. Most of technical surfaces are not well-defined. These materials may contain impurities, flaws, dislocations as well as internal stresses. It causes significant problems in their characterization as the methods of surface physics require well defined crystalline surfaces and high vacuum conditions during experiments. Therefore, the fundamental processes of adsorption, wetting and adhesion are poorly described. Adsorption isotherm is most often used in the description of adsorption equilibrium.

Proper characteristic of the heterogeneous particles should include various methods taking into account different aspects of the composition and properties of the surface layer. Surface character of the examined nanomaterials was described by the dispersive component of surface free energy, parameters describing surface ability to specific intermolecular / interparticle interactions. The examined nanomaterials were also characterized with the use of Hansen solubility parameters determined by the modified sedimentation procedure. All these parameters were used in the description of the behavior of aqueous and non-aqueous dispersions of iron nano oxides.

Schultz and Lavielle method was used for determination of γ_s^d (the dispersive component of surface free energy [mJ/m^2]) by IGC at infinite dilution conditions.

The estimated surface characteristics were used to observe the changes occurring during chemical modification of the examined nanomaterials as well as during their application in dispersed systems.

APPLICATION OF HPLC ASSAY TO THE MEASUREMENTS OF TOTAL ANTIOXIDANT POTENTIAL OF HONEY AND MEAD

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Free radicals and antioxidants are crucial from the physiological and pathological point of view of human being. They change during the aging, most of diseases and after food uptake. They can analysed using many HPLC assays. Nevertheless, it turned out that frequently more information is obtaining measuring the global value – Total Antioxidant Potential (TAP). It is equal to the sum of products of concentrations of all species in the sample by their kinetic constants.

Generally, HPLC assay can be used to measure free radicals indirectly, after they reaction with the spin-trap compound called *detector*. The TAP measurements are based on the generation of proper radicals (in our case the hydroxyl ones) and their reaction with the detector. In the second step the investigated sample is added. TAP values are expressed as the differences of peak areas of the products of reaction of free radical with detector in both cases.

As a spin trap *p*-hydroxybenzoic acid (pHBA) can be used. The product of its reaction with the hydroxyl radicals, 3,4-dihydroxybenzoic acid (3,4DHBA), are separated using ion exclusion or, more frequently, reversed phase HPLC. Previously, there were described systems based on the electrochemical or fluorescence detectors. The aim of the paper is check if UV detector can be used in this assay. The new assay has been applied the TAP estimation of different honey and mead samples. The alternative assay is based on the measurements of total surface area of all peaks recorded using electrochemical detector. Selectivity of the last method can be easily controlled by changing the potential difference.

ANALYSIS OF LIGHT HYDROCARBONS ON CAPILLARY COLUMNS COATED WITH ADSORBENT OF ELECTRON-DONOR –ACCEPTOR PROPERTIES

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Gas chromatography is challenged with increasingly complex analytical problems and one way of approaching them is through the search for new column packings. As already over a hundred years ago prof. Cwiet noted that the column packing has a dominant role in the separating system, development of the technology of columns and their packings has been definitely improving the chromatographic parameters such as the rate and efficiency of separation. At present of increasing interest are the chemically bonded phases obtained as a result of silica modification. The packings of this type are characterised by good thermal stability, high reproducibility of results and small loss of the stationary phase on exploitation. An important group of chemically bonded phases are those used in the complexation gas chromatography (CGC) and permitting introduction of a transition metal into the phase structure. The development of these phases opened the possibility of employing the specific interactions of the complex with unsaturated hydrocarbons and heteroatom containing compounds.

The paper concerns the adsorbents formed as a result of silica surface modifications with silylpropyl phase with diphenylketoimine groups. Because of the surface functional groups, the adsorbents obtained were characterised by electron-donor-acceptor properties. The groups permitted formation of complexes containing Cu^{2+} metal cations on the surface.

Thanks to the presence of the empty orbital in the transition metal cation it was possible to study the specific interactions between the stationary phase and the adsorbate of electron-donor properties. The study was performed on a group of volatile hydrocarbons having unsaturated bonds so capable of specific interactions with the adsorbents tested. The retention parameters were determined in order to characterise the specificity of the adsorbate – adsorbent interactions. To test the procedure proposed, selected mixtures of such volatile hydrocarbons of similar molecular mass and structure were separated.

COMPARATIVE ANALYSIS OF CHROMATOGRAPHIC DATA

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Comparative analysis of samples aims revealing differences among the samples being studied. This is done on the basis of their chemical content that can be described using many analytical techniques. Chromatographic techniques are frequently selected to achieve this goal. In general, two types of chromatographic data exist: chromatographic signals and data tables where peak areas or concentration of selected components are reported. Chromatographic data are by nature multivariate and they contain from several to hundreds of variables. The comparative analysis of samples is greatly facilitated by the use of chemometric methods. They can help to visualize differences among samples, construct calibration and classification models and to point out relevant variables that contribute most to phenomena being studied.

In this study, we focus our attention on the variable selection issue in the context of the comparative analysis of samples described by chromatographic data. To date, different variable selection techniques were introduced to distinguish between relevant and irrelevant variables, e.g. permutation test [1], the significance analysis approach [2] and uninformative variable elimination partial least squares [3]. It is, however, well known that any calibration or classification model with the variable selection scheme is prone to overfitting. Therefore, the model built has poor prediction power for new samples. The step of variable selection has to be validated carefully. Moreover, importance of a variable is scored using a certain statistical test at a given significance level. In such a case, variable selection approaches have to take into account a simultaneous evaluation of all variables (the so-called multiple hypothesis testing principle) [4]. The major problem faced in multiple testing is the increase of the number of variables selected wrongly.

In our study we illustrate performance of different variable selection approaches focusing on their validation and incorporating the multiple hypothesis testing principle into variable selection procedures. Usefulness of variable selection methods is discussed and illustrated using experimental chromatographic data.

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COMPARISON OF RETENTION BEHAVIOUR OF SELECTED ANALYTES IN HYPERSIL GOLD™ AND HYPERSIL GOLD aQ™ LC COLUMNS

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Hypersil GOLD columns are based on improved, highly pure silica and a nove proprietary derivatization and endcapping procedure using alkyl chain chemistry [1]. These columns offering in many cases more symmetrical peaks of chromatographed compounds in comparison with traditional C18 columns. Hypersil GOLD aQ™ stationary phase is built upon the technology of the Hypersil GOLD™ silica. In this column a polar endcapped C18 phase was used, what offers superior retention, especially of polar compounds. The polar endcapping of Hypersil GOLD aQ media also makes it usable in 100% aqueous mobile phases without the risk of loss of performance or poor stability [1].

In this work the retention behaviour of the several compounds such as flavonoids, phenols and aromatic hydrocarbons as test analytes in RP-HPLC with methanol – water as binary mobile phase was analysed. The investigations were performed in the Hypersil GOLD (150x4.6 [mm], particle size 3 [μm]) and Hypersil GOLD aQ (150x4.6 [mm], particle size 3 [μm]) columns. The second purpose of this study is to analyse of the retention mechanisms in the presented above chromatographic systems. These investigations were conducted with the use of the five valuable retention models assumed as the partition, adsorption/partition and adsorption mechanism of retention. All the models were verified for different RP-HPLC systems by four statistical criteria: the sum of squared differences between the experimental and theoretical data, approximation of the standard deviation, Fisher test and F-test ratio.

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DEPENDENCE OF CHROMATOGRAPHIC RETENTION INDICES ON A RATIO BETWEEN AMOUNTS OF TARGET AND REFERENCE COMPOUNDS

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Retention indices (RI) are the chromatographic invariants of highest interlaboratory reproducibility. With fixed stationary phase, RI values depend slightly on the temperature ($\beta = d\mathrm{RI}/d\mathrm{T} > 0$) and on the content of the phase in the packed columns (PC) that is caused by sorption effects. However, there is an additional factor influencing the constancy of RI values, namely the ratio between amounts of target analytes and reference compounds in the samples.

If we express the ratio of target (x) and reference ($r1$, $r2$) compounds by the ratio of their peak areas (S) by the following relation:

$$\gamma = S_x / (S_{r1} + S_{r2}) , \qquad (1)$$

the dependence RI(γ) can be approximated by the linear regression:

$$\mathbf{RI} = \mathbf{RI_0} + k \ln(\gamma) , \qquad (2)$$

where RI_0 is the retention index value at $\ln(\gamma) = 0$ which is equivalent to $\gamma = 1$, i.e. to the equality of peak areas, $S_x = (S_{r1} + S_{r2})$; the value of coefficient k reflects the "sensitivity" of RI values to the variations of γ.

Some model experiments permit us to conclude that the values of the coefficient k in the Eq. (2) for compounds analyzed with PC exceed those for WCOT columns in few times. Thus, the range of a variations RI(γ) for PC can be *ca*. 50-60 i.u. that exceeds the influence of all other factors. At the same time, "standardized" RI_0 values remain to be high reproducible.

The results of RI(γ) data processing for acetophenone measured with different GC columns are presented below.

Column	Sample dilution	T, ^{0}C	RI_0	k	r	S_0	γ_{min}	γ_{max}
Packed	**1 : 10**	**70**	**1047.6 ± 0.4**	**6.4 ± 0.3**	**0.985**	**1.5**	**0.05**	**4.8**
(SE-30)	**1 : 100**	**70**	**1048.0 ± 1.0**	**4.0 ± 1.2**	**0.745**	**2.9**	**0.04**	**6.8**
WCOT	**1 : 1**	**80**	**1033.2 ± 0.3**	**1.6 ± 0.4**	**0.973**	**0.5**	**0.5**	**2.6**
(OV-101)	**1 : 100**	**80**	**1032.6 ± 0.1**	**1.0 ± 0.0**	**0.999**	**0.1**	**1.2**	**11.5**
	1 : 1	**140**	**1051.4 ± 0.5**	**1.3 ± 0.6**	**0.583**	**1.3**	**0.11**	**1.7**
	1 : 10	**140**	**1049.8 ± 0.5**	**1.7 ± 0.7**	**0.669**	**1.7**	**0.28**	**4.3**

CHROMATOGRAPHIC INVESTIGATION OF THE FORMATION OF 5-(4-ACETAMIDOPHENYL)-10,15,20-TRIPIRYDYLOPORPHYRIN

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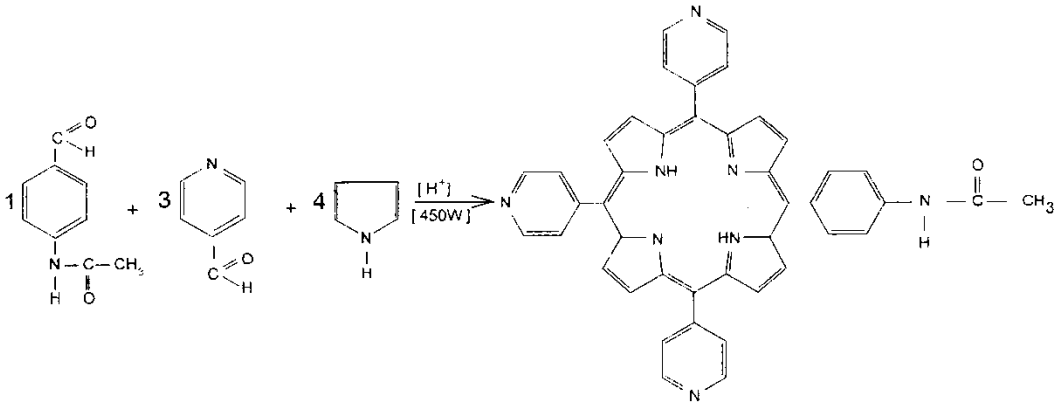
Meso-Tetraaryloporphyrins are compounds which don't occur in nature as opposed to some porphyrinoides .They are used as a model compounds of the different processes for instance :breathing , photosynthesis,dehalogenation ,and as photosensitizer in PDT method[1]. Adler's method which consists on condensation of aromatic aldehydes with pyrrole in acid medium is one of methods of their synthesis. .This method and its some modifications use a large volumes of solvents such as propionic acid ,dichlorometane .The synthesis lasts about 1 hour[2].To receive 5-(4-acetamidophenyl)-10,15,20-tripirydylo-porphyrin were used 4- acetcarboxybenzaldehyde ,4-pyridinecarboxaldehyde in mixture with pyrrole.



Application of microwave radiation for this reaction reduces considerably solvent consumption about 100 times and reaction time about 10 times. This is with agreement perform the green chemistry rules referring to environmental protection[3] .The reaction mixtures were tested by TLC on silica gel plates.Mobile phase was the mixture chloroform: methanol v/v 9:1.In order to confirm the qualitatative TLC results densitometry was used .Densitograms were obtained by means of the Desaga CD 60 densitometer controlled by Pentium computer .The plates were scanned at 420 nm[4].

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CHROMATOGRAPHIC INVESTIGATION OF THE FORMATION OF 5-(4'-CARBOXYPHENYL)-10,15,20-TRIS-(4PYRIDYL)-PORPHYRIN

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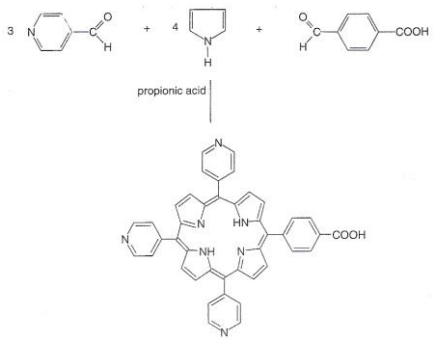
The synthesis and characterization of 5-(4'-carboxyphenyl)-10,15,20-tris-(4pyridyl)-porphyrin is described.This class of compounds could serve as potential photosensitizers for the PDT method[1].

This synthesis is consist with method Adler-Longo which is commonly used to synthesis of the meso-tetraaromatic porphyrins, by the condensation of aromatic aldehydes and pyrrole in acid medium[2].



The condensation of carboxybenzaldehyd, 4-pyridinecarboxaldehyde and pyrrole (molar ratio 1:3:4) in propionic acid for about 6 min in the domestic microwave oven give 5-(4'-carboxyphenyl)-10,15,20-tris-(4pyridyl)-porphyrin.Applied of microwave energy was 450 W. After each 15 second of heating the reaction mixture were cooled to room temperature[3].

The reaction mixtures were tested by TLC on silica gel plates. Mobile phase was the mixture chloroform: methanol v/v 9:1. In order to transfer of the qualitative TLC results to quantitative ones, densitometry method was used. Densitograms were obtained by means of the Desaga CD 60 densitometer controlled by Pentium computer. The plates were scanned at 420 nm[4].

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Normal and reversed phases TLC determination of rosmarinic and valerenic acids

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The chromatographic properties of valerenic and rosmarinic acids were studied by using normal- and reversed-phase TLC plates with eight developing solvent systems (methanol-water, ethanol-water, propanol-water, acetonitrile-water, acetonitrile-methanol, THF-water, acetonitrile-buffer, methanol-buffer) in which the concentration of organic modifier was varied from 0 to 100% *(v/v).*

Chromatographic retention of these acids was highly dependent on the mobile phase used. In reversed-phase chromatography rosmarinic acid was eluted better. The length of the carbon chain of the alcohol component of the mobile phase affected elution of the acids - increasing the length of the carbon chain increased the eluting power of the alcohols. For valerenic acid the eluting power of the mobile phase depended on the amount of water added to the mobile phase. On Kieselgel 60W and RP-18 TLC transport of valerenic acid required addition of an organic modifier to the mobile phase. Chromatographic retention of both acids on normal-phase plates was significantly dependent on mobile phase pH when pure buffer phase was used as mobile phase or when the mobile phase contained only a small amount of methanol or acetonitrile. In RP TLC, on the other hand, elution of rosmarinic acid depended on mobile-phase pH for methanol-buffer and acetonitrile-buffer mobile phases containing from 0 to 10 % *(v/v)* organic modifier.

AN APPLICATION OF HPLC FOR QUANTITATIVE ANALYSIS OF SELECTED GLUCOCORTICOIDS IN PLASMA AND URINE SAMPLES (poster)

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Cortisol, cotrisone and corticosterone are a class of glucocorticoids produced by the adrenal cortex which are involved in a wide range of physiologic systems such as stress response, immune response and regulation of inflammation, glucose utilization, fat metabolism, bone development and blood electrolyte levels. They may be used for replacement of natural hormones in patients with their deficiency, as well as for treatment of arthritis, asthma, anemia, various cancers, and skin inflammations. Cortisol is usually referred to as the "*stress hormone*" as it is involved in response to stress and anxiety. Cortisone can be considered an inactive metabolite of cortisol. However 11-β-steroid dehydrogenase can catalyze the reverse reaction as well and thus cortisone is also the inactive precursor molecule of cortisol. Corticosterone is converted to aldosterone by aldosterone synthase. The analysis of glucocorticoids in biological samples such as plasma and urine is an essential source of information for pharmacological, diagnostic and ***clinical studies which*** should result in a better understanding of preclinical and clinical data.

The aim of this work was to developed a simple method for the determination of cortisol, cortisone and corticosterone in human plasma and urine by high-performance liquid chromatography with UV detection. Sample pretreatment involved solid phase extraction (SPE). Chromatographic separations were performed on a Nucleosil 100 C-18 analytical column using a mixture of acetonitrile and water (30:70, v/v) as mobile phase. The flow-rate was 1 min/ml. The analytes were monitored by UV detector at 240 nm. The method has been validated for accuracy, precision, selectivity, linearity, recovery and stability. The assay was linear over the concentration range of 2-300 ng/ml and 2-200 ng/ml for all analysed glucocorticoids in human plasma and urine, respectively ($r > 0.9998$). The average recoveries from plasma and urine were above 93,2 and 82.8%, respectively. The limits of detection and quantification were found to be 1 and 2 ng/ml for all compounds in both biological matrix, respectively. The results of validation confirm that the method was highly suitable for the clinical and the diagnostic investigations. This presented method has been applied successfully for the determination of endogenous glucocorticoid levels in human plasma and urine of female and male volunteers.

SEX DIFFERENCES OF HUMAN STRESS HORMONES

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The primary goal of the study was to evaluate sex differences in the levels of endogenous cortisol and cortisone as *stress hormones* in human volunteers. Evidence from recent investigations in people [1,2] suggest the relationship between cortisol or cortisone/cortisol ratio and stress may be sex-specific.

Steroids hormones were collected from human plasma and urine samples and analysed by reversed-phase high-performance liquid chromatography (RP HPLC) method with UV detection. The reported studies were performed on a group of 140 volunteers of both sex. Stress hormones were isolated from both biological materials using solid-phase extraction (C18, 500 mg).

Statistic methods as parametric tests as well as non-parametric tests have been used for the evaluation of the analytical data. Level of significance in each case was 0.05.

We have observed large inter-subject variability existing in volunteers. However, the range of plasma cortisol values in the present study distinguished men from women, with the highest values among women. Nevertheless, the application of statistic methods identified the lack of unambiguous relationship between concentrations of endogenous cortisol and sex of volunteers. It may be that women were more likely to feel some apprehension or "stress " regarding the blood test, which resulted in greater variability in cortisol levels among the women in plasma at arrival to laboratory. Consistent with the latter suggestion is the cortisol levels of the women dropped over the stay in laboratory in compare to men. Thus, it is possible that the relationship between cortisol levels and perspective error scores as successive of stress would be the same in men and women.

In summary, our results are consistent with recent evidence indicating large individual variability in the levels of cortisol, cortisone, their free forms or cortisone/cortisol ratios influences human mood, but no significant sex differences between male and female group even though higher cortisol values in women [3].

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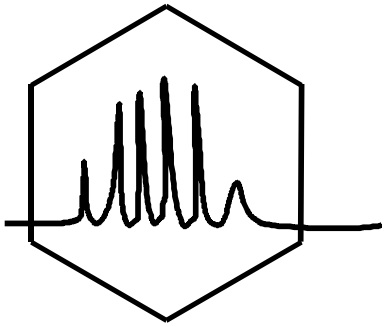

THE XXXIInd SYMPOSIUM

'CHROMATOGRAPHIC METHODS OF INVESTIGATING THE ORGANIC COMPOUNDS'

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E X T E N D E D A B S T R A C T S

CONTENTS

Interactions among electron-donor compounds and packings modified with cyclam-transition metal complexes

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1. Introduction

Nowadays there is continuous need for selective gas chromatography stationary phases, to be applied not only to analytical chemistry, but also to the pharmacy and other similar application domains. Development of such phases can help to achieve better resolution parameters in many difficult chromatographic separations. Recently, significant attention has been drawn to macrocycles. Macrocycles are useful in the determination of large group of analytes, mainly due to their ability to form specific interactions with many other molecules. For this reason macrocyclic stationary phases are highly specific towards many organic and inorganic molecules.

Cyclam (1,4,8,11-tetraazacyclotetradecane) (Fig. 1) is known for its strong affinity to mono and divalent metal ions. Complexes of cyclam with copper, nickel and cobalt are very stable, even in high temperatures, which determines their application in catalysis [1]. Both cyclam and its derivatives as well as cyclam complexes show interesting interactions with many ions and neutral molecules, so can be applied in LC, chemical sensors [2], and pharmacy [3].

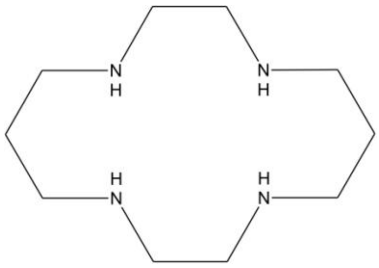

Figure 1. The structure of 1,4,8,11-tetraazacyclotetradecane (cyclam).

Researches carried out on Complexation Gas Chromatography (CGC) stationary phases, such as chemically bonded diketones and ketoimines [4,5], showed very good separation of aromatics and unsaturated olefins differing only with fine structure properties.

Supramolecular chiral selectors are also widely used in CGC instead of chelate complexes. Schurig in his work presented a wide variety of chiral separations carried on macrocyclic-based stationary phases [6].

2. Reagents and apparatus

Preparation of packings was based on commercial Unibeads 3S 80/100 (Grace Davison, Deerfield, USA). Solvents: hexane, kerosene fraction, POCH, (Gliwice, Poland); ethanol, analytically pure, POCH, (Gliwice, Poland); xylene, mixture of isomers, analytically pure, Chempur, (Piekary Śląskie, Poland). (3-chloropropyl)-triethoxysilane 95% was delivered by Sigma-Aldrich, (St. Louis, USA). Copper(II) chloride, pure, was manufactured by POCH, (Gliwice, Poland). 1,4,8,11-tetraazacyclotetradecane 98% was obtained from Aldrich, (Steinheim, Germany).

Chromatographic measurements were provided using Varian CP-3800 gas chromatograph equipped with FID detector and standard stainless steel columns 1/8 inch diameter, 150 cm length.

3. Preparation of packings

Synthesis of modified silica's was realized in four stages (Fig. 2). 12 g of dried silica (130 ºC, under vacuum) was placed in a round-bottom flask and kept under reflux with continuous stirring with anhydrous xylene (75 cm^3) and 8.8 cm^3 (3-chloropropyl)-triethoxysilane for 12 hours. 3-chloropropyl functionalized derivative was extracted with anhydrous xylene using soxhlet apparatus. In the second stage the silica was stirred in boiling xylene under reflux for 12 hours with addition of 1,4,8,11-tetraazacyclotetradecane (1.7 g). Product was extracted in soxhlet extractor with anhydrous xylene. Third stage was the end-capping reaction with hexamethyldisilazane in anhydrous xylene under reflux. After that, the cyclam functionalized silica was extracted in soxhlet apparatus with xylene, hexane and ethanol. In the fourth stage silica with chemically bonded cyclam groups was stirred in $CuCl_2$ ethanol solution (20 mg Cu^{2+}/mL), for one day and then rinsed with ethanol several times. Products after third and fourth stages (CFS – silica with chemically bonded cyclam groups and CCFS – silica with bonded cyclam-$CuCl_2$ complexes) were dried in 100 ºC.

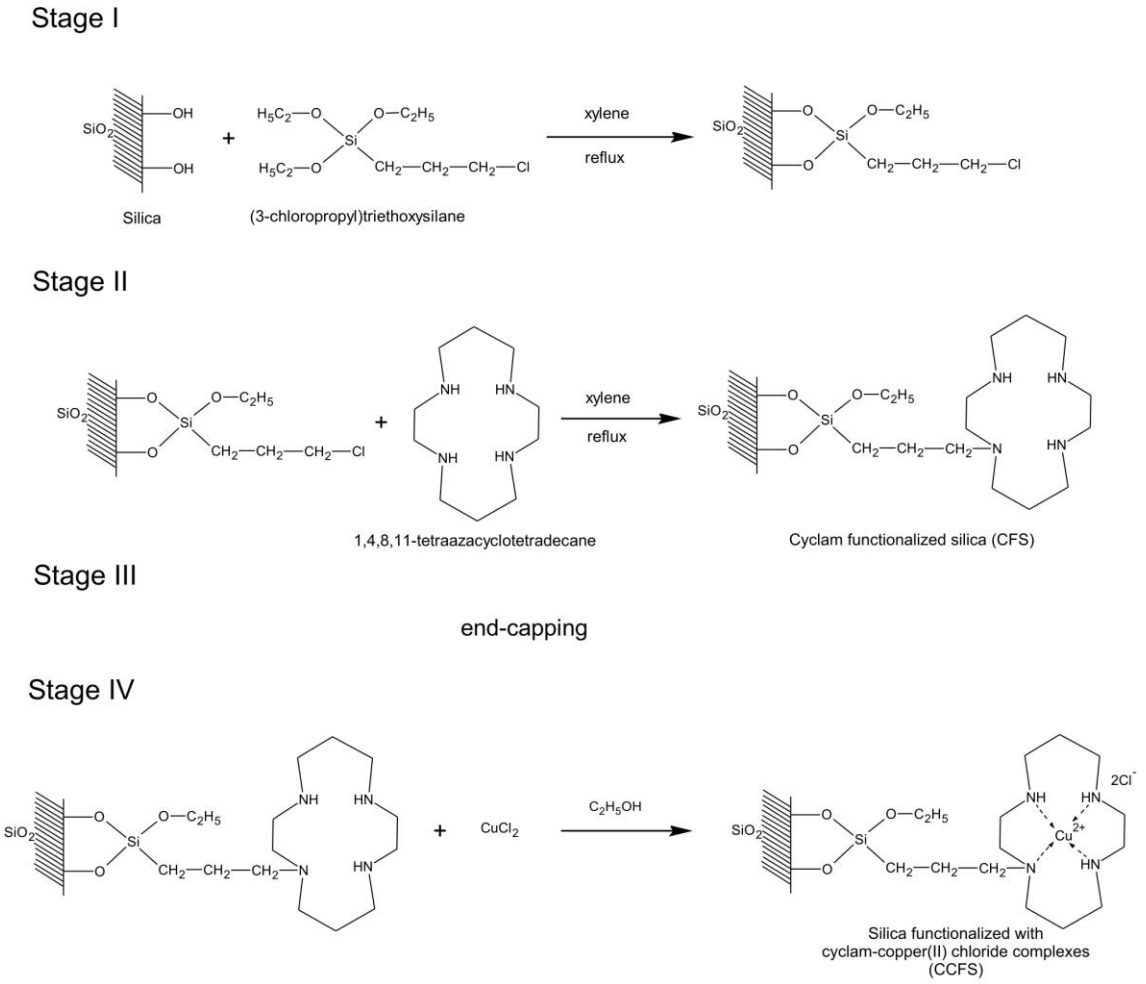

Figure 2. Scheme of silica modification reactions.

4. Results and discussion

4.1. Investigation of packings

Prepared packings were investigated using elemental analysis (Tab. 1). Surface concentration of bonded silanes was calculated based on the carbon content for CPFS (silica with bonded 3-chloropropyl functionalities) and on the nitrogen content for CFS. Amount of complexed copper was determined using ICP-OES. For CCFS results show that 77.9% of superficial cyclam groups were complexing copper ions.

Table 1. Results of elemental analysis.

Packing	C [%]	H [%]	N [%]	Surf. Coverage [μmol/m^2]
Unibeads 3S	0.170	0.482	0.017	-
CPFS	3.997	1.034	0.023	2.59
CFS	6.335	1.385	1.245	0.86

Thermogravimetric methods were applied to determine the thermal stability of prepared packings. Obtained thermograms (Fig. 3) show acceptable maximum operation temperatures for gas chromatography; 220 °C for CFS and 200 °C for CCFS.

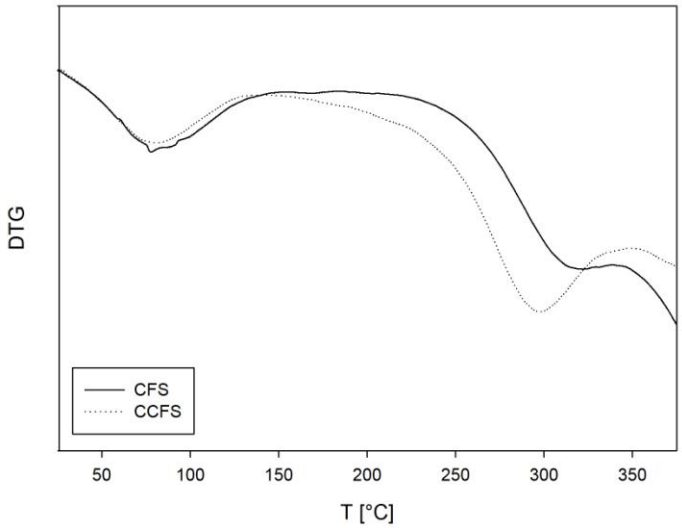

Figure 3. DTG curves of CFS and CCFS packings.

Figure 4 shows three Diffuse Reflectance UV-Vis spectra's. The presence of bonded copper in CCFS is represented by the absorption band with maximum at about 640 nm.

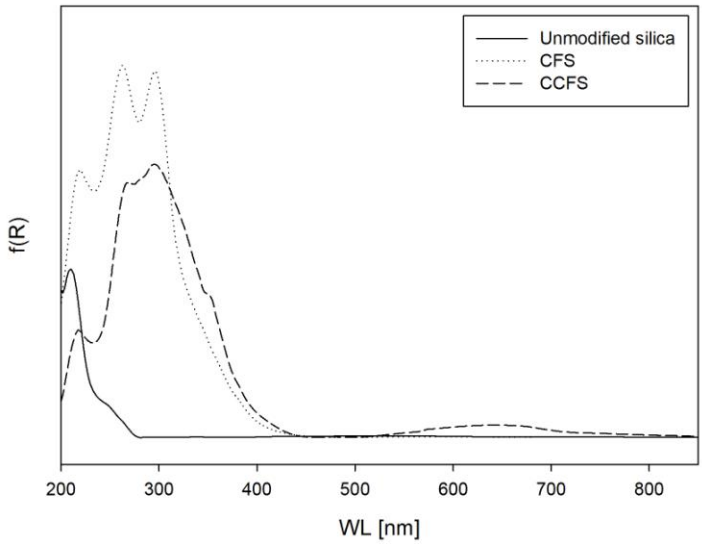

Figure 4. DRUV-Vis spectra's of prepared packings.

4.2. Interactions of packings with electron-donor compounds.

Both CFS and CCFS packings were tested in gas chromatography systems. To shorten the retention times and simultaneously increase the interactions with volatile compounds we used silica with high specific surface (about 280 m^2/g) and a short column (1.5 m). The injection volume was 0.1 μL of vapors. Tested compounds are listed in tables 2 – 5.

Table 2. Ethers. I_a, I_b – Kovàts indices; ΔM_e – molecular retention index; $\Delta I = I_b - I_a$.

Oven temperature: 140 °C.

Adsorbate	**CFS**		**CCFS**		
	I_a	ΔM_e	I_b	ΔM_e	ΔI
butyl ethyl ether	716	0.32	761	6.56	45
butyl methyl ether	645	4.28	698	11.72	53
tert-butyl ethyl ether	660	-7.57	695	-2.64	35
furan	466	-0.65	520	6.91	54
2-methylfuran	570	-0.16	622	7.12	52
tetrahydrofuran	654	21.59	739	33.55	85
thiophene	620	4.80	682	13.49	62
tetrahydrothiophene	745	18.39	831	30.37	86

Table 3. Linear aliphatic hydrocarbons. Oven temperature: 100 °C.

Adsorbate	CFS		CCFS		
	I_a	ΔM_e	I_b	ΔM_e	ΔI
hexane	600	0.00	600	0.00	0
1-hexene	591	0.79	611	3.49	20
cis-2-hexene	603	2.42	618	4.55	15
trans-2-hexene	599	1.89	611	3.54	12
1,5-hexadiene	582	1.49	616	6.30	34
2,3-hexadiene	621	6.91	643	10.05	22
1-hexyne	622	7.14	690	16.62	68
3-hexyne	645	10.29	705	18.80	60
heptane	700	0.00	700	0.00	0
1-heptene	692	0.84	711	3.52	19
cis-2-heptene	703	2.46	719	4.67	16
trans-2-heptene	700	2.02	712	3.72	12

Table 4. Branched aliphatic hydrocarbons. Oven temperature: 100 °C.

Adsorbate	CFS		CCFS		
	I_a	ΔM_e	I_b	ΔM_e	ΔI
2,2-dimethylbutane	560	-5.59	564	-5.10	4
2,3-dimethylbutane	577	-3.26	579	-2.90	2
2,3-dimethyl-1-butene	577	-1.24	590	0.66	13
3,3-dimethyl-1-butene	546	-5.56	560	-3.54	14
3-methylpentane	587	-1.85	588	-1.73	1
3-methyl-1-pentene	573	-1.77	587	0.16	14
cis-3-methyl-1-pentene	603	2.37	612	3.64	9
trans-3-methyl-1-pentene	607	3.05	614	4.00	7
2,2,4-trimethylpentane	729	-9.94	734	-9.22	5
2,4,4-trimethyl-1-pentene	742	-6.18	758	-3.86	16

Table 5. Cyclic hydrocarbons. Oven temperature: 100 °C.

Adsorbate	CFS		CCFS		
	I_a	ΔM_e	I_b	ΔM_e	ΔI
cyclohexane	605	2.78	602	2.30	-3
methylcyclopentane	590	0.58	589	0.53	-1
cyclohexene	617	6.45	626	7.65	9
1,3-cyclohexadiene	620	8.80	647	12.68	27
1,4-cyclohexadiene	643	12.06	670	15.82	27
benzene	632	12.54	688	20.42	56
1-methyl-1-cyclopentene	602	4.29	609	5.29	7
1-methyl-1-cyclohexene	712	5.78	717	6.36	5
4-methyl-1-cyclohexene	697	3.66	708	5.22	11
cycloheptane	723	5.20	719	4.62	-4
cycloheptene	720	6.80	731	8.40	11

Interpretation of interactions between volatile electron-donor organic compounds and the *d*-orbitals of copper in cyclam complex was made on the basis of the difference between Kovàts indices of CCFS and CFS stationary phases. When the difference (ΔI) is higher, the influence on the retention time by transient metal is stronger. The influence is more positive when density of electron orbital of analyzed compound is higher and the orbital is more stericaly accessible. In case of ethers (Tab. 2) positive interactions are the greatest from all studied compounds, cause of their high electron density. Linear and branched ethers attract weaker with the CCFS phase, than the cyclic ones. That is because of the high repulsive steric effect (the copper ion in cyclam complex has a big steric surrounding). Electron-donor orbitals in cyclic ethers are more available, so the interactions are stronger. For aromatic ethers (furan, thiophene) the *n*-orbitals of the heteroatom take part in delocalized π-orbital formation, so they are less accessible for *d*-orbital of copper ion, than the *n*-orbitals of cyclic ethers (tetrahydrofurane, tetrahydrothiophene).

Interactions between aliphatic hydrocarbons (Tab. 3, 4) and the copper ion depend on the double bond count, type of bond (single, double, triple) and the steric effect. Electron density of triple bond is higher than double bond, in result alkynes attract with the phase stronger than alkenes. Furthermore more double bonds in the molecule affects in stronger affinity to the copper ion. Negative steric effect can be explained on the example of *cis* and

trans isomers. *Cis* isomers attract stronger than *trans*, because their double bond is more exposed. In some cases the negative steric effect is stronger than positive electron donor effect and the compound (ex. 2,4,4-trimethyl-1-pentene, has negative ΔM_e value) is actually repulsed from the stationary phase. For cyclic hydrocarbons (Tab. 5) all effects are approximately the same as for aliphatic hydrocarbons, but they have more in common with branched hydrocarbons, because annularity affects the interactions just the same as branching.

5. Conclusion

Results show that obtained stationary phases can be applied for gas chromatography in separation of many electron-donor organic compounds revealing only slight structure differences. Thanks to the combination of positive electron effects and negative steric effects it is possible to separate structure isomers using packed columns. In order to maximize the efficiency of columns and make prepared stationary phases applicable for commercial use it is need to transfer cyclam complexes into capillary chromatography, which will be done in our future researches. Obtained set of chromatographic data including explanation of specific host-guest interactions can be utilized in pharmacy, and many analytical methods [7].

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Thermodynamics of interactions of olefins with stationary phases containing Co(II) and Ni(II) salts chemically bonded to silica in gas chromatography

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Key words: thermodynamic parameters, supported metal complexes, specific interactions, aliphatic linear and branched hydrocarbons

Summary

Thermodynamic research was performed on packings containing cobalt (II) and nickel acetylacetonate (acac), hexafluoroacetylacetonate (hfac), and chloride, chemically bonded via β-diketonate groups. The specific retention volume (V_g) for aliphatic linear and branched hydrocarbons was determined and used to calculate the following thermodynamic parameters: free energy of adsorption (ΔG_a), heat of adsorption (ΔH_a), and entropy of adsorption (ΔS_a). These parameters enable characterization of specific interactions between adsorbate molecules and metal complexes chemically bonded to the silica surface.

Introduction

In complexation gas chromatography the most commonly used modifiers of packings are salts and complexes of Ni and Co [1-3]. Such compounds were initially used either in the "coated on support" form, or dissolved in liquid stationary phase, or as pure metal salts (e.g. $CoCl_2$, $NiCl_2$, tetra(-4-methylpyridino)nickel dithiocyanate or tetra(γ-picoline) dithiocyanate complexes of Co and Ni [4,5].

Packings modified with the above-mentioned metal complexes are characterized by high selectivity, thermal stability and resistance to different external factors [1, 6-8]. These packings form a good base to study interactions between stationary phases and the adsorbate molecules showing electron-donor properties. A possibility of influencing the above parameters in a simple way, by changing the composition of the chemically bonded phase, makes these packings important from both analytical and physicochemical points of view.

The gas chromatography can be successfully used to investigate the kinetics and thermodynamics of metal-ligand interactions and to determine the complex stability constants. The chromatographic properties of sorbents modified with the surface layers of pure chelates and with the stationary phases containing metal complexes depend on many factors, the most essential of which are the nature of the metal, distribution of the electron density in the complex, and geometry of the complex after its bonding to the support surface or its inclusion into the liquid phase [9].

In [10] broadened research is described devoted to physico-chemical characteristics of chemically bonded metal complexes used as stationary phases in gas chromatography. The aims of this investigation were to characterize the stationary phases by the solubility parameter, dispersive force parameters, and thermodynamic parameters of solutions, as well as to determine the influence of the compounds on the parameters considered.

A discussion on physico-chemical aspects of a sorption and a separation by means of complexes present at the surface of a stationary phase was given by Slizhov [9]. Special attention was given to the physicochemical aspect of sorption and separation with participation of the complex that forms sites of the surface.

In [11] interesting research results are described regarding thermodynamic studies on 12 pairs of N-trifluoroacetyl-*O*-alkyl nipecotic acid ester enantiomers on diluted permethylated β-cyclodextrin stationary phase (CP Chirasil-Dex CB). Similar, interactions between aroma compounds (d-limonene, ethyl hexanoate, octanal and 1-hexanol) and high amylase cornstarch were studied by Baoutboul et. al [12].

Packings in which transition metals were immobilized on a carrier by means of β-diketonate groups were determined as very effective towards a separation of several groups of organic compounds [13-19]. Separation properties of the packings were controlled by applying different metals at the silica surface, in the form of a salt, e.g., MCl_2, as well as complexes of types $M(acac)_2$ and $M(hfac)_2$, where M means Cu(II) [13-15], Co(II), and Ni(II) [16-19]. Based on physicochemical and spectroscopic research it was determined that the metal ion is complexed mainly by a single group bonded to the silica [20]. For the chromatographic applications, such metal-bonding type is very profitable due to the accessibility of the metallic center towards the sorbate [20].

This paper presents results of studies of packings containing Co(II) and Ni(II) salts chemically bonded to silica via β-diketonate groups β-diketonate groups. Cobalt and nickel complexes were bonded to the silica surface by means of β-diketonate silanes, using 3-[(3-(trimethoxysilyl)propyl)]-2,4 pentanodione.

We concentrated our research on the packings containing such metal salts as chloride (Cl), acetylacetonate (acac) and hexafluoroacetylacetonate (hfac). Special attention was paid to fluorinated acetylacetonate, because replacement of hydrogen atoms of methyl groups, present in the acetylacetonate ligand, by fluorine atoms results in a decrease in the σ-donor and π-donor properties of the ligands. At the same time the capability of bonding additional ligands to the metal increases.

To characterize the packings investigated, the specific retention volume (V_g) was measured and used to calculate the flowing thermodynamic parameters: free energy of adsorption (ΔG_a), heat of adsorption (ΔH_a), and entropy of adsorption (ΔS_a). These parameters enable characterization of specific interactions between adsorbate molecules and metal complexes chemically bonded to a silica surface. The study was performed using the above-mentioned adsorbates for the analysis of aliphatic linear and branched hydrocarbons.

Experimental

Reagents

Silica (Porasil C, 80-100mesh) was obtained from Waters Associates (Milford, MA, USA). 3-(3-trimethoxysilylpropyl)-pentanodione-2,4 (TMSPP) was obtained from the Laboratory of Organometallic Chemistry (AMU, Poznań, Poland). Transition metal salts were obtained from POCH (Gliwice, Poland). Xylene, hexane and tetrahydrofuran (THF) were distilled over metallic sodium prior to use. These chemicals were also obtained from POCH (Gliwice, Poland). Chromatographic standards were purchased from different producers, including Fluka (Buchs, Switzerland), Aldrich (Milwaukee, WI, USA) and ICN (Plainvie, USA).

Apparatus

Chromatographic measurements were carried out on a gas chromatograph CHROM 5 (Czech Republic) equipped with a flame-ionization detector. The temperature in the oven was determined using a DT 2000 thermometer (Digital Thermometer, Slandi, Warsaw, Poland) and the pressure at the column inlet was measured with a mercury manometer. Helium, dried on molecular sieve 4A, was used as carrier gas. The flow rate of the carrier gas was measured with a digital flowmeter (J&W Scientific, Falsom, CA, USA). Stainless steel columns were used (2 m in length, 3 mm in I.D.).

Elemental analysis was performed on 2400 CHN Elemental Analyzer (Perkin-Elmer, Norfolk, USA). The obtained results are presented in Table I. Surface areas determined by BET method are listed in Table I.

Table I. Physicochemical characteristics of the investigated packings

No	Packing	Elemental analysis [%]			Specific surface area [m^2 g^{-1}]	Silane surface concentration [μmol m^{-2}]
		C	H	Metal		
1.	No metal	2.54	0.61	-	75	3.30
2.	$CoCl_2$	1.06	0.38	0.71	94	1.10
3.	$Co(acac)_2$	2.26	0.46	0.10	79	2.11
4.	$Co(hfac)_2$	2.17	0.49	0.15	76	2.02
5.	$NiCl_2$	1.69	0.40	0.69	97	1.65
6.	$Ni(acac)_2$	2.46	0.47	0.11	91	2.88
7	$Ni(hfac)_2$	1.84	0.33	1.73	71	2.33

Results and discussion

The schematic structure of the packings studied in the paper is show in Figure 1. Detailed procedure of modification of these packings was given in our previous publications [17].

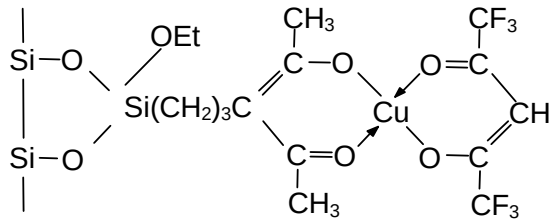

Fig. 1. *Schema of proposed silica modification*

The interactions between adsorbate and a packing modified by complexes of transition metals are the combined result of specific and non-specific interactions. The specific interactions depend on the π-electron properties of an adsorbate and its configuration. Such interactions results in, e.g., different retention behavior of aromatic hydrocarbons having either electron-

donor (-CH_3) or electron-acceptor (-Cl) substituents, as well as the fact that *trans*-isomers of n-olefins are eluted before *cis* isomers. Such behavior is reflected in different retention volumes, that in turn permits to determine some thermodynamic such as the heat of adsorption (-ΔH_a), the free energy of adsorption (ΔG_a), and the entropy of adsorption (ΔS_a).

These parameters enable characterization of specific interactions between adsorbate molecules and metal complexes chemically bonded to a silica surface.

The adsorbates used in our research were provided in doses of 0.01 μl of vapor, and the peaks obtained were symmetrical. Thus, we assumed that the measurement was taken in the linear range of the adsorption isotherm. As a result, we were able to compute given thermodynamic parameters [21-23].

The calculation of the heat of adsorption ΔH_a was based upon the relationship between the logarithm of the specific retention volume ln (V_g) and reciprocal of temperature (1/T) in [°K]. Heat of adsorption values were obtained for temperatures from 120°C to 150°C. Retention measurements for all adsorbates studied were obtained at three temperatures.

The reactions of creation of π-type complexes of olefins and transient-metal cations, which took place under such conditions, were characterized by full reversibility. Such behavior is one of the basic requirements for the application of these reactions in complexation gas chromatography.

The heat of adsorption (ΔH_a) was calculated from the slope of the retention diagram, which is equal to (- ΔH_a/R), according to equation (1):

$$\Delta H_a = -R \cdot \frac{\partial(\ln V_g)}{\partial(1/T)} \qquad (1)$$

The free energy of adsorption (ΔG_a) was calculated from the equation (2)

$$\Delta G_a = RT \ln K = RT \ln V_s \qquad (2)$$

where: R is the molar gas constant (8.314 J mol^{-1} K^{-1}), T – is the temperature, K is the partition constant, and V_s the specific retention volume (cm^3 m^{-1}).

The entropy of adsorption (ΔS_a) was calculated by use of the equation (3):

$$\Delta S_a = (\Delta H_a - \Delta G_a) \cdot T^{-1} \qquad (3)$$

In Table II some values are given of the heat of adsorption ($-\Delta H_a$), as well as the entropy of adsorption (ΔS_a), for the packings modified with cobalt (II). Similar results for nickel (II) are given in Table III.

Table II. Entropy ΔS_a [J mol^{-1}K^{-1}], free energy ΔG_a [kJ mol^{-1}] at 140°C and heat of adsorption $-\Delta H_a$ [kJ mol^{-1}] for aliphatic linear and branched hydrocarbons

Adsorbate	Packings with											
	-acac			$CoCl_2$			$Co(acac)_2$			$Co(hfac)_2$		
	ΔS_a	ΔG_a	$-\Delta H_a$	ΔS_a	ΔG_a	$-\Delta H_a$	ΔS_a	ΔG_a	$-\Delta H_a$	ΔS_a	ΔG_a	$-\Delta H_a$
Hexane	33.75	10.07	25.11	88.01	10.19	32.79	31.46	10.34	30.63	35.97	10.87	36.84
1-hexene	38.37	10.20	26.80	74.86	10.25	38.61	34.14	10.56	31.16	69.28	10.98	37.83
cis-2-hexene	42.82	10.35	28.30	61.87	10.43	33.24	35.68	10.73	24.04	73.68	11.03	39.43
trans-2-hexene	37.87	10.24	26.48	59.94	10.37	32.68	40.37	10.68	25.97	71.14	11.00	38.49
1,3-hexadiene	50.45	9.39	30.69	80.85	9.65	39.41	54.40	9.74	30.51	73.64	10.02	38.64
1,4-hexadiene	43.63	9.84	28.64	77.64	9.99	38.37	45.73	10.07	28.07	70.00	10.20	37.75
1,5-hexadiene	41.74	9.75	28.05	71.77	10.04	36.66	45.96	10.27	28.05	68.71	10.48	37.53
1,3,5-hexatriene	36.36	9.33	25.91	79.76	9.87	38.49	42.77	9.97	26.80	69.41	10.23	37.85
2-methylpentane	30.72	10.30	24.07	43.96	10.55	27.83	38.48	10.79	25.97	39.97	12.05	25.15
2-methyl-1-pentene	37.03	10.08	26.19	58.24	10.76	31.95	52.37	10.83	30.79	43.26	10.90	27.97
4-methyl-1-pentene	33.39	10.11	25.06	50.65	10.96	29.68	33.95	11.05	23.74	35.13	11.35	25.22
2-methyl-2-pentene	33.39	10.28	24.74	65.78	10.82	34.54	41.55	10.95	26.34	45.62	10.75	28.75
cis-3-methyl-2-pentene	37.31	9.74	26.19	21.20	10.05	16.39	44.63	10.49	27.35	46.31	10.69	28.97
trans-3-methyl-2-pentene	36.78	9.67	25.92	65.28	9.81	34.28	43.62	10.19	26.91	44.36	10.53	28.14
cis-4-methyl-2-pentene	30.19	9.18	23.84	55.35	9.45	31.21	39.33	10.31	25.97	35.42	10.45	25.29
trans-4-methyl-2-pentene	32.67	9.17	24.79	55.17	9.40	31.18	38.33	10.29	25.53	35.09	10.40	25.15
2,3-dimethylbutane	15.34	9.30	18.44	44.27	10.19	24.99	40.14	9.68	23.70	40.85	10.38	26.55
2,3-dimethyl-1-butene	29.52	10.11	23.55	57.69	10.31	31.99	50.69	10.46	30.46	41.99	11.14	27.71
2,3-dimethyl-2-butene	34.93	10.22	25.10	71.35	10.59	36.34	48.86	10.68	28.96	50.90	11.38	30.46
3,3-dimethyl-1-butene	24.36	11.45	22.13	47.06	11.79	28.69	36.11	11.85	25.40	22.37	11.94	20.76

Table III. Entropy ΔS_a [J mol^{-1}K^{-1}], free energy ΔG_a [kJ mol^{-1}] at 140°C and heat of adsorption $-\Delta H_a$ [kJ mol^{-1}] for aliphatic linear and branched hydrocarbons

Adsorbate	Packings with											
	-acac			$NiCl_2$			$Ni(acac)_2$			$Ni(hfac)_2$		
	ΔS_a	ΔG_a	$-\Delta H_a$	ΔS_a	ΔG_a	$-\Delta H_a$	ΔS_a	ΔG_a	$-\Delta H_a$	ΔS_a	ΔG_a	$-\Delta H_a$
Hexane	33.75	10.07	24.11	41.87	10.10	29.82	27.13	10.42	24.83	39.39	10.67	30.08
1-hexene	38.37	10.20	26.80	57.50	10.52	33.05	65.77	10.62	35.98	62.04	11.07	35.09
cis-2-hexene	42.82	10.35	28.30	50.18	10.89	30.63	71.81	11.63	37.89	66.78	11.34	36.62
trans-2-hexene	37.87	10.24	26.48	52.68	10.54	31.81	70.03	11.46	37.38	66.56	11.05	36.68
1,3-hexadiene	50.45	9.39	30.69	70.34	9.56	36.88	78.35	9.65	39.66	66.57	9.83	35.84
1,4-hexadiene	43.63	9.84	28.64	66.55	10.43	35.64	74.76	10.52	38.73	65.73	10.68	36.08
1,5-hexadiene	41.74	9.75	28.05	61.35	10.68	34.18	72.27	10.94	38.08	70.19	11.05	38.04
1,3,5-hexatriene	36.36	9.33	25.91	65.45	9.46	35.19	81.57	9.59	40.29	79.55	9.87	40.17
2-methylpentane	34.72	10.30	25.07	42.59	11.30	28.05	28.45	11.35	25.56	40.47	11.89	31.83
2-methyl-1-pentene	37.03	10.08	26.19	53.16	10.42	31.32	42.99	10.42	27.37	41.40	11.41	32.71
4-methyl-1-pentene	33.39	10.11	25.06	48.05	10.88	29.77	34.43	10.93	24.51	37.86	11.74	36.65
2-methyl-2-pentene	33.39	10.28	24.74	55.35	10.76	32.02	6.01	10.87	13.24	41.89	11.25	37.75
cis-3-methyl-2-pentene	37.31	9.74	26.19	61.32	10.21	32.67	47.01	10.11	28.64	42.63	11.20	37.99
trans-3-methyl-2-pentene	36.78	9.67	25.92	54.66	10.14	31.63	45.87	10.04	28.12	44.15	11.13	38.51
cis-4-methyl-2-pentene	30.19	9.18	23.84	49.40	10.80	30.22	35.21	10.79	24.67	39.20	11.69	37.13
trans-4-methyl-2-pentene	32.67	9.17	24.79	51.49	10.84	31.09	37.53	10.81	25.61	38.72	11.73	36.98
2,3-dimethylbutane	35.34	9.30	26.44	43.99	11.33	32.45	29.58	11.38	26.46	34.85	11.94	29.66
2,3-dimethyl-1-butene	36.52	10.11	23.55	49.92	10.59	33.32	38.72	10.69	26.96	38.76	11.68	36.95
2,3-dimethyl-2-butene	39.93	10.22	25.10	58.00	10.91	35.71	51.72	10.82	30.22	44.13	11.26	38.39
3,3-dimethyl-1-butene	35.36	10.69	22.13	42.09	11.43	33.95	26.32	11.46	28.84	35.77	12.27	36.35

The heat of adsorption on the packings was measured in the temperature range 120-150°C. The formation of π-type complexes between aliphatic linear and branched hydrocarbons and transition metal cations under these conditions is characterized by full reversibility, which is a prerequisite for the use of this phenomenon in complexation gas chromatography. The heat of adsorption was usually below 58÷63 kJ mol^{-1}, which led to the conclusion that chemisorption did not proceed in the systems studied [24]. The data listed in Table III show that ($-\Delta H_a$) values are highest for the packing with bonded Ni(II) and Co(II) hexafluoroacetylacetonates. The other two packings (i.e. those with bonded Ni(II) and Co(II) chloride and Ni(II) and Co(II) acetylacetonates) have similar ($-\Delta H_a$) values. A comparison of values of adsorption heat for metal-containing packings and for the packings without a metal shows that this parameter is higher for the packings with bonded metal salts.

ΔH_a and ΔG_a values allowed to determine an influence of steric factor, both for the unsaturated, and saturated hydrocarbons. For example, adsorption heat and free energy values are being lowered while a level of branching of a molecule is decreasing:

hexane > 2-methylopentane > 2,3-dimethylobutane

These values are less for the alkenes in comparison with corresponding olefins.

In Table IV and Table V beside values of adsorption free energy ΔG_a, a difference is given of free energy between a packing with a bonded metal, and a packing with free functional group. Taking into account the fact that the retention research was performed under similar conditions for all the packings under study, one may draw a conclusion that the difference of adsorption energy is related with a factor differentiating the packing, e.g., with metal complex bonded to the surface. Assuming absolute values of ΔG_a computed from the formula (4):

$$\delta\Delta G_a = \Delta G_{a(\text{with metal})} - \Delta G_{a(\text{no metal})}$$

the packings may be ordered by increasing values of $\delta\Delta G_a$:

$CoCl_2 < Co(acac)_2 < Co(hfac)_2$ and $NiCl_2 < Ni(acac)_2 < Ni(hfac)_2$

Table IV. Free energy ΔG_a [kJ mol^{-1}] at 140°C and differences in free energy of adsorption $\delta\Delta G_a$ [kJ mol^{-1}] for aliphatic linear and branched hydrocarbons

Adsorbate	Packings with						
	-acac	$CoCl_2$	$Co(acac)_2$	$Co(hfac)_2$	$CoCl_2$	$Co(acac)_2$	$Co(hfac)_2$
	ΔG_a				$\delta\Delta G_a = \Delta G_{a(\text{with metal})} - \Delta G_{a(\text{no metal})}$		
Hexane	10.07	10.19	10.34	10.87	0.12	0.27	0.80
1-hexene	10.20	10.25	10.56	10.98	0.05	0.36	0.78
cis-2-hexene	10.35	10.43	10.73	11.03	0.08	0.38	0.68
trans-2-hexene	10.24	10.37	10.68	11.00	0.13	0.44	0.76
1,3-hexadiene	9.39	9.65	9.74	10.02	0.26	0.35	0.63
1,4-hexadiene	9.84	9.99	10.07	10.20	0.15	0.23	0.36
1,5-hexadiene	9.75	10.04	10.27	10.48	0.29	0.52	0.73
1,3,5-hexatriene	9.33	9.87	9.97	10.23	0.54	0.64	0.90
2-methylpentane	10.30	10.55	10.79	12.05	0.25	0.49	1.75
2-methyl-1-pentene	10.08	10.76	10.83	10.90	0.68	0.75	0.82
4-methyl-1-pentene	10.11	10.96	11.05	11.35	0.85	0.94	1.24
2-methyl-2-pentene	10.28	10.82	10.95	10.75	0.54	0.67	0.47
cis-3-methyl-2-pentene	9.74	10.05	10.49	10.69	0.78	0.75	0.95
trans-3-methyl-2-pentene	9.67	9.81	10.19	10.53	0.14	0.52	0.86
cis-4-methyl-2-pentene	9.18	9.45	10.31	10.45	0.27	1.13	1.27
trans-4-methyl-2-pentene	9.17	9.40	10.29	10.40	0.23	1.12	1.23
2,3-dimethylbutane	9.30	9.39	9.68	10.38	0.09	0.38	1.08
2,3-dimethyl-1-butene	10.11	10.31	10.46	11.14	0.20	0.35	1.03
2,3-dimethyl-2-butene	10.22	10.59	10.68	11.38	0.37	0.46	1.16
3,3-dimethyl-1-butene	11.45	11.79	11.85	11.94	0.34	0.40	0.49

Table V. Free energy ΔG_a [kJ mol^{-1}] at 140°C and differences in free energy of adsorption $\delta\Delta G_a$ [kJ mol^{-1}] for aliphatic linear and branched hydrocarbons

Adsorbate	Packings with						
	-acac	$NiCl_2$	$Ni(acac)_2$	$Ni(hfac)_2$	$NiCl_2$	$Ni(acac)_2$	$Ni(hfac)_2$
	ΔG_a				$\delta\Delta G_a = \Delta G_{a(\text{with metal})} - \Delta G_{a(\text{no metal})}$		
Hexane	10.07	10.10	10.42	10.67	0.03	0.35	0.60
1-hexene	10.20	10.52	10.62	11.07	0.32	0.42	1.14
cis-2-hexene	10.35	10.89	11.63	11.34	0.54	1.28	0.99
trans-2-hexene	10.24	10.54	11.46	11.05	0.30	1.22	0.81
1,3-hexadiene	9.39	9.56	9.65	9.83	0.17	0.26	0.44
1,4-hexadiene	9.84	10.43	10.52	10.68	0.59	0.68	0.84
1,5-hexadiene	9.75	10.68	10.94	11.05	0.93	1.19	1.30
1,3,5-hexatriene	9.33	9.46	9.59	9.87	0.13	0.26	0.54
2-methylpentane	10.30	11.30	11.35	11.89	1.00	1.05	1.59
2-methyl-1-pentene	10.08	10.42	10.42	11.41	0.34	0.34	1.33
4-methyl-1-pentene	10.11	10.88	10.93	11.74	0.77	0.82	1.63
2-methyl-2-pentene	10.28	10.76	10.87	11.25	0.48	0.59	0.97
cis-3-methyl-2-pentene	9.74	10.21	10.11	11.20	0.47	0.37	1.46
trans-3-methyl-2-pentene	9.67	10.14	10.04	11.13	0.47	0.37	1.46
cis-4-methyl-2-pentene	9.18	10.80	10.79	11.69	1.62	1.61	2.51
trans-4-methyl-2-pentene	9.17	10.84	10.81	11.73	1.67	1.64	2.56
2,3-dimethylbutane	9.30	11.33	11.38	11.94	2.03	2.08	2.64
2,3-dimethyl-1-butene	10.11	10.59	10.69	11.68	0.48	0.58	1.57
2,3-dimethyl-2-butene	10.22	10.91	10.82	11.26	0.69	0.60	1.04
3,3-dimethyl-1-butene	10.69	11.43	11.46	12.27	0.74	0.77	1.58

The difference between the adsorbates in the scope of an application of a single packing is quite important while testing the packing towards efficient separation of isomers. The applicability of the packings towards such separation is the following:

$$CoCl_2 > Co(acac)_2 > Co(hfac)_2 \quad \text{and} \quad NiCl_2 > Ni(acac)_2 > Ni(hfac)$$

The order is influenced by the fact that too strong interactions limit packing selectivity. In Fig. 2 sample separation is presented of a mixture of *trans*/*cis* isomers of hexane. The separation was achieved by means of the packing with $Co(hfac)_2$ bonded to the silica surface.

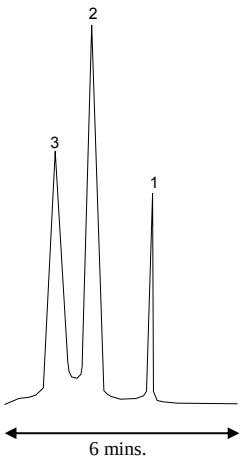

Fig. 2. *Separation of a mixture of cis/trans isomers of hexene; column: $Co(hfac)_2$; V_{Ar}=28 ml/min.; temp=152.2 ℃; peaks: 1) Hexane, 2) trans-2-hexene, 3) cis-2-hexene*

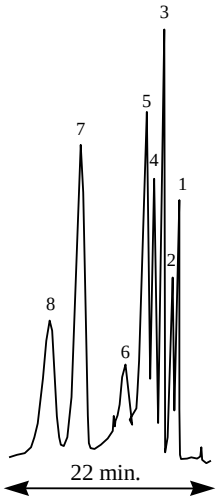

Fig. 3. *Separation of mixture of linear and branched aliphatic hydrocarbons. Packing with $NiCl_2$:column temperature, 101^0C; V_{helium}= 27,0 ml min^{-1} Peaks: 1) 3-methyl-1,2-butadiene 2) 3,3-dimethylbutene-1 3) 2,3-dimethylbutene-1 4) 2,3-dimethylbutene-2 5) cis-1,4-hexadiene 6) 1,3-hexadiene 7) 2,2,4-trimethylpentane 8) 2,4,4-trimethylpentene-1*

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Chromatographic separation and isolation of arylstilbazolium *cis*/*trans* isomers

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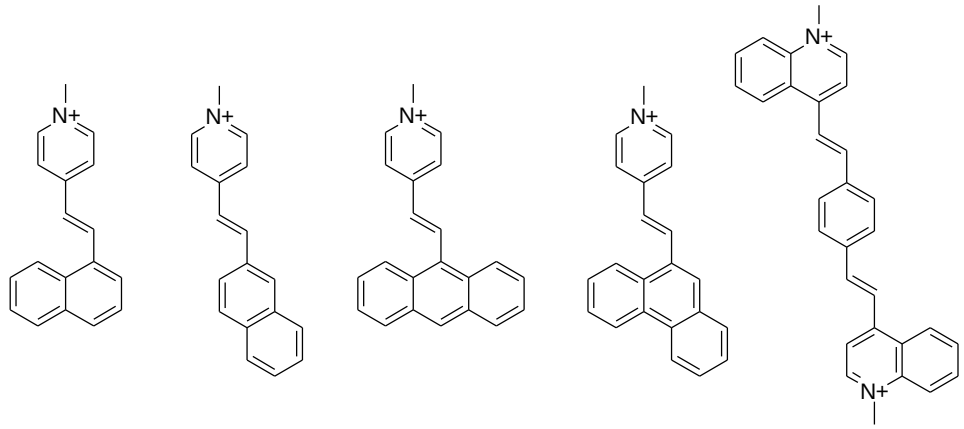
Introduction

Anticancer therapy is the most developing field in medicinal chemistry. Chemotherapy, being part of this area, is commonly applied on variety cancer diseases. A majority of chemical medications (ligands) interact with DNA structure, affecting cell poliferation. When DNA/ligands reaction concerns four-stranded DNA (G-quadruplex, tetraplex), the G-quadruplex structure is consequently stabilized. Stabilization of tetraplexes on a telomeric DNA strand inhibits telomerase activity, the enzyme which operates in cancer cells. Moreover, four-stranded form of gene promoters can also be stabilized by these ligands. As a result, oncogene activation is inhibited [1-2]. Our previously investigation showed that *trans* isomers of arylstilbazolium derivatives stabilized four-stranded DNA, therefore, ligands 1-5 are expected to be useful in anticancer therapy [3]. On the other hand, photoisomarization of arylstilbazolium derivatives was reported [4-6]. With an assumption that different isomers reacting with DNA will show different binding affinity, the DNA binding studies with *cis* isomers of arylstilbazolium ligands should be performed.



1 2 3 4 5

Figure 1. *Arylstilbazolium ligands.*

In order to carry out DNA/ligands interaction studies, *trans* and *cis* isomers need to be separated and isolated. HPLC technique is generally used for these processes. Here, we report an optimization study in order to separate *cis* and *trans* isomers of arylstilbazolium ligands using HPLC approach.

Experimental

Compounds 1-5 were previously synthesized in our laboratory as *trans* forms. HPLC separations was carried out on a Hitachi LaChrom HPLC system composed of a L-7100 pump, L-7455 diode array detector and D-7000 interface. Seperations were performed on a ODS column (Inertsil, ODS-3, GL Sciences Inc.) using gradient or isocratic elution with a flow rate of 1 ml/min. The mobile phase contained acetonitryle (HPLC gradient grade, J. T. Baker), ammonium acetate (P. A., POCH) and Millipore-filtered water. Chromatograms for each isomer were monitored at the isosbestic point of ligand. For sample preparation, an aqueuos solution of *trans* isomer of ligand ($1x10^{-5}$M) was exposed to sun-light for two hours for extensive *trans*→*cis*

isomerization. An aliquot of 50 μL of isomeric mixture was then injected on the column.

Results and discussion

As expected in chromatography, good separation of peaks and short retention time are desirable Therefore, the optimization of separation process of *cis/trans* isomers (of arylstilbazolium derivatives 1 - 5) was carried out using three different methods. Two gradient elution methods with different composition of mobile phases and one isocratic elution method with two mobile phases (Table 1).

Table 1. Elution methods used for the separation of *cis*/*trans* isomers.

Name of mathod	Type of method	Components of mobile phase (ACN = acetonitryle)	Elution conditions Time [min]
Method 1	Isocratic elution	A = 70 mM ammonium acetate in 65% ACN B = 70 mM ammonium in water	Time: 0 30 %A: 50 50 %B : 50 50
Method 2	Gradient elution	A = 70 mM ammonium acetate in 65% ACN B = 70 mM ammonium in water	Time: 0 15 20 %A 10 100 10 %B 90 0 90
Method 3	Gradient elution	A = 70 mM ammonium acetate in 65% ACN B = 70 mM ammonium acetate in 35% ACN	Time: 0 10 20 25 %A 0 40 40 0 %B 100 60 60 100

Cis/*trans* isomers of ligand 1 are eluted using all three methods. The best separation is received with Method 1 and 2, but the shortest retention times (11.52 min and 14.75 min for cis and trans isomers, respectively) is obtained exploiting Method 3 with satisfactory separability.

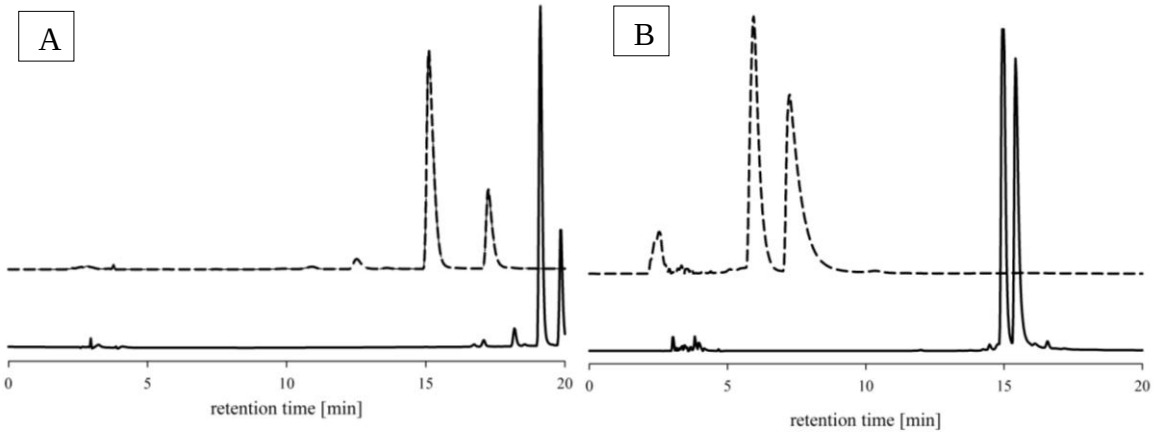


Figure 2. *Separation of isomers of ligand 2 (A) monitored at 275 nm and ligand 5 (B) monitored at 386 nm. Chromatogram A shows separation with Method 3 (broken line) and with Method 2 (solid line). Chromatogram B demonstrates separation using Method 1 (broken line) and using Method 2 (solid line).*

Similarly, for derivative 2 the shortest retention time (for *cis* form t = 15.12 min, for *trans* form t = 17.25 min) is obtained using Method 3. The Method 2 leads to very sharp peaks with only modest separation (Figure 2).

Ligands 3 and 4 are eluted with good separation only using Method 3. For *cis* isomer of derivative 3 retention time is 16.51 minutes (for *trans* isomer t = 18.96 min) and for *cis* isomer of 4 derivative retention time is 16.96 min (for *trans* isomer t = 20.03 min).

Elution of cis/trans isomers of ligand 5 was achieved with all methods, but the best results with satisfactory separation and short retention time was obtained with Method 1. For this method absorption spectra of *cis* and *trans* isomers of 5 are included (Figure 3). Method 3 gives very sharp picks, although retention time is relatively long (t = 14.99 and t = 15.41 for *cis* and

trans isomers, respectively) (Figure 2). Retention data for all ligands are presented in Table 2.

Table 2. Retention times for the *cis* and *trans* isomers, separated with the most satisfactory method.

ligands / isomers	1[a]	2[a]	3[a]	4[a]	5[b]
cis	11.52 min	15.12 min	16.51 min	16.96 min	5.25 min
trans	14.75 min	17.25 min	18.96 min	20.03 min	7.25 min

[a] Method 3

[b] Method 1

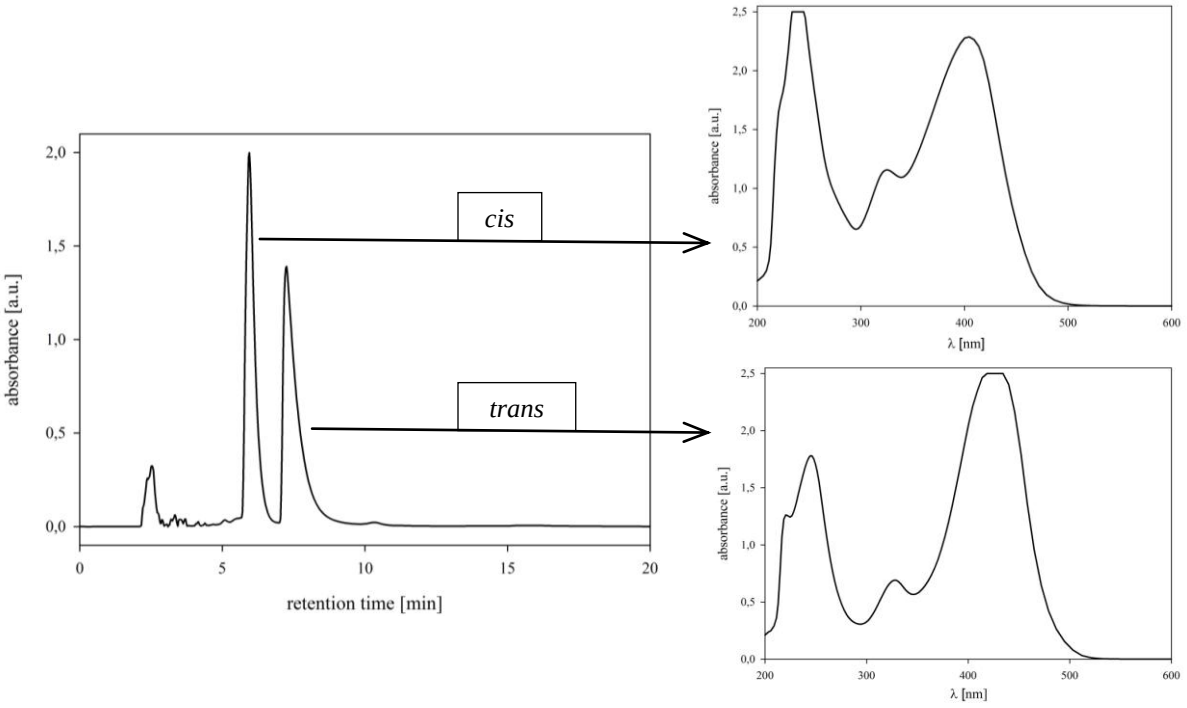


Figure 3. *Chromatogram of the separation of cis/trans isomers (ligand 5) monitored at wavelength of 386 nm (left side). Right side panels show absorption spectra of cis-5 (top) and trans-5 (bottom).*

Conclusion

The current study provides the method for sufficient separation of isomers of arylstilbazolium derivatives 1-5 by HPLC technique. The next step will include preparative scale separation of *cis* isomers for each ligand. Finally, interaction study between DNA and *cis* isomers will be performed.

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NEW SPME FIBER FOR QUALITATIVE ANALYSIS OF VOLATILE COMPOUNDS IN PRINTING INKS

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Keywords: SPME, Solid-phase microextraction, optical fiber, SPME fiber, quartz fiber, novel SPME fiber, SPME fiber preparation

Introduction

Solid-phase microextraction (SPME) is an effective, a simple, fast, sensitive, newly technique for sample preparation in chemical analysis. The microextraction process is composed of two main stages: first, an adsorption of the analytes (on) the sorbent located on the surface of the glass fiber, and second, an desorption of the analytes in injector port of the GC.

This technique do not require organic solvents and has many advantages like e.g. low costs, simplicity, possibility of combining with other analytical techniques. The stationary phases used as coatings of the SPME fiber can be of various types. The most often used are: polydimethylosiloxane (PDMS) for preconcentration of non polar organic compounds [1]; polyarylic (PA) [2-4] for analysis of polar organic compounds. There are also some fibers with coating containing two or more ingredients e.g: polydimethylosiloxane-divinylobenzene (PDMS-DVB), carboxen-polydimethylosiloxane (CAR-PDMS), carbowax-divinylbenzene (CW-DVB) [5-6] which are used for preconcentration of polyaromatic hydrocarbons (PAH's), volatile aromatic compounds, polar aromatic solvents or aromatic

amines. All those fibers are prepared by depositing a thin layer of polymer on the surface of fused silica or quartz fiber.

The main function of solid-phase microextraction is the sorption of analytes from solid, gaseous or liquid samples on sorbent immobilized on fused silica or quartz fibers.

An equilibrium between sample and the sorbent is reached under specified conditions like e.g. optimal extraction and desorption time, temperature, agitation and pH of water samples. The quantity of extracted compound depends on its concentration, affinity towards the sorbent and on the length or thickness of the sorbent film. The speed of extraction process depends also on the sorbent properties. Right selection of the appropriate sorbent guarant fast and high value of extraction capacity with short extraction time. The volume and surface area of the stationary phase depends on the coating thickness. When the coating thickness increases, the sensitivity of the analytical method is being improved. Unfortunately, the thickness of the coating material is limited by outer diameter of SPME fiber, which is usually 100– 125μm.

This work is devoted to a preparation of SPME fibers covered with silica modified with ketoimine groups, as well as an optimization of the conditions for the qualitative analysis of some volatile compounds in printing inks.

Experimental section

Reagents and apparatus. SPME-GC analysis was carried out on an Varian CP-3800 gas chromatograph equipped with a flame ionization detector (FID). The column used was a Varian WCOT Fused Silica CP-Sil5 CB, 30 m x 0,32 mm, 0,25 μm film thickness.

The flow rate of the carrier gas was set at 1 cm^3/min. Carrier gas split ratio was set at 1:20. The modified SPME device used in analysis is shown in Fig. 1.

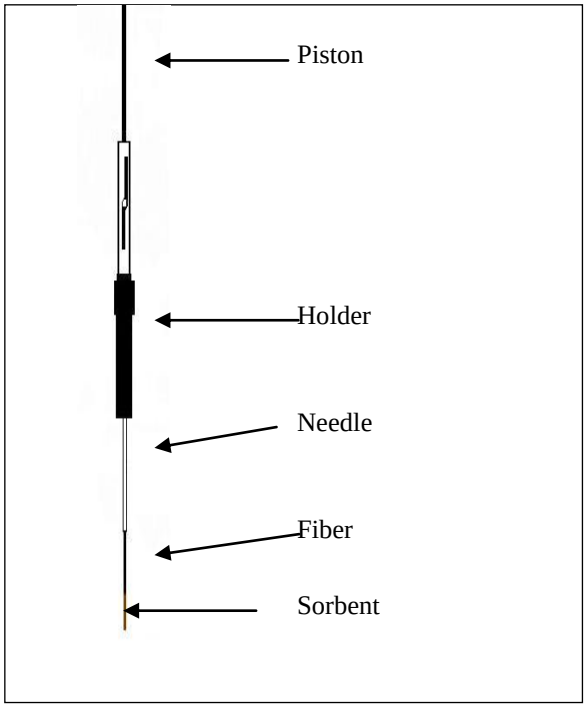

Fig. 1. SPME device.

The quartz fiber (100mm X 100µm), used for preparation a SPME fiber was obtained from OPTEC (Białystok, Poland). The standards solution of volatile compounds (Hydroquinone, 2,6-Di-tert-bytyl-4-methylphenol, Mesitaldehyde, Phenylglycol, Ethylene glycol phenyl ether acrylate) were purchased from Sigma-Aldrich. Nucleosil 50-3 (Baker Analyzed, 3~µm particle size) was purchased from J.T.Baker. 3 pentano-2,4-dione derivatives, which were used for the modification of the silica gel [7], were obtained from The Metaloorganics Department of the Adam Mickiewicz University, Poznań, Poland. Organic solvents used for silica gel modification or fiber

purification were purchased from POCh (Gliwice, Poland) or Fluka (Buchs, Switzerland).

SPME Fiber Preparation

Pretreatment of the fiber. A 8cm-long glass fiber was used to prepare the SPME fiber. Firstly, surface of the fiber was purified from potential contaminations by immersion in acetone for 1 an hour. Secondly, the fiber was dipped in 1M NaOH solution for 1 an hour, to expose the maximum number of silanol groups on the fiber surface and cleaned in water. Subsequently, fiber was inserted into 0,1 M HCl solution for 30 min, to neutralize excess of NaOH, cleaned in water, and then dried on air in room temperature.

Preparation of OV1 solution. The 3% OV1 solution was prepared by dissolving 37,5 mg of OV1 in 1,25 ml of chloroform. 3% OV1 solution was leaved to dissolve for 48 hours.

Procedure of sorbent immobilization on the surface of a glass fiber. A 1cm-end segment of glass fiber was immersed in 3% OV1 solution, assuming thin film thickness. Subsequently, the glass fiber was coated with the ketoimino-modified silica gel in order to obtain sorbent monolayer and heated in oven in 180°C.

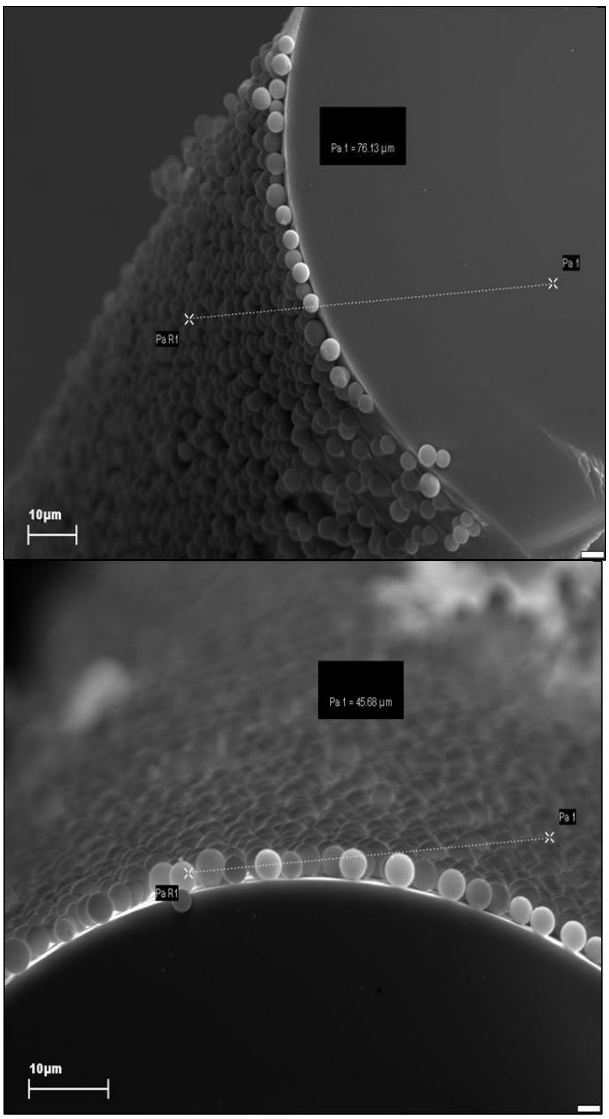

Fig. 2. The SEM of the SPME fiber

HS-SPME procedure. Printing ink analysis. HS-SPME was performed, to avoid direct contact with the sample matrix. The 2-3cm DVD disc pieces were placed into a 20 ml glass vials and tightly capped. The DVD sample was putted into a water bath to stabilize its temperature. Subsequently, the needle was driven through the vial septa and the fiber was exposed to the headspace above the sample. After extraction, the fiber was

removed from the sample vial and immediately inserted into the injector port of the gas chromatograph to perform desorption of the analytes from the fiber. After analysis, the sample vials was cooled to minimize the loss of the volatile printing ink components.

Results and discussion

Firstly, SPME-GC-MS was used in qualitative analysis of the volatile compounds present in the DVD disc overprint. Secondly, HS-SPME-GC analysis was performed to identify the volatile compounds in light-hardenable inks. Fig 3 shows the chromatogram of a DVD disc sample using novel SPME fiber.

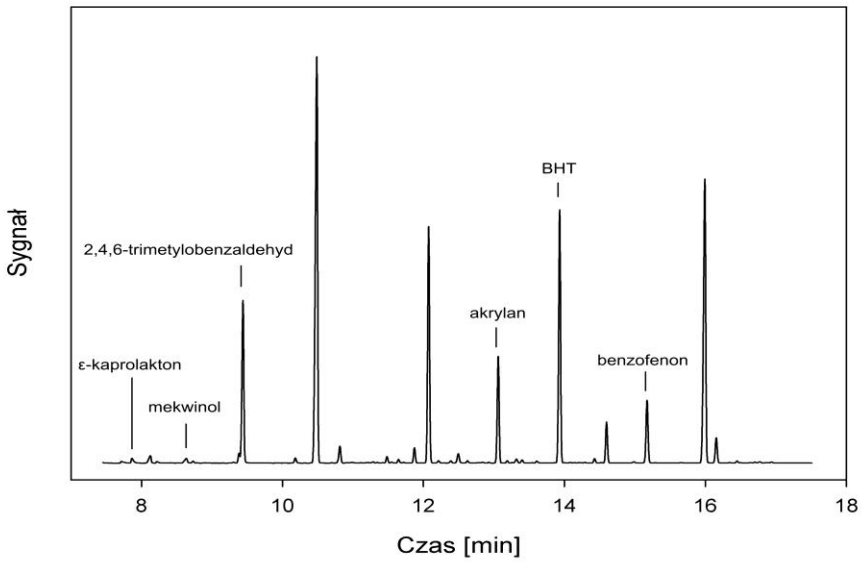

Fig. 3. The chromatogram of DVD disk sample

Optimization of SPME process. The time and temperature of the extraction and desorption process were investigated for their effect on the extraction process. The solution of mekwinol standard was 60 mg/ml.

Effect of extraction time. The extraction time is the main parameter affecting the extraction capacity. The water bath was set at 56°C while the sample was heated. Extraction time was spread between 2 and 48 min to examine the extraction profile. Fig.4. shows, that the signals increases from 2 to 16 min, when the extraction equilibrium was reached. Further analysis was performed in 16 min extraction time.

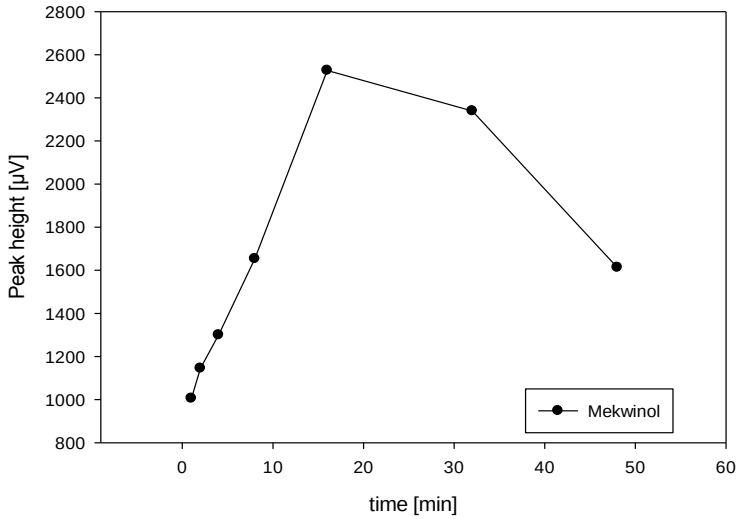

Fig.4. Effect of extraction time for mekwinol

Effect of extraction temperature. Extraction temperature was maintained between 30°C and 90°C. Sample was putted into water bath to heat to the established temperature. The obtained profile is illustrated in Fig.5.

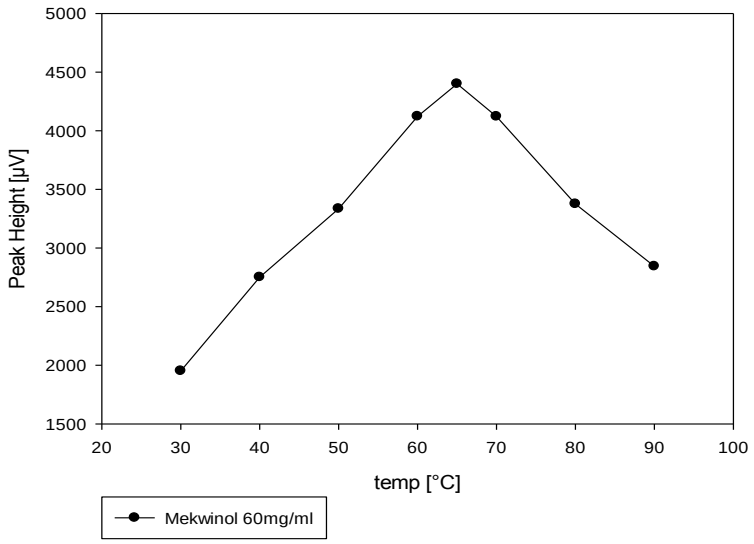

Fig.5. Effect of extraction temperature for mekwinol

The extraction capabilities for mekwinol increased when the sample temperature grows to 65°C. Beyond 65°C, the extraction capabilities decreases, when the sample temperature drops. The extraction temperature of 65°C was admitted as the optimal extraction temperature and used for further analysis.

Effect of desorption time. The desorption time also plays an important role during the extraction process. The effect of desorption time was investigated between 7s and 120s. Figure 6. shows the influenced of the desorption time on the extraction capacity.

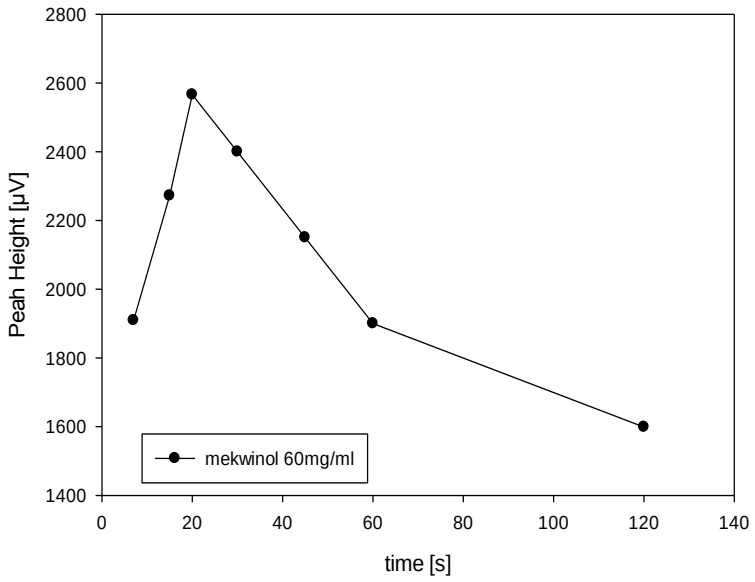

Fig. 6. Effect of the desorption time for mekwinol.

Desorption time of 20s was admitted as the optimal for qualitative analysis.

Conclusions

A novel SPME fiber was successfully applied in the qualitative analysis of volatile components of printing inks. The novel SPME fiber could be used for the qualitative analysis volatile organic compounds from liquid or solid samples. The HS-SPME-GC technique is a simple, sensitive and fast method for environmental sample analysis.

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Analysis of light hydrocarbons on capillary columns coated with adsorbent of electron-donor –acceptor properties

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Key words: capillary columns, PLOT, complexation gas chromatography, transitions metals

ABSTRACT

Gas chromatography is challenged with increasingly complex analytical problems and one way of approaching them is through the search for new column packings. As already over a hundred years ago prof. Cwiet noted that the column packing has a dominant role in the separating system, development of the technology of columns and their packings has been definitely improving the chromatographic parameters such as the rate and efficiency of separation. At present of increasing interest are the chemically bonded phases obtained as a result of silica modification. The packings of this type are characterised by good thermal stability, high reproducibility of results and small loss of the stationary phase on exploitation. An important group of chemically bonded phases are those used in the complexation gas chromatography (CGC) and permitting introduction of a transition metal into the phase structure. The development of these phases opened the possibility of employing the specific interactions of the complex with unsaturated

hydrocarbons and heteroatom containing compounds. The paper concerns the adsorbents formed as a result of silica surface modifications with silylpropyl phase with diphenylketoimine groups. Because of the surface functional groups, the adsorbents obtained were characterised by electron-donor-acceptor properties. The groups permitted formation of complexes containing Cu^{2+} metal cations on the surface. Thanks to the presence of the empty orbital in the transition metal cation it was possible to study the specific interactions between the stationary phase and the adsorbate of electron-donor properties. The study was performed on a group of volatile hydrocarbons having unsaturated bonds so capable of specific interactions with the adsorbents tested. The retention parameters were determined in order to characterise the specificity of the adsorbate-adsorbent interactions. To test the procedure proposed, selected mixtures of such volatile hydrocarbons of similar molecular mass and structure were separated.

INTRODUCTION

For the first time metal cations were applied in gas chromatography by Bradford in 1950 [1]. Since that time the use of metal cations in analysis of compounds of similar structure and properties has been a subject of continuous research. The metals that can be used for chromatographic purposes are those that reveal deficit of electrons and form electron-donor-acceptor complexes with organic compounds. The complexes are called the charge transfer type ones. In the chromatography of key importance are the interactions between the analyte molecules and the chromatographic column packing. When liquid stationary phases are applied, the interactions are assumed to take place according to the following two mechanisms: that based on non-specific van der Waals interactions and that based on electron transfer between the interacting molecules. The nature of adsorption interactions

between the adsorbate molecules and the solid surface of the adsorbent is in practice the same as for the liquid stationary phases. Covalent interactions or the interactions between strong acids and bases leading to stable products have no practical use in chromatography. Only relatively weak interactions characterised by energy of the order of a few kcal/mol are suitable for separation of compounds [2]. All specific interactions taking place in the liquid stationary phase and on the solid state surface can be explained on the basis of the acid-base interactions where the electron donor species act as bases and electron acceptor species act as acids or on the basis of molecular orbitals theory. In the latter case the electron-donor-acceptor interactions are classified according to the interactions of molecular orbitals: *n* are nonbonding orbitals, σ and π are the bonding (occupied) orbitals, while σ^* and π^* are the antibonding (empty) orbitals. In such situation, the electron-donor-acceptor complexes are the species in which the metal acts as an electron acceptor and the molecules of organic compounds having π bonds or free electrons *n* are electron donors. In complex chromatography two types of complexes are distinguished: the organic ones (e.g. π-π^* or n-n^*) and coordination complexes between metal cations and organic ligands [2].

Metal cations making electron-donor-acceptor complexes used for modification of column packings in gas chromatography can be divided into two groups: liquid superselective phases and superselective adsorbents. The second group includes the salts and oxides of transition metals, organometallic polymers and salts of transition metals bound to the support surface.

The study reported in this paper was undertaken to synthesise two new adsorbents from the group of superselective ones. The first of them was obtained as a result of bonding diphenylketoimine groups to the support surface and the second one was a modification of the first. The modification

involved bonding copper(II) cations to diphenylketoimine groups. The effect of the adsorbates properties and spatial structure on their interactions with the adsorbents studied.

ANALYTICAL PART

1. Reagents

The reagents used for the synthesis of adsorbent and column preparation were purchased from Merck (Darmstad, Germany) and Aldrich (Milwaukee, USA) and they were of analytical grade. Methylhydrogenpolysiloxane (SL 6020-D1) was manufactured by GE Silicones (Waterford, USA). The silica of particle size 5 μm and average pore size of 300 Å was produced by Macherey-Nagel (Düren, Germany). The capillary columns were purchased from Quadrex (Woodbridge, USA).

2. Apparatus

The chromatographic measurements were made on a gas chromatograph Hewlett-Packard Series II, equipped with a split-splitless type injecting device and a flame-ionisation detector (FID). The atmospheric pressure was measured by a mercury barometer PS (Warszawa) to an accuracy of 0.1mm Hg. At the inlet to the column the pressure was measured by a mercury manometer to an accuracy of 1mm.

3. Preparation of the adsorbent containing amine groups bonded to the silica surface

A portion of 3.5g of Nucleosil 300-5 silica was dried at 180˚C under vacuum to remove the water adsorbed. Then 1.1ml of 3-aminopropyltriethoxysilane was added and the total content was heated till

boiling under reflux for 15h in xylene environment (Fig. 1). After this time the unreacted silane was extracted with xylene using a Soxhlet apparatus. The processes was completed with the end-capping reaction by hexamethyldisilazane (Fig. 2).

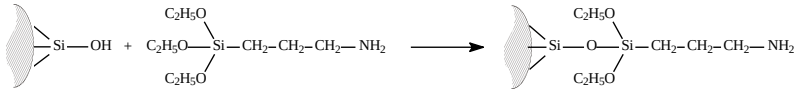

Fig. 1 Scheme of silane bonding to the silica surface

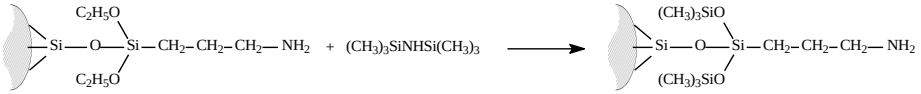

Fig. 2 The end-capping reaction

4. Preparation of the adsorbent containing ketoimine groups bonded to the silica surface

To the adsorbent obtained according to the above procedure, 1.1ml 1,3-diphenyl-1,3-propanedione was added and the content was heated and stirred under reflux for 15h in xylene environment. Unreacted 1,3-propanedione was extracted with xylene using a Soxhlet apparatus.

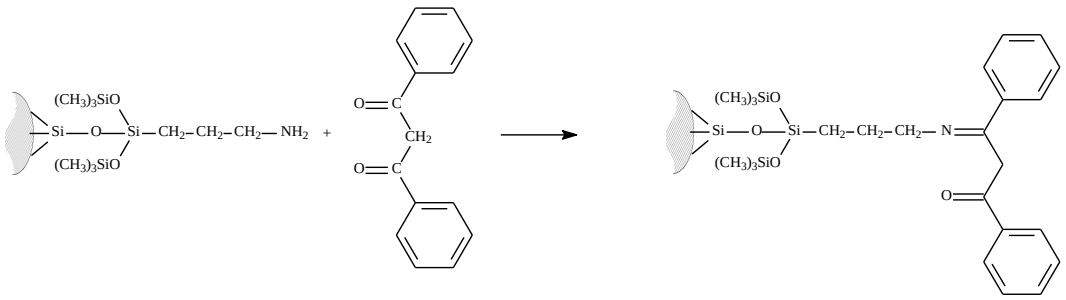

Fig. 3 Scheme of 1,3-diphenyl-1,3-propanedione bonding to amine groups

5. Preparation of the adsorbent containing copper(II) chlorides bonded by ketoimine groups

To the adsorbent containing diphenylketoimine groups obtained according to the earlier described procedure, a solution of ethanol with copper(II) chloride was added, with $CuCl_2$ in 100% excess relative to the content of ketoimine groups. The mixture was left to rest for 7 days. After this time the solution was decanted and the unreacted copper chloride was extracted with ethyl alcohol in a Soxhlet apparatus.

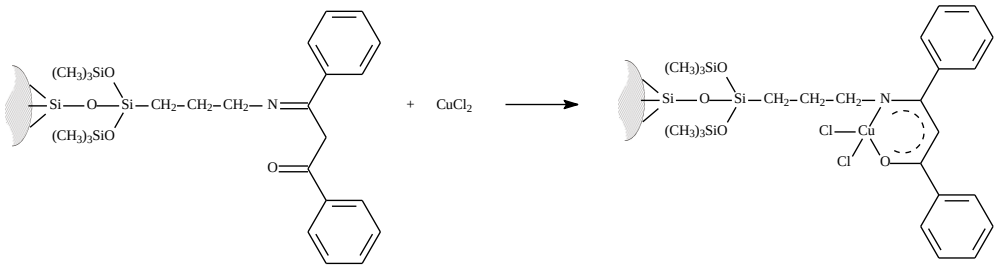

Fig. 4 Scheme of the complex formation reaction

6. Column preparation

A quartz column (30m x 0.32mm) was washed with methylene chloride, dried with a stream of argon and by a static method a 5µm thick film of methylhydrogenpolysiloxane was coated on the column inner wall. The adsorbent was introduced into the column by the dynamic method. To ensure homogeneity of the adsorbent layer, the column was subjected to vibrations [3].

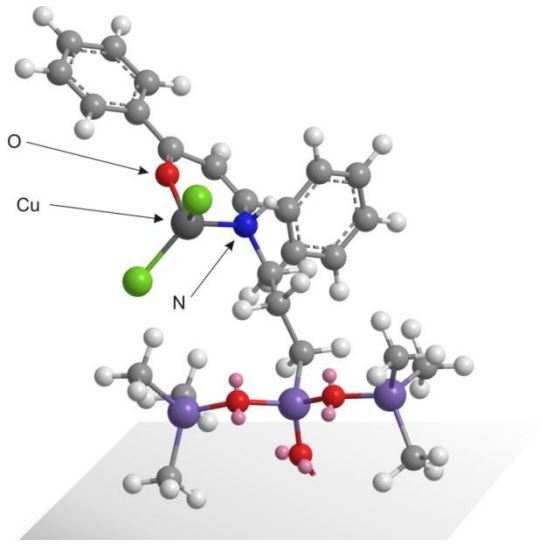

Fig. 5 Scheme of the spatial structure of the chemically bonded phase containing diphenylketoimine groups

RESULTS AND DISCUSSION

The retention parameters: retention factor (k), retention index (I), molecular retention index (ΔMe)[4] and the specific retention volume (Vg) determined for aliphatic linear hydrocarbons, are collected in Table 1. These parameters characterise the specific interactions between the adsorbate and the column packing containing stationary phase with chemically bonded ketoimine complexes with copper(II) chloride. For the sake of comparison, the values of analogous parameters obtained for the reference column with the packing containing free ketoimine groups, are also presented. The strength of the specific interactions depends on the structure and configuration of the adsorbate molecules, the number, type and position of the unsaturated bond. The presence of *d*-block metals in the adsorbent complexes determines the characteristic sequence of elution of alkanes and alkenes, which was a consequence of the metal ability to get involved in the coordination interactions with the π-electrons of the unsaturated bond. For

the columns with the packing containing stationary phase with chemically bonded ketoimine complexes with copper(II) chloride, the first eluted compound is alkane (higher boiling point) followed by alkene (lower boiling point). The strength of the specific interactions and hence the values of the retention parameters depend on the type of unsaturated bond (double or triple), which can be easily illustrated for hexene-1 and hexyne-1. The sequence of elution depends also on the position of the unsaturated bond or bonds, which can be illustrated for hexynes and hexadienes. As follows from the values of ΔMe, the adsorbent-adsorbate interactions are stronger for the column packing containing copper ions than for the column with the reference packing. The strength of interactions between the stationary phase modified with $CuCl_2$ and hexynes depends considerably on the position of the triple bond.

For hexadienes, the strength of adsorbent-adsorbate interactions increases with decreasing distance between the unsaturated bonds, and the sequence of elution in this group of compounds is:

1,5-hexadien > 1,4-hexadien > 2,3-hexadien

The effect of the adsorbate molecule structure on the strength of the π type interactions can be observed for geometric isomers. The *trans* isomer is eluted before the *cis* one, which is a consequence of different accessibility of the unsaturated bond in the two isomers.

Fig. 6 The sequence of elution of cis/trans isomers of hexene-2

Analysis of the retention parameters obtained for branched hydrocarbons, given in Table 2, has shown that the presence of substituents in the main chain of alkenes should be considered from at least two viewpoints. On the one hand, they have negative effect being an additional steric obstacle hindering direct contact of the adsorbate with the electron-acceptor centre. However, on the other hand, alkyl substituent because of its properties by induction effect increases the electron density of the unsaturated bond, thus enhancing the specific interactions. The effect of the substituent is the greater the closer the substitution site to the double bond. This effect can be illustrated on the example of 2,3-dimethyl-1-butene, 2,3-dimethyl-2-butene and 3,3-dimethyl-1-butene, whose sequence of elution is:

3,3-dimethyl-1-butene > 2,3-dimethyl-1-butene > 2,3-dimethyl-2-butene

A similar situation is observed for 2-methyl-1-pentene, 3-methyl-1-pentene, 4-methyl-1-pentene and 2-methyl-2-pentene. For the group of unsaturated branched hydrocarbons, the sequence of elution of cis/trans isomers of olefins is the reverse than that for *n*-olefins. For *n*-olefins the first isomer eluted is *trans*, while for olefins the first isomer eluted is *cis*. This observation was made also by Hively [5] and by Wasiak[6]. A good example to illustrate this change is a pair of isomers of 3-methyl-2-pentene.

Fig. 7. The sequence of elution of cis/trans isomers of 3-methyl-2-pentene

Fig. 8 and 9 illustrate the separation of mixtures of linear hydrocarbons. A very interesting possibility is the separation of isomers of C_5, C_6 and C_7 hydrocarbons. Fig. 10 illustrates an exemplary separation of a mixture of branched hydrocarbons.

Table 1 The retention factor (k), retention index (I), molecular retention index (ΔMe) and specific retention volumes (Vg) for linear hydrocarbons determined at 80˚C

Adsorbate	Column packing without metal				Column packing Modified with $CuCl_2$			
	k	*I*	Δ*Me*	*Vg*	*k*	*I*	Δ*Me*	*Vg*
1-pentene	0.2184	496	1.46	0.31	0.2993	501	2.19	0.40
cis-2- pentene	0.2432	509	3.31	0.34	0.3639	528	5.90	0.49
trans-2-pentene	-	-	-	-	0.3241	512	3.71	0.44
1-pentyne	0.2841	529	8.07	0.40	0.5660	587	16.28	0.76
1-hexene	0.4832	596	1.44	0.68	0.6275	601	2.19	0.85
cis-2-hexene	0.5333	608	3.16	0.75	0.7455	624	5.35	1.00
trans-2-hexene	0.5149	604	2.55	0.72	0.7256	620	4.86	0.98
cis-1,4-hexadiene	0.5007	600	4.08	0.70	0.7360	622	7.13	0.99
trans-1,4-hexadiene	0.5090	602	4.36	0.71	0.6921	614	6.00	0.93
1,5-hexadiene	0.4552	588	2.39	0.64	0.6469	605	4.76	0.87
2,3-hexadiene	0.6428	631	8.41	0.90	1.0484	668	13.62	1.41
1-hexyne	0.6400	631	8.34	0.90	1.1912	685	15.97	1.60
2-hexyne	0.8326	663	12.90	1.17	1.5728	722	21.13	2.12
3-hexyne	0.7729	654	11.61	1.08	1.6071	725	21.54	2.17
1-heptene	1.0892	696	1.52	1.53	1.3359	700	2.03	1.80
cis-2-heptene	1.1968	708	3.17	1.68	1.5398	719	4.70	2.07
trans-2-heptene	1.1687	705	2.75	1.64	1.4394	710	3.43	1.94
cis-3-heptene	1.1287	701	2.75	1.64	1.4964	715	4.16	2.02
trans-3-heptene	1.1154	699	1.94	1.57	1.3486	701	2.21	1.82
1-heptyne	1.4786	735	8.88	2.08	2.9620	806	18.97	3.99
1-octene	2.4432	797	1.61	3.43	2.9127	804	2.61	3.92
1-octyne	3.3729	837	9.27	4.73	5.7830	896	17.44	7.79
1-nonene	5.4648	898	1.67	7.67	6.0353	901	2.20	8.13

Table 2 The retention factor (k), retention index (I), molecular retention index (ΔMe) and specific retention volumes (Vg) for branched hydrocarbons determined at 80˚C

Adsorbate	Column packing without metal				Column packing Modified with $CuCl_2$			
	k	*I*	Δ*Me*	*Vg*	*k*	*I*	Δ*Me*	*Vg*
2-methyl-1,3-butadiene	0.0950	508	5.15	0.09	0.3347	524	7.44	0.46
3-methyl-1,2-butadiene	0.0950	508	5.15	0.09	0.3729	539	9.47	0.52
2,2-dimethylbutane	0.1308	558	-5.58	0.12	0.3828	542	-8.10	0.53
2,3-dimethylbutane	0.1496	579	-2.89	0.14	0.4741	571	-4.10	0.66
2,3-dimethyl-1-butene	0.1474	577	-1.19	0.14	0.4912	576	-1.42	0.68
2,3-dimethyl-2-butene	0.3830	732	20.53	0.36	2.0481	767	25.51	2.84
3,3-dimethyl-1-butene	0.1385	567	-2.57	0.13	0.3365	525	-8.50	0.47
2-methylpentane	0.1521	582	-2.51	0.14	0.4750	571	-4.06	0.66
3-methylpentane	0.1586	587	-1.84	0.15	0.5290	585	-2.05	0.73
2-methyl-1-pentene	0.1585	589	0.41	0.15	0.5744	596	1.51	0.80
3-methyl-1-pentene	0.1444	574	-1.64	0.14	0.4647	568	-2.46	0.64
4-methyl-1-pentene	0.1470	577	-1.25	0.14	0.4606	567	-2.62	0.64
2-methyl-2-pentene	0.1457	575	-1.44	0.14	4.1040	862	38.70	0.69
cis-3-methyl-2-penten	0.1726	602	2.30	0.16	0.6676	616	4.33	0.93
trans-3-methyl-2-pentene	0.1772	606	2.92	0.17	0.7031	623	5.30	0.98
trans-4-methyl-2-pentene	0.1491	579	-0.93	0.14	0.4921	576	-1.38	0.68
2,2,4-trimethylpentane	0.3715	727	-10.22	0.35	1.2303	698	-14.29	1.71
2,4,4-trimethyl-1-pentene	0.4001	739	-6.55	0.37	1.5020	725	-8.47	2.08
2,4,4-trimethyl-2-pentene	0.4061	741	-6.22	0.38	1.6158	735	-7.08	2.24

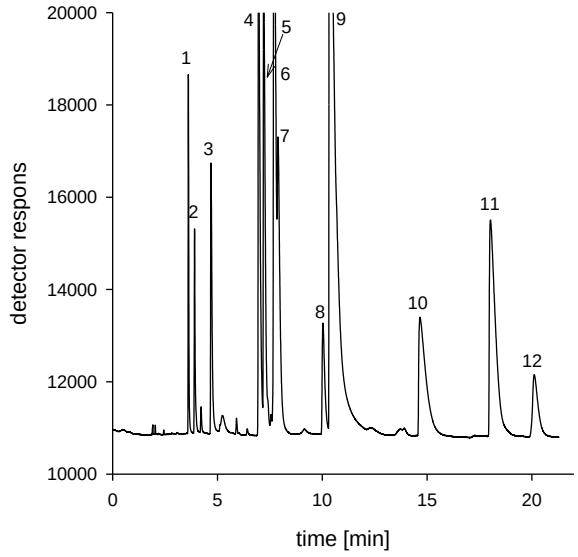

Fig. 8 Separation of a mixture of linear hydrocarbons compounds; the packing modified without metal. Column temperature: 35°C (10 min) → 10°C·min^{-1} → 90°C; V_{He} = 1.76ml·min^{-1}; 1 = 1-pentene; 2 = cis-2-pentene; 3 = 1-pentyne; 4 = 1,5-hexadiene; 5 = 1-hexyne; 6 = cis-2-hexene; 7 = ?; 8 = 2-hexyne; 9 = 2,3-hexadiene; 10 = 1-heptene; 11 = cis-2-heptene; 12 = 2-heptyne;

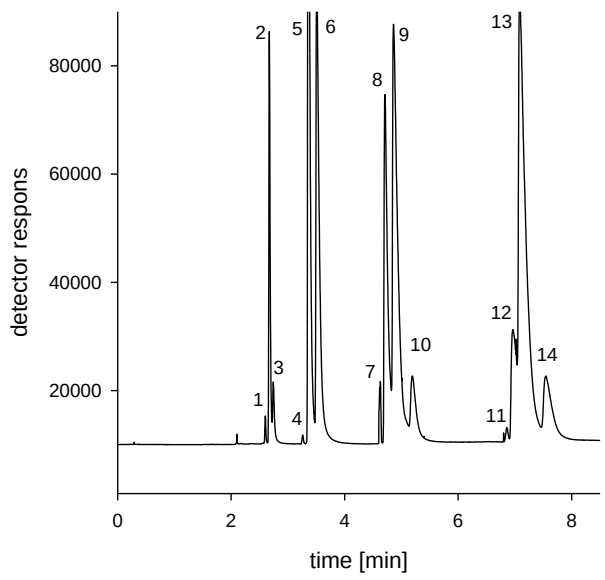

Fig. 9 Separation of a mixture of linear hydrocarbons compounds; the packing modified with $CuCl_2$. Column temperature: 80°C (4,5 min) → 10°C·min^{-1} → 100°C; V_{He} = 1.50ml·min^{-1}; 1 = pentane; 2 = trans-2-pentene; 3 = cis-2-pentene; 4 = hexane; 5 = trans-2-hexene; 6 = cis-2-hexene; 7 = heptane; 8 = trans-3-heptene; 9 = cis-3-heptene + trans-2-heptene; 10 = cis-2-heptene; 11 = octane; 12 = 1-octene; 13 = trans-2-octene; 14 = cis-2-octene

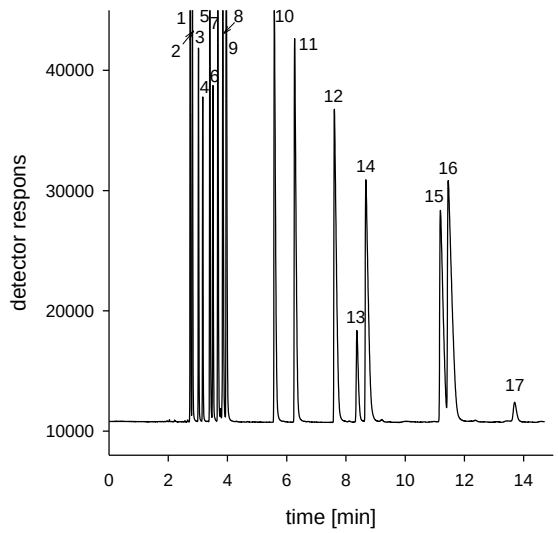

Fig. 10 Separation of a mixture of branched hydrocarbons; the packing without metal complexes. Column temperature: 60°C; V_{He} = 1.41ml·min^{-1};
1 = 2-methyl-1,3-butadiene; 2 = 3-methyl-1,2-butadiene; 3 = 3,3-dimethyl-1-butene;
4 = 2,2-dimethylbutane; 5 = 2,3-dimethylbutane; 6 = trans-4-methyl-2-pentene;
7 = 2-methyl-1-pentene; 8 = cis-3-methyl-2-pentene;9 = trans-3-methyl-2-pentene;
10 = 2-methyl-1-hexene; 11 = 2,3-dimethylpentane; 12 = 2,2,4-trimethylpentane;
13 = 2,4,4-trimethyl-1-pentene; 14 = 2,4,4-trimethyl-2-pentene;
15 = 4-methylheptane; 16 = 2-methylheptane; 17= 2-methyl-2-heptene

For aromatic hydrocarbons the main type of interactions is that between the electron cloud of the aromatic ring and the empty orbital of the transition metal. The retention parameters characterising the specific interactions between the aromatic compounds and the adsorbents studied are given in Table 3. On the basis of the retention parameters it was possible to establish the effect of the type of chain (linear or branched, saturated or unsaturated) and the position of the substituent at the ring on the strength of the

interaction. The reference compound was benzene. The presence of substituents can have different effects on the specific interactions as on the one hand they hinder the access to the ring but on the other hand their presence can induce the increase in the density of the electron cloud of the aromatic compound. According to the definition of the molecular retention index, its positive values point to the presence of interactions between the adsorbent and the analyte. The value of ΔMe for benzene has been assumed as the reference value for the strength of the adsorbent-adsorbate interactions. To estimate the effect of the type of chain of the substituent on the strength of adsorbent-adsorbate interactions, the retention parameters of butylbenzenes were analysed. Depending on the degree of branching of the saturated chain of the butyl group, the strength of the specific interactions of the analyte and the electron-acceptor centres changes leading to the values of Δ*Me* increasing in the following sequence:

tert-butylbenzene < *iso*-butylbenzene < *sec*-butylbenzene < *n*-butylbenzene

These compounds illustrate the domination of the steric hindrance effect over the inductive effect of the substituent on the strength of interactions.
At the next stage of the study, the effect of the number and position of the substituents in the ring on the strength of the specific interactions was tested on the example of a series of mono-, di- and tri-substituted derivatives of benzene. The strongest adsorbent-adsorbate interactions were found for trimethylbenzenes. The sequences of elution of these compounds were different for the two columns studied. For the column with the packing without copper(II) chloride complexes the sequence of elution was:

1,3,5-trimethylbenzene < 1,2,4-trimethylbenzene < 1,2,3-trimethylbenzene

while for the column with the packing with copper(II) chloride the sequence was:

1,2,3-trimethylbenzene < 1,3,5-trimethylbenzene < 1,2,4-trimethylbenzene

The change in the sequence was a result of the rigid and planar structure of the copper(II) chloride complex whose presence changed the adsorbate accessibility to the electron-donor-acceptor centre.

The effect of saturated and unsaturated substituents on the adsorbent-adsorbate interactions can be illustrated by the results obtained for ethylbenzene and styrene. The molecular retention index for styrene is much higher than for ethylbenzene, which is related to the metal capability of involvement in the coordination interactions with π electrons of the double bond. For the column with the packing containing copper(II) chloride complexes the difference in ΔMe for styrene and ethylbenzene reached as much as 10 units. The differences in the structure of these two compounds is illustrated in Fig. 11.

Fig. 11. Structures of ethylbenzene and styrene

The values of retention factors (k), molecular retention indices (ΔMe), specific retention volumes (Vg) and retention indices (I) of selected cyclic hydrocarbons are given in Table 4. Analysis of these data indicates that the

presence of a methyl or ethyl substituent in a ring leads to decrease in the strength of adsorbent-adsorbate interactions. It is apparent e.g. from comparison of the retention parameters of methylcyclopentane and the corresponding cycloalkanes. The ΔMe values of the substituted compounds are lower than for cyclopentane and cyclohexane, which is probably due to the steric factor. The weakening of the interactions is smaller for ethyl substituent (ethylcyclohexane) than for methyl one (methylcyclohexane). The presence of a substituent directly at the double bond (1-methyl-1-cyclohexene) has resulted in strengthening the interactions relative to those with the compound in which the double bond was opposite to the substituent (4-methyl-1-cyclohexene). The above-discussed relations are more pronounced for the column with the packing with copper(II) chloride.

As far as cyclic hydrocarbons with more than one double bond are concerned, the interactions with those with isolated double bonds (1,4-cyclohexadiene, 1,5-cyclooctadiene) is stronger than with those having accumulated double bonds (1,3-cyclohexadiene, 1,3-cyclooctadiene).

Table 3 The retention factor (k), retention index (I), molecular retention index (ΔMe) and specific retention volume (Vg) for selected aromatic hydrocarbons determined at 90˚C

Adsorbate	Column packing without metal				Column packing Modified with $CuCl_2$			
	k	*I*	Δ*Me*	*Vg*	*k*	*I*	Δ*Me*	*Vg*
benzene	0.4692	633	12.66	0.53	0.9810	699	21.94	1.11
ethylbenzene	2.1415	837	13.28	2.43	4.0134	901	22.27	4.54
propylbenzene	4.3219	932	12.58	4.90	7.5009	992	20.93	8.49
1,3,5-trimethylbenzene	4.0863	925	11.53	4.63	7.9977	1001	22.23	9.05
1,2,4-trimethylbenzene	5.3577	961	16.64	6.07	9.9708	1032	26.62	11.29
1,2,3-trimethylbenzene	6.1643	980	19.29	6.99	6.9166	980	19.28	7.83
tert-butylbenzene	6.2356	982	5.48	7.07	9.4940	1025	11.61	10.75
iso-butylbenzene	7.0683	998	7.85	8.01	10.6293	1041	13.86	12.03
sec-butylbenzene	6.8308	994	7.20	7.74	10.9008	1045	14.36	12.34
n-butylbenzene	9.2647	1035	12.91	10.50	15.0473	1091	20.78	17.04
toluene	1.0397	740	12.69	1.18	2.0686	805	21.74	2.34
2-ethylbenzene	4.9094	949	14.99	5.57	8.9921	1018	24.56	10.18
3-ethylbenzene	4.5056	938	13.37	5.11	7.8429	998	21.83	8.88
p-xylene	2.2346	843	0.07	2.53	4.1001	904	8.68	4.64
m-xylene	2.2724	845	0.39	2.58	4.1599	906	8.97	4.71
o-xylene	2.5594	861	2.65	2.90	4.9981	933	12.70	5.66
styrene	2.7261	870	19.89	3.09	5.9467	958	32.26	6.73
2-methylstyrene	5.5007	965	19.15	6.24	11.1196	1048	30.80	12.59
3-methylstyrene	5.7916	972	20.12	6.57	11.9643	1058	32.26	13.55
α-methylstyrene	5.0010	952	17.35	5.67	10.0579	1033	28.80	11.39
cumene	3.6796	911	9.55	4.17	-	-	-	-
3-phenyl-1-propene	4.2409	930	14.24	4.81	8.1404	1003	24.59	9.22
trans-1-phenyl-1-propene	7.1905	1001	24.21	8.15	14.5005	1085	36.09	16.42
p-cymol	7.6917	1010	9.43	8.72	13.3573	1074	18.41	15.12

For some molecules the ketoimine groups and phenyl radical can make a significant steric hindrance. The access of the adsorbates to the electron-acceptor centre is also influenced by the π electrons of the aromatic ring of benzyl group. Their effect is particularly well seen for cyclic hydrocarbons, which is reflected in the strength of the specific interactions. According to increasing strength of the adsorbate-adsorbent interactions the cyclic hydrocarbons C6 are ordered as:

Fig. 12. The sequence of elution of cyclic C6 hydrocarbons

Because of its planar configuration, the molecule of benzene needs the most space to incite the interactions between π electrons and the metal complex. The molecules of cyclohexadienes, although of the shape similar to that of benzene, can interact with the electron-acceptor centre in two positions: planar and side. The weakest interactions with the adsorber shows cyclohexane whose molecule is the most "deformed" and it is the first to be eluted. The columns with the packings obtained were used for analysis of cyclic and aromatic hydrocarbons. The results obtained on the reference column and on these with the stationary phase modified with copper(II) chlorides were very interesting. The chromatograms obtained were characterised by sharp and well-separated peaks. Exemplary chromatograms are presented in Figs. 13-14.

Table 4 The retention factor (k), retention index (I), molecular retention index (ΔMe) and specific retention volumes (Vg) for cyclic hydrocarbons determined at 90˚C

Adsorbate	Column packing without metal				Column packing Modified with $CuCl_2$			
	k	*I*	Δ*Me*	*Vg*	*k*	*I*	Δ*Me*	*Vg*
cyclopentane	0.1978	513	3.84	0.23	0.3726	566	11.34	0.43
cyclopentene	0.1908	508	5.15	0.23	0.3717	566	13.30	0.43
cyclohexane	0.3979	610	3.42	0.47	0.7331	663	10.91	0.84
cyclohexene	0.4414	624	7.44	0.52	0.8771	689	16.50	1.01
cycloheptane	0.9940	735	6.88	1.17	1.8975	800	16.03	2.18
cykcloheptene	0.9727	732	8.50	1.15	1.9792	806	18.90	2.28
cyclooctane	2.2932	846	8.41	2.71	4.3835	921	18.92	5.04
cyclooctene	2.1065	834	8.83	2.49	4.4499	923	21.24	5.12
methylcyclopentane	0.3474	591	0.78	0.41	0.5736	629	6.02	0.66
methylcyclohexane	0.9088	723	5.22	1.07	0.9589	702	2.24	1.10
ethylcyclohexane	1.5743	795	1.33	1.86	2.4688	838	7.31	2.84
1-methyl-1-cyclopentene	0.3840	605	4.74	0.45	0.6947	656	11.85	0.80
1-methyl-1-cyclohexene	0.8949	721	6.95	1.06	1.6278	778	14.94	1.87
4-methyl-1-cyklohexene	0.7842	703	4.51	0.93	1.3600	752	11.31	1.56
1,3-cyclohexadiene	0.4289	620	8.90	0.51	0.8681	687	18.31	1.00
1,4-cyclohexadiene	0.5308	650	13.03	0.63	1.1287	725	23.59	1.30
benzene	0.4558	629	12.10	0.54	0.9092	694	21.25	1.05
1,3-cyclooctadiene	2.1172	835	10.94	2.50	4.5389	919	22.75	5.14
1,5-cyclooctadiene	2.6447	865	15.14	3.13	6.9417	987	32.28	7.98
trans-decahydronaphtalene	6.5327	986	2.14	7.72	11.0678	1054	11.63	12.73
cis-decahydronaphtalene	7.7990	1010	5.49	9.22	11.6608	1062	12.68	13.41

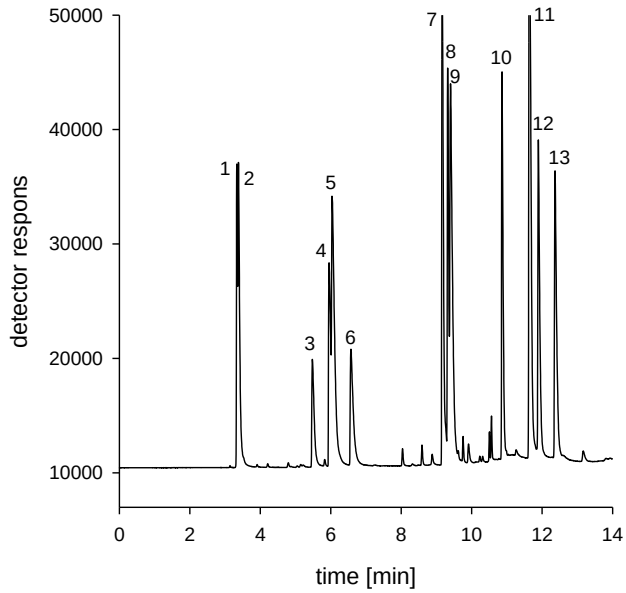

Fig. 13 Separation of a mixture of cyclic compounds; the packing without metal complexes.

Column temperature: 40°C(5 min) → 12°C·min^{-1} → 110°C; V_{He} = 1.40ml·min^{-1};

1 = cyclopentene; 2 = cyclopentane; 3 = methylcyclopentane;

4 = 1-methyl-1-cyclopentene; 5 = cyclohexane; 6 = cyclohexene;

7 = 1-methyl-1-cyclohexene; 8 = cycloheptene; 9 = cycloheptane;

10 = 1,3-cyclooctadiene; 11 = cyclooctene; 12 = cyclooctane;

13 = 1,5-cyclooctadiene;

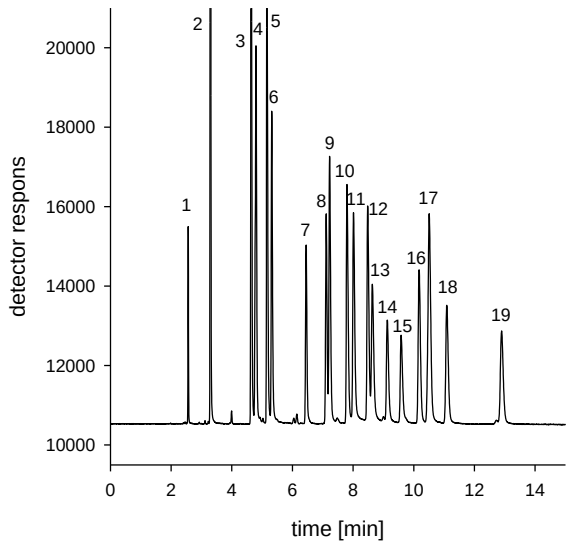

Fig. 14 Separation of a mixture of aromatic compounds; the packing modified with $CuCl_2$. Column temperature:: 100°C; V_{He} = 1.10ml·min^{-1}; 1 = benzene; 2 = toluene; 3 = ethylbenzene; 4 = m-xylene; 5 = o-xylene; 6 = styrene; 7 = cumene; 8 = 3-phenyl-1-propene; 9 = 1,3,5-trimethylbenzene; 10 = propylbenzene; 11 = α-methylstyrene; 12 = 1,2,4-trimethylbenzene; 13 = 2-methylstyrene; 14 =2-methylstyrene; 15 = 1,2,3-trimethylbenzene; 16 = sec-butylbenzene; 17 = iso-butylbenzene; 18 = trans-1-phenyl-1-propene; 19 = n-butylbenzene

CONCLUSIONS

On the basis of the retention parameters and chromatograms obtained it can be concluded that the adsorbents used can be successfully applied in analysis of unsaturated linear or branched, cyclic or aromatic compounds. The chromatograms recorded show sharp peaks and the interactions of the column stationary phase with the compounds analysed are selective enough to permit separation of geometric cis/trans isomers. The interactions with the compounds having π electrons were stronger with the stationary phase containing copper(II) chloride complexes than the reference phase with free ketoimine groups. Modification of the stationary phase with copper(II)

chloride complexes with diphenylketoimine for many compounds resulted in the sequence of elution different than on the reference column. The reason for this change was a different approach to the EDA centre which had planar and rigid structure in the phase with copper(II) chloride complexes.

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APPLICATION OF HPLC ASSAY TO THE MEASUREMENTS OF TOTAL ANTIOXIDANT POTENTIAL OF HONEY AND MEAD

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ABSTRACT

Free radicals and antioxidants are crucial from the physiological and pathological point of view of human being. They change during the aging, most of diseases and after food uptake. They can analysed using many HPLC assays. Nevertheless, it turned out that frequently more information is obtaining measuring the global value – Total Antioxidant Potential (TAP). It is equal to the sum of products of concentrations of all species in the sample by their kinetic constants.

Generally, HPLC assay can be used to measure free radicals indirectly, after they reaction with the spin-trap compound called *detector*. The TAP measurements are based on the generation of proper radicals (in our case the hydroxyl ones) and their reaction with the detector. In the second step the investigated sample is added. TAP values are expressed as the differences of peak areas of the products of reaction of free radical with detector in both cases.

As a spin trap *p*-hydroxybenzoic acid (pHBA) can be used. The product of its reaction with the hydroxyl radicals, 3,4-dihydroxybenzoic acid (3,4DHBA), are separated using ion exclusion or, more frequently, reversed phase HPLC. Previously, there were described systems based on the electrochemical or

fluorescence detectors. The aim of the paper is check if UV detector can be used in this assay. The new assay has been applied the TAP estimation of different honey and mead samples. The alternative assay is based on the measurements of total surface area of all peaks recorded using electrochemical detector. Selectivity of the last method can be easily controlled by changing the potential difference.

INTRODUCTION

Free radicals and antioxidants are important in many processes of living organisms. They change is observed during the aging, diseases and after food uptake. HPLC can be used to the measurements of free radicals (indirectly, after they reaction with the spin-trap compound called *detector*) as well as antioxidants [1]. It turned out that frequently more information is obtaining measuring the global value – Total Antioxidant Potential (TAP) [1-6]. It is defined as the sum of products of concentrations of all species in the sample by their kinetic constants.

TAP measurements are based on the generation of proper radicals (in our case the hydroxyl ones) and their reaction with the detector. In the second step the investigated sample is added. In the *kinetic* (photometric [5, 6] or fluorometric [25, 26]) assays the induction time is measured. In the *static* (HPLC [8 – 11] or MS [27]) assays TAP values are expressed as the differences of peak areas of the products of reaction of free radical with detector in both cases.

Previously, we have described *p*-hydroxybenzoic acid (pHBA) used as spin-trap [21, 22]. The product of its reaction with the hydroxyl radicals, 3,4-dihydroxybenzoic acid (3,4DHBA), are separated using ion exclusion [4] or, more frequently, reversed phase HPLC [16]. Previously, there were described systems based on the electrochemical [34] or fluorescence [33] detectors.

Aim of the paper is testing of possible application of UV detector in this assay.

Antioxidants are called by chemists reducers. The second proposed assay is based on the measurements of total surface area of the sample analysed using electrochemical detector. It is worth to note that in this case we can easy control selectivity by changing the working electrode potential.

It is well known that honeys, and obtained from them meads, contain a lot of antioxidants (poly-phenols and flavanoids). Especially dark-colour honeys are characterised by strong anti-oxidative properties. The new assays have been applied to the TAP estimation of different honey and mead samples. It turned out that results were correlated with the concentration of flavanoids and/or anthocyanides in the sample.

EXPERIMENTAL

Instrumentation

Measurements were performed by means of a chromatograph comprising a Interface Box, 4 channel Degasser K-5004, Solvent Organizer K-1500, Dynamic Mixing Chamber,

HPLC Pump K-1001, Diode Array UV Detector K-2600, ClinLab (RECIPE, Germany) Amperometric detector EC 3000, Eurochrom 2000 chromatographic data acquisition and analysis software (all from Knauer GmbH, Berlin, Germany), Basic+ marathon Autosampler (Spark Holland B.V., Emmen, The Netherlands), Jet-Stream Plus Column Thermostat (Industrial Electronics, Langenzersdorf, Austria). Samples were separated on a Eurospher RP-18 5 μm, 250x4 mm I.D. (Knauer GmbH, Berlin, Germany) column.

Reagents

Methanol, pHBA, 3,4DHBA and phosphate buffered saline (PBS) tablets were obtained from Sigma (St. Louis, MO, USA). All other reagents (Sigma, St. Louis, USA; Fluka, Buchs, Switzerland; and POCh, Gliwice, Poland) were of analytical-reagent grade and were used without further purification. Honey and mead samples were obtained from different sources. Water was passed through Millipore (Bedford, USA) Milli-RO4 and Milli-Q water purification systems. Mobile phases were filtered through a 0.22-μm membrane filter (Millipore, Bedford, USA).

Procedures

All chromatographic experiments were performed at flow rate 1 ml/min. Column was stabilized at 20ºC by passing the mobile phase for 1 h prior to the chromatographic measure-ments. Phosphate buffer (pH 6.6) was used as mobile phase. Stock solutions of the analyzed compounds were prepared in Milli-Q water and diluted to the required concentration before use. 20 μL samples were injected using autosampler. Out-put signal from the photometric detector working simultaneously at 205, 254 and 280 nm or electrochemical detector, working at different potential of working electrode, were continuously displayed on the computer. Every sample was injected six times and the average was taken for further elaboration.

Hydroxyl radicals were generated in Fenton-like reaction. 1 min incubation of 0.5 mM Fe^{2+} and 2 mM H_2O_2 in 50 mM phosphate buffer (pH 7.4) in the presence of 1 mM pHBA and analyzed sample at 37ºC has been performed. Product of reaction of pHBA with hydroxyl radicals, namely 3,4DHBA was detected photometrically. The same samples were detected electrochemically, at different working electrode potentials.

RESULTS AND DISCUSSION

It turned out pHBA and 3,4DHBA can be easily separated on the standard RP-18 column (Fig. 1). For the both acids linear calibration curves were obtained (Fig. 2).

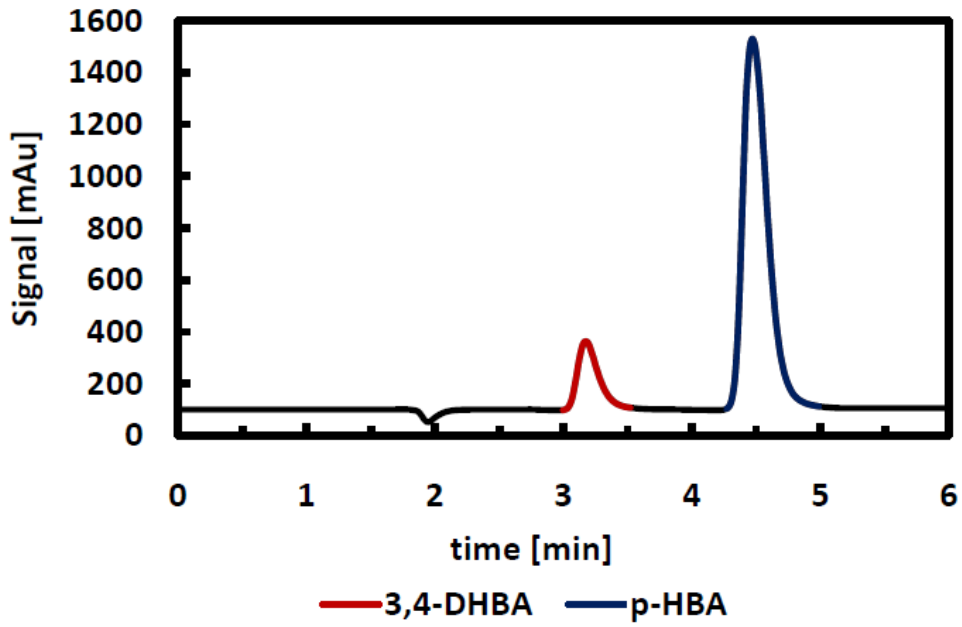

Fig. 1. HPLC chromatogram of pHBA after its re action with hydroxyl radicals. Chromatographic conditions: column - Eurospher - C18 250 x 4 mm (Knauer), temp. - 20°C, mobile phase – phosphate buffer pH-6.6, flow rate - 1 ml/min, detector - UV-205 nm.

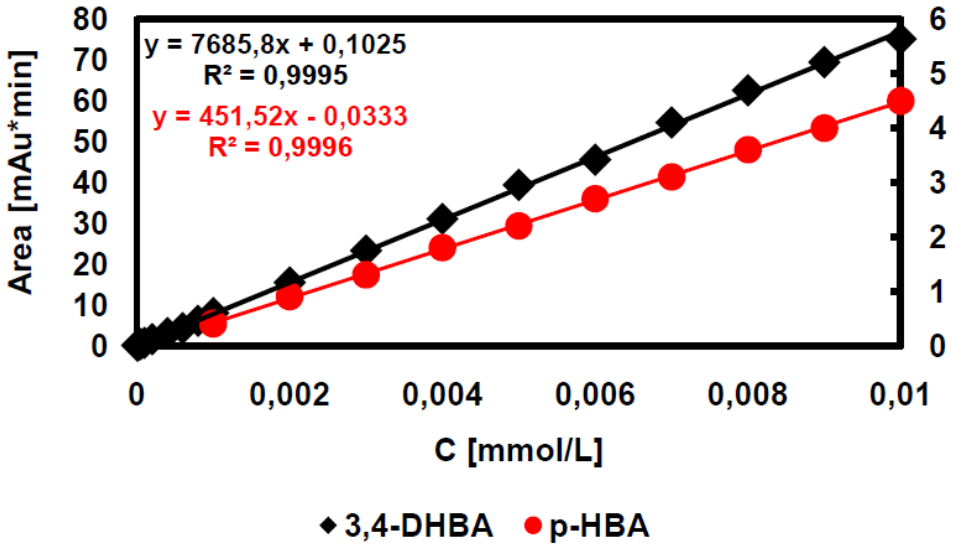

Fig. 2. Calibration curves of pHBA and 34DHBA. Chromatographic conditions as on Fig. 1.

It turned out that among traditional honeys the buckwhead one is characterized by the strongest antioxidant properties (Fig. 3). For herbal honeys the nettle one has been characterized by highest TAP values (Fig. 4). Similar dependences for meads is presented on Fig. 5. In this case we can see the proportion between their TAP values and concentration of honey in the mead. The same measurements have been repeated using electrochemical detector (Fig. 6 and 7). It was found different line-ups using different methods. In the case of meads the same line-ups were obtained. The results were, probably, correlated with the concentration of the ethanol in the sample.

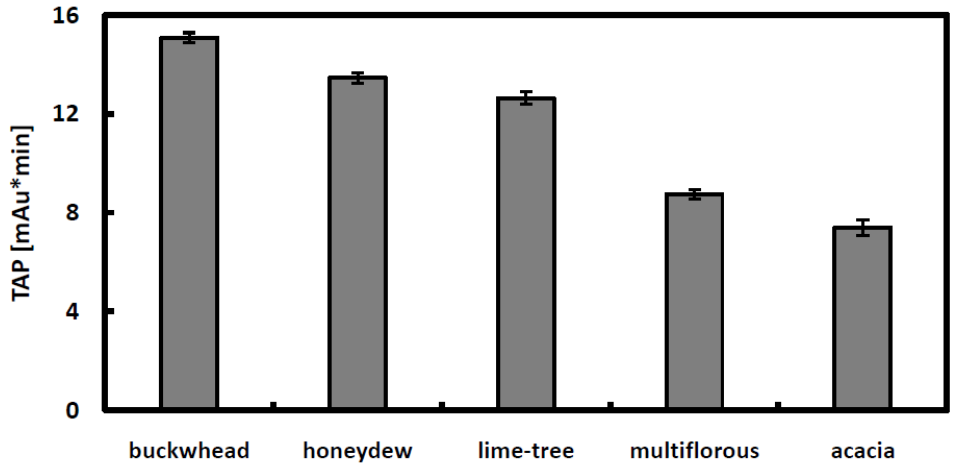

Fig. 3. TAP values of some traditional honeys at concentration 1 mg/ml, obtained from "Sądecki Bartnik". Chromatographic conditions as on Fig. 1.

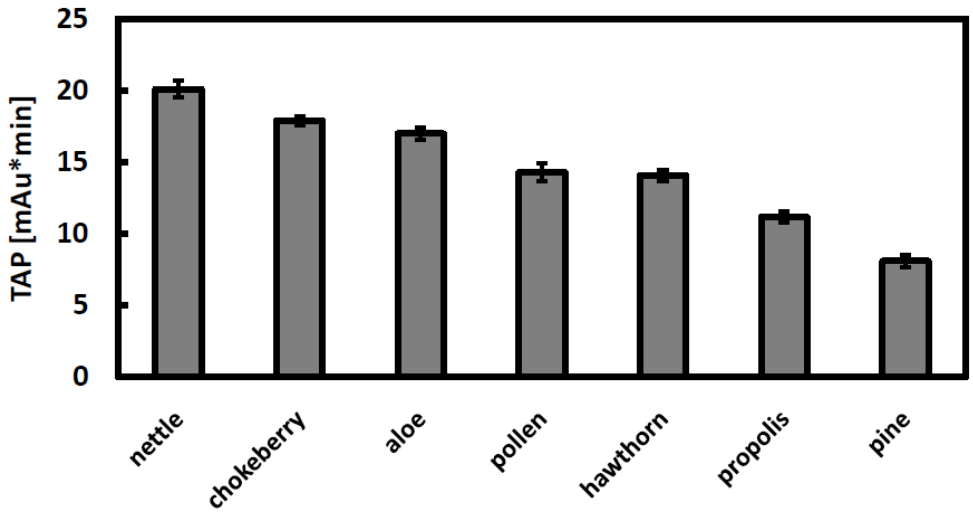

Fig. 4. TAP values of some herbal honeys at concentration 1 mg/ml. Chromatographic conditions as on Fig. 1.

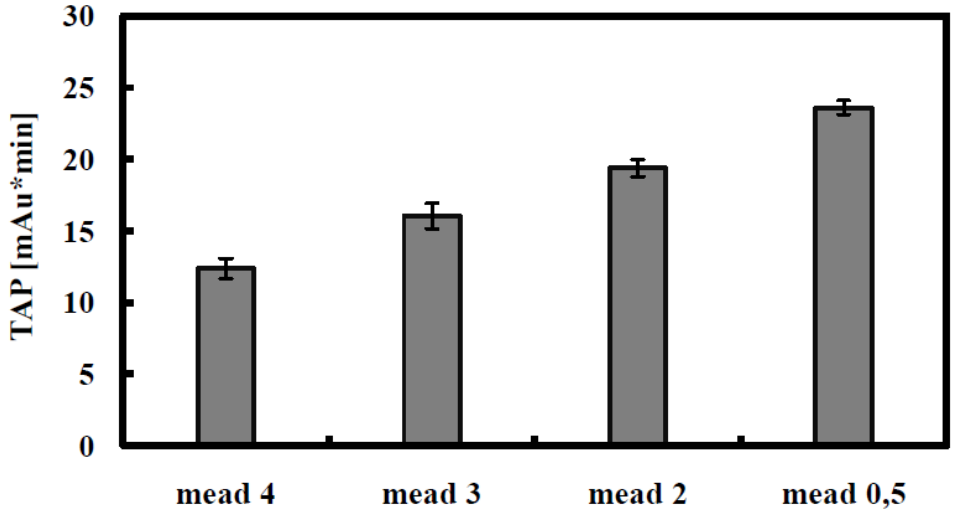

Fig. 5. TAP values of some meads at concentration 1 mg/ml. Chromatographic conditions as on Fig. 1.

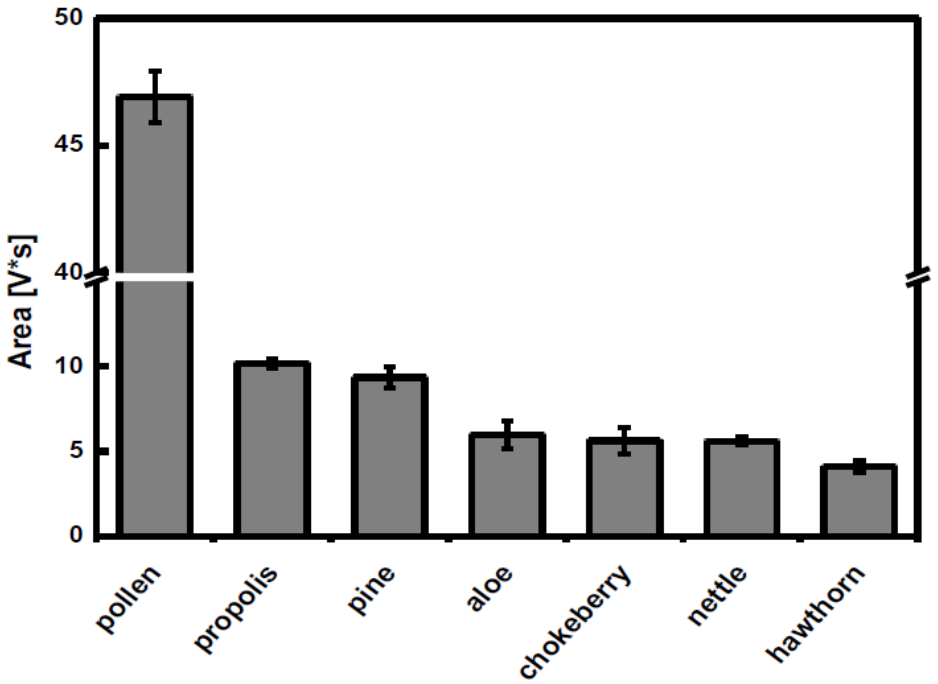

Fig. 6. TAP values of some herbal honeys, at concentration 1 mg/ml, obtained using electrochemical detector working at 0.8 V vs. Ag/AgCl electrode. Chromatographic conditions as on Fig. 1.

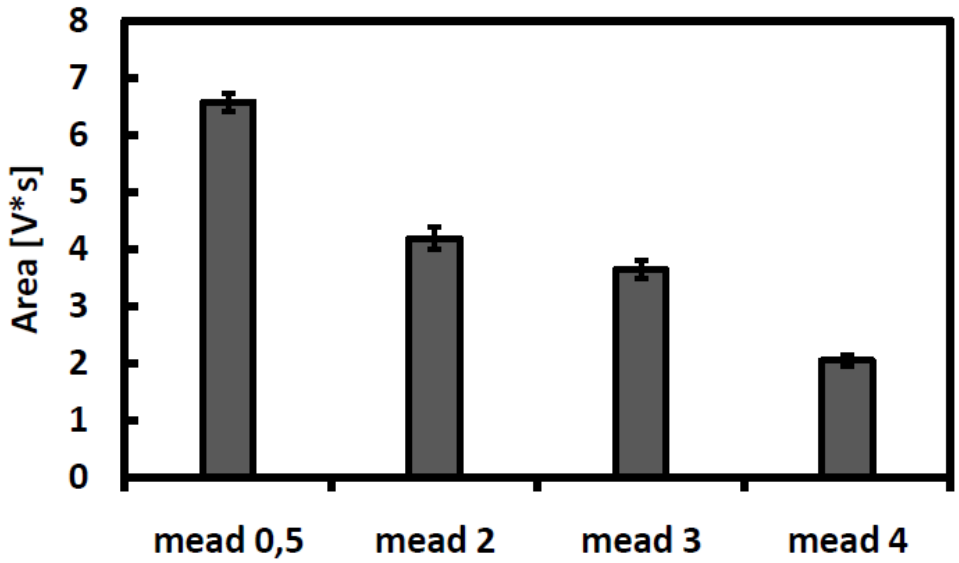

Fig. 7. TAP values of some meads, at concentration 1 mg/ml, obtained using electrochemical detector working at 0.8 V vs. Ag/AgCl electrode. Chromatographic conditions as on Fig. 1.

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RETENTION MECHANISM OF CARBOXYLIC ACIDS ON DIFFERENT HPLC PHASES

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ABSTRACT

Carboxylic acids plays important role as flavors (aliphatic) or preservatives (aromatic) components of food products, antioxidants, medicines and environmental contaminations [1-3]. Therefore, their analysis is crucial for a lot of scientific disciplines. Frequently High Performance Liquid Chromatography (HPLC) is used for this purpose [1, 4].

In the presentation the retention mechanism on different columns is discussed. In reversed phase HPLC the main retention mechanism is hydrophobic adsorption. It can be assumed that dissociated forms of carboxylic groups do not interact with the stationary phase. Increase of their retention is observed when ion-pairing reagent is added to the mobile phase. From the other side inclusion compounds, like cyclodextrines, decrease their retention [5].

In ion exclusion chromatography (IEC) the dissociated forms of acids are repulsed from the stationary phase, characterized by the same sign of the electric charge. Additional effects, like hydrophobic adsorption and screening effect increase or decrease retention, respectively. Aromatic acids are much stronger retained then aliphatic ones, because of their π-electron interaction with the resin skeleton [4, 6, 7].

Very strong π-electron interactions, and in consequence retention, are observed on the Porous Graphitized Carbon (PGC) column. Additionally, induced dipoles are responsible for interaction of the polar (or ionic) compounds with the PGC phase. It is so called PREG-effect (*Polar Retention Effect by Graphite*) [8, 9].

The described effects are discussed, and experimentally confirmed, for aliphatic and aromatic acids.

EXPERIMENTAL

Instrumentation

Measurements were performed by means of a chromatograph comprising a Interface Box, 4 channel Degasser K-5004, Solvent Organizer K-1500, Dynamic Mixing Chamber, HPLC Pump K-1001, Diode Array UV Detector K-2600, Eurochrom 2000 chromatographic data acquisition and analysis software (all from Knauer GmbH, Berlin, Germany), Basic+ marathon Autosampler (Spark Holland B.V., Emmen, The Netherlands), Jet-Stream Plus Column Thermostat (Industrial Electronics, Langenzersdorf, Austria) and fluorometric detector (Shimadzu, Tokyo, Japan) (Fig. 1).

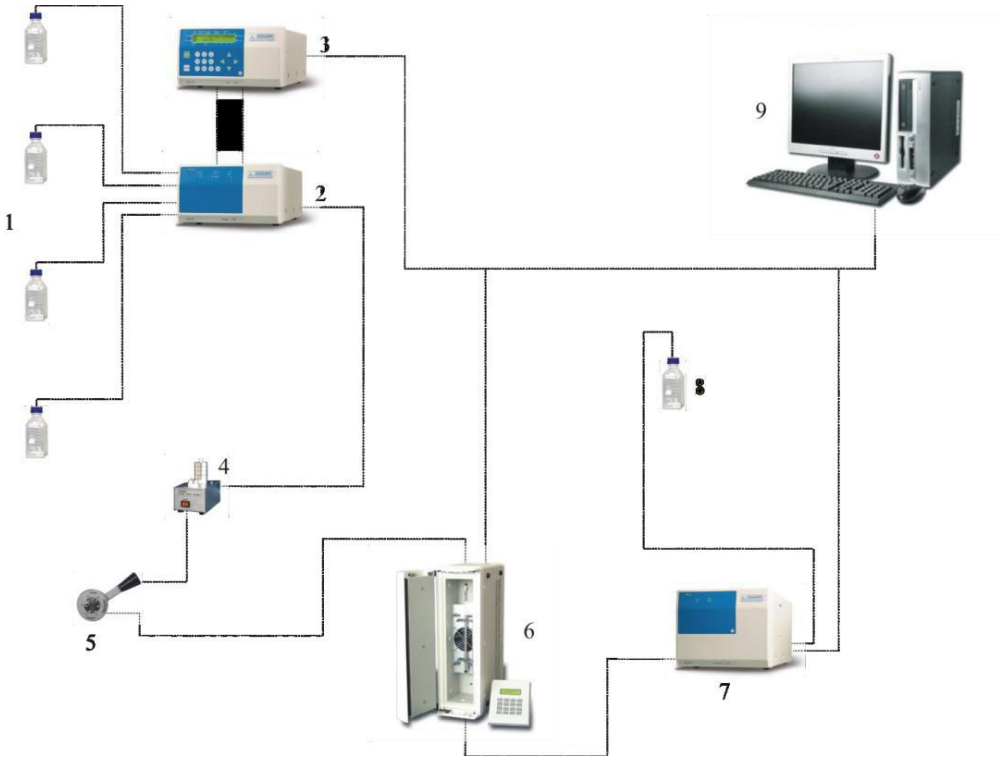

Fig. 1. Scheme of HPLC system used.

Retention has been investigated using columns presented in Table I.

Table I.

Column		Dimensions		Support	
Name	***Producer***	***L [mm]***	***d [mm]***	***Type***	***Particle [µm]***
BIO-RAD HPX-87H	Aminex	300	7.8	PS-DVB	9
Eurospher C18	Knauer	250	4	Silica-gel	5
PGC	Thermo	100	4.6	Graphite	5

Reagents

Methanol and investigated acids were obtained from Sigma (St. Louis, MO, USA). All other reagents (Sigma, St. Louis, USA; Fluka, Buchs, Switzerland; and POCh, Gliwice, Poland) were of analytical-reagent grade and were used without further purification. Water was passed through Millipore (Bedford, USA) Milli-RO4 and Milli-Q water purification systems. Mobile phases were filtered through a 0.22-μm membrane filter (Millipore, Bedford, USA).

RESULTS AND DISCUSSION

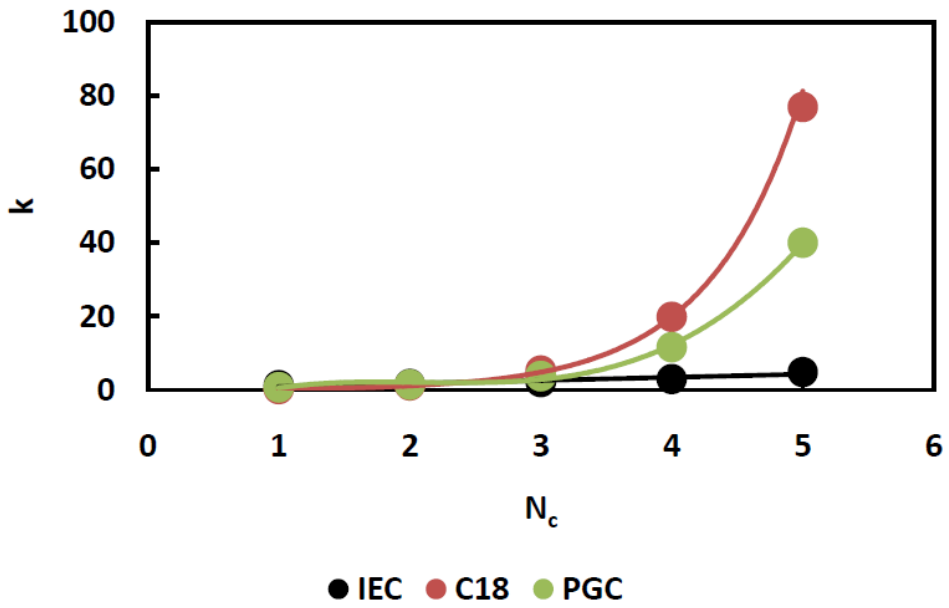

Fig.2. Influence of number of carbon atoms, NC, in the acid molecule on its retention coefficient, k.

It turned out that retention of aliphatic AIDS increased with the number of carbon atoms in the molecule (Fig. 2). It is worth to note that the retention was nearly independent on their dissociation constants. It means that the main retention mechanism is hydrophobic adsorption, what was confirmed by

linear dependence between the logarithm from retention coefficient and NC (Fig. 2). This can explain also shorter retention observed for isomers (*e.g.* isovaleric *vs.* valeric acid), characterized by smaller molecular area.

Retention of acids was also strongly dependent on the presence (and number) of functional groups. For example, much smaller retention on RP-18 and IEC phases has been observed for dicarboxylic acids. The second, partially dissociated, carboxylic group prevent hydrophobic adsorption of the aliphatic chain (the so called *screening effect* [4, 6, 7]). Similar effect was observed for hydroxy acids (Table II), because of small dissociation of hydroxyl group. However, opposite result has been observed on PGC phase (Fig. 3). Probably interactions between dipole (analyzed acid) and induced dipole (PGC phase) are responsible for this phenomena. It confirms the PREG effect. The presence of aromatic ring in the molecule increased retention of acids in all cases. However, the highest retention has been observed on PGC because of π-electron interactions.

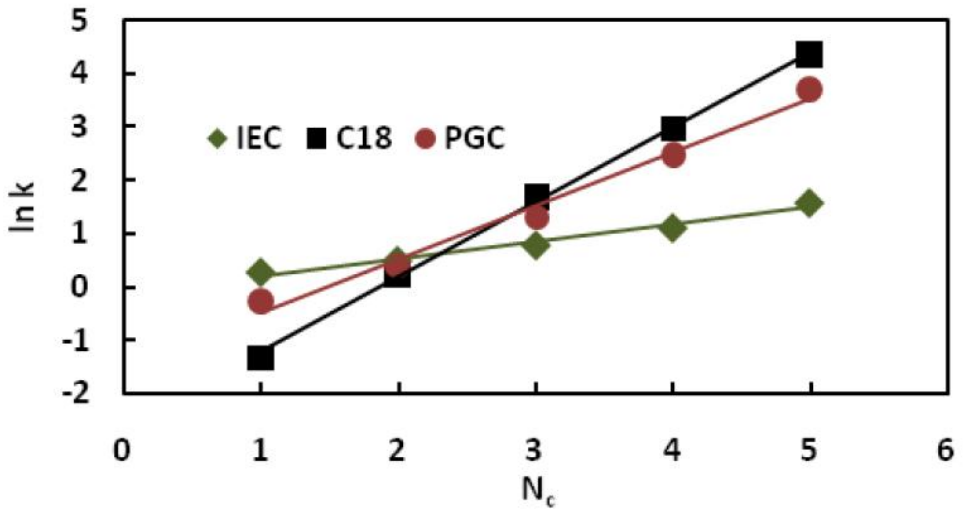

Fig.3. Influence of number of carbon atoms, NC, in the acid molecule on the logarithm from its retention coefficient, k.

Table II.

ACID		pKa	IEC	PGC	RP C18
name	formula		t_r [min]	t_r [min]	t_r [min]
propionic	CH_3-CH_2-COOH	4,87	11,31	5,96	14,85
lactic	CH_3-CH(OH)-COOH	3,86	7,51	2,96	4,51
succinic	HOOC-CH_2-CH_2-COOH	4,21	7,56	37,53	10,39
malic	HOOC-CH_2-CH(OH)-COOH	3,4	5,64	9,84	3,86
glutaric	HOOC-CH_2-CH_2-CH_2-COOH	4,34	9,49	91,82	23,45
citric	HOOC-CH_2- COH(COOH)-CH_2-COOH	3,13	4,75	70,31	8,44
trans-aconitic	HOOC-CH_2- C(COOH)=CH-COOH	2,8	5,85	-	15,81

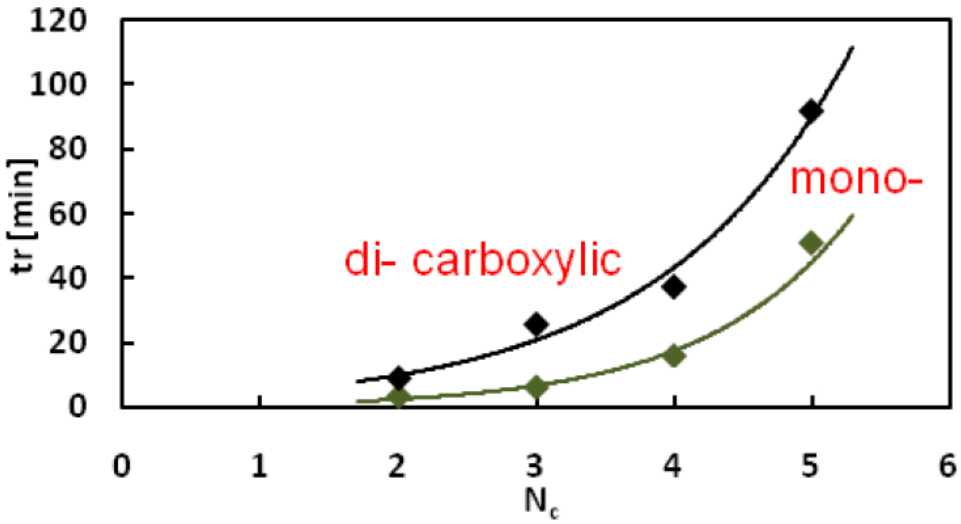

Fig.4. Influence of number of carbon atoms, NC, in the acid molecule on its retention time, tR, for mono- and di-carboxylic acids. Chromatographic conditions: column - PGC Hypercarb 100 x 4,6 mm I.D. (ThermoQuest), temperature - 20 ºC, mobile phase - 1 mM H2SO4, flow rate - 1 ml/min.

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ANALYSIS OF NICKEL COMPLEXES WITH SCHIFF BASE LIGANDS USING HPLC ASSAY WITH UV AND ELECTROCHEMICAL DETECTORS

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ABSTRACT

Schiff complexes are widely investigated since the end of nineteen centaury. They are important in the coordination chemistry as well as in metabolism processes in living organisms. From the chemical point of view they are imines RCH=NR` with the characteristic >C=N– group [1]. They are able to coordinate many of the transient metals creating many different structures (many different donors) and stabilize they different oxidation steps [2-6].

In the presentation separation of five nickel (II) complexes with Schiff base ligands has been investigated using RP-HPLC assay with UV and amperometric detectors. It turned out that good separation of all complexes was obtained at low (5°C) temperature. From the other side, at this temperature very wide (broaded) peak of the complex with salpn ligand was obtained. Probably it was caused by kinetic effects.

It was found that UV (DAD) detector can be used to monitor separation. Because central ion, Ni(II), as well as ligands can be easily oxidized/reduced we have also tested the amperometric detector. However, it turned out that non-sensitive, irreversible results were obtained. Therefore, the electrochemical behavior of separated complexes has been investigated in

strong and weak donating solvents by cyclic voltammetry. Nickel (II) species can be reduced to nickel (I) in several solvents. Characteristic feature has been observed in weak donating solvents, nickel (II) complexes with Schiff bases ligands create conducting polymeric films at the electrode surface. The properties of nickel (II) complexes was compared in different solvents.

INTRODUCTION

Metal complexes of Schiff bases have been widely studied. It is known that nickel (II) complexes with tetradentate N_2O_2 Schiff base ligands are reversibly reduced to nickel (I) species, in several solvents. It was found as application in homogenous electrocatalysis [8, 9]. The oxidative electrochemistry of nickel (II) extend more attractive and interesting. It was demonstrated that nickel (II) complexes are oxidized to nickel (III) complexes in strong donating solvent [10, 11]. In weak donor solvents nickel (II) with Schiff base ligands complexes polymerized at the electrode surface to generate electroactive films [12-14]. The application of nickel modified electrode as heterogeneous catalyst in redox reaction was found as a subject of investigations for several groups [15, 16]. The preparation of metal *salen*-based polymeric films by oxidative electropolymerisation of the monomers has prompted their use as sensor devices. These novel materials have a very interesting feature: its redox processes are not localized at a specific centre, but rather delocalized through the polymer backbone.

In the paper we discuss HPLC analysis of nickel complexes with Schiff base ligands. Their separation has been monitored using UV and amperometric detectors. It turned out that additional cyclic voltammetry measurements were necessary to better understand reactions going on the amperometric detection.

EXPERIMENTAL

Instrumentation

Measurements were performed using a chromatograph comprising a Interface Box, 4 channel Degasser K-5004, Solvent Organizer K-1500, Dynamic Mixing Chamber, HPLC Pump K-1001, Diode Array UV Detector K-2600, ClinLab (RECIPE, Germany) Amperometric detector EC 3000, Eurochrom 2000 chromatographic data acquisition and analysis software (all from Knauer GmbH, Berlin, Germany), Basic+ marathon Autosampler (Spark Holland B.V., Emmen, The Netherlands), Jet-Stream Plus Column Thermostat (Industrial Electronics, Langenzersdorf, Austria) and photometric and electrochemical detectors. Samples were separated on a Kromasil 100 RP-18 5 μm, 250x4 mm I.D. (Besta-Technik GmbH, Wilhelmsfeld, Germany) column.

Electrochemical measurements were performed using the Autolab PGSTAT20 potentiostat /galvanostat. The electrochemical cell was a closed standard three-electrode cell that was connected to a solution reservoir through a Teflon tube. A Pt disk electrode with an area of 0.0314 cm^2 was used as working electrode and a Pt gauze electrode as the counter electrode. All potentials refer to the Ag/AgCl (1 mol dm^{-3} NaCl) reference electrode. Prior to use, the Pt working electrode was polished with an aqueous suspension of 0.05 mm alumina on polishing pad, then rinsed with water. All solutions were deareated and delivered to the cell by a stream of Ar.

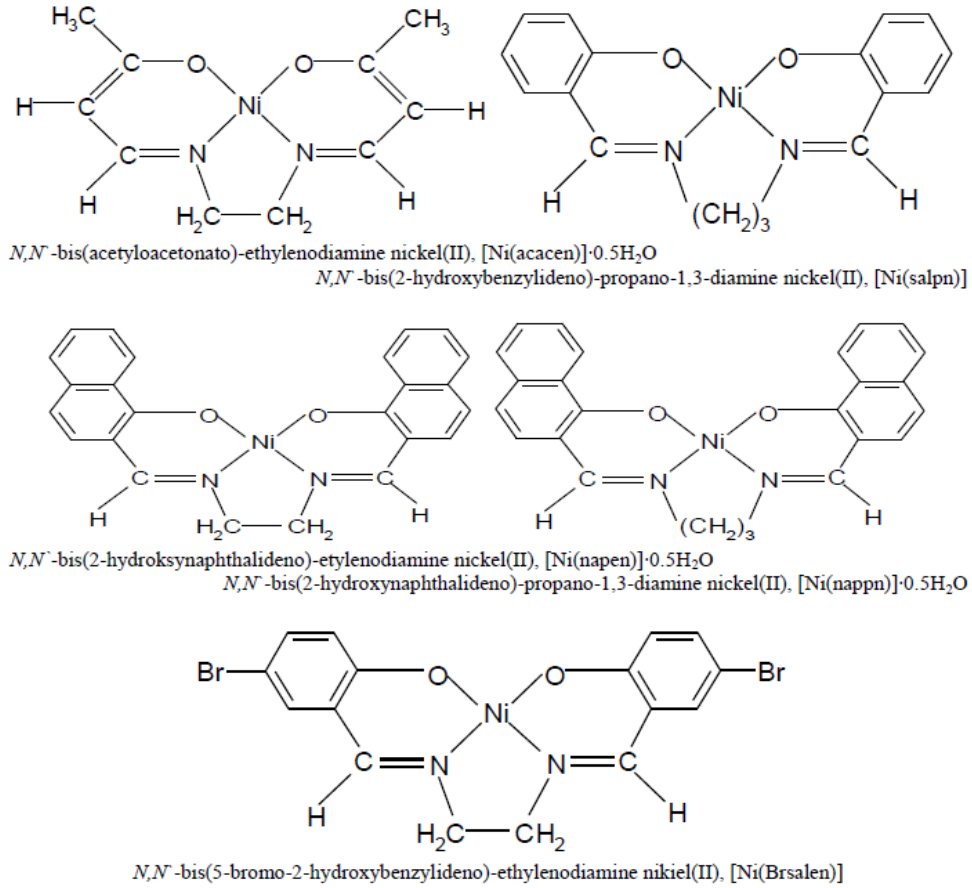

Fig. 1. Structures of nickel complexes with Schiff base ligands.

Reagents

HPLC grade methanol and acetonitrile, DMSO, DMF and tetra-alkyl salts (TBAP and TEAP) were obtained from Sigma (St. Louis, MO, USA). All other reagents (Sigma, St. Louis, USA; Fluka, Buchs, Switzerland; and POCh, Gliwice, Poland) were of analytical-reagent grade and were used without further purification. Quaternary distilled from quartz water has been used.

Procedures

The complexes ([Ni(acacen)] $0.5H_2O$, [Ni(nappn)] 0.5 H_2O, [Ni(napen)] H_2O, [Ni(Nsalen)] H_2O, [Ni(salpn)], [Ni(Brsalen)] were prepared by standard methods [7] and re-crystallized from CH_3CN (*Fig. 1*).

All chromatographic experiments were performed at flow rate 1 ml/min. Column was stabilized at 20ºC by passing the mobile phase for 1 h prior to the chromatographic measure-ments. Pure methanol was used as a mobile phase. Stock solutions of the analyzed compounds were prepared in Milli-Q water and diluted to the required concentration before use. 20 μL samples were injected using autosampler. Out-put signal from the photometric detector working simultaneously at 210, 254 and 280 nm or electrochemical detector, working at different potential of working (Pt) electrode, were continuously displayed on the computer. Every sample was injected six times and the average was taken for further elaboration.

The voltammetric characterization of the reduction process of investigated complexes were performed in several solvents: acetonitrile, dimethyl sulfoxide, dimethylformamide, dichloromethane, methyl chloride and dimethyl ether. All voltammograms were recorded at several scan rates: 1, 0.8, 0.5, 0.2, 0.1, 0.05 and 0.02 V s^{-1}.

The recognition of ion by electroactive polymer film were investigated under electrochemical control [18]. The role of the nickel is structural but Ba^{2+} probably is trapped in a pseudo-crown ether. Using electrochemical methods it was found that some of Schiff bases polymers was able to detect Ba^{2+} ion at micromolar levels. It is interesting that Ni^{2+} ion is electrochemically inactive. The reactivity of the film is ligand centered and the structural changes are associated with the electroinactive Ba^{2+}. We tried to confirm those investigations using electrochemical method and Na^{+} cation. The voltammetric response of our poly-films modified electrodes to successive

addition of Na^+ to the supporting electrolyte solutions shows that Na^+ give increasing peaks in the anodic features. This response promises much for sensing applications.

RESULTS AND DISCUSSION

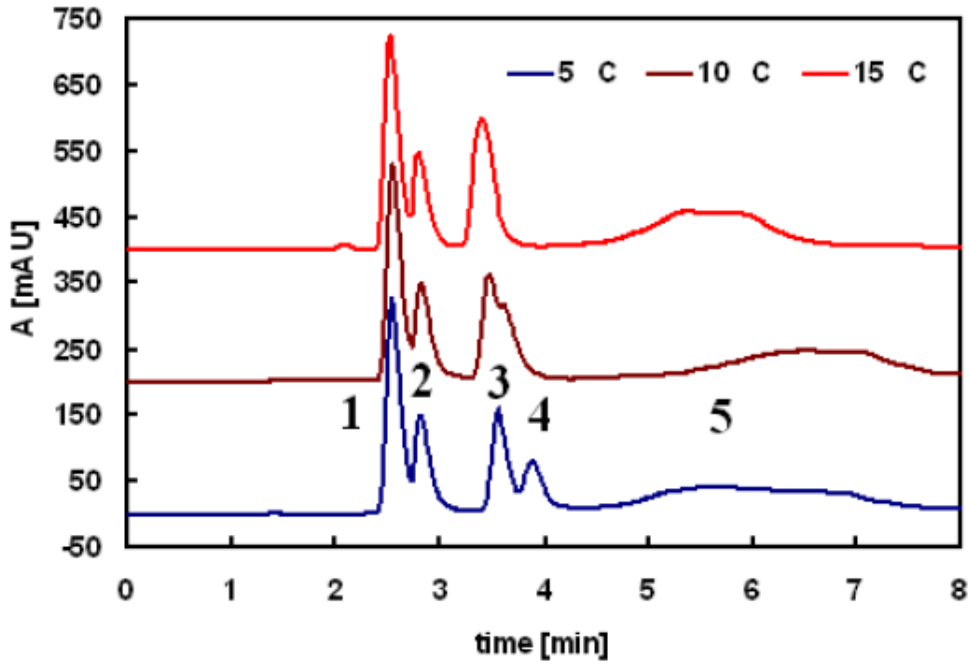

Fig. 2. HPLC chromatograms of nickel complexes with Schiff base ligands: salen (1), acacen (2), nappn (3), napen (4) and salpn (5) recorded at different temperatures. Chromatographic conditions: column - Kromasil 100 C18 column, 5 μm, 250 x 4.0 mm I.D.; mobile phase – methanol; detector - DAD UV-254 nm; flow rate – 1.0 ml/min.; injection volume – 20 μl.

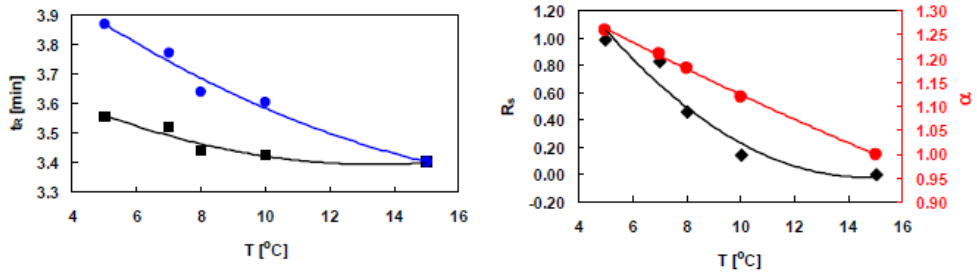

Fig. 3. Influence of temperature on the retention times, resolution and selectivity of ***nappn*** *and* ***napen****.*

It turned out that nickel complexes with Schiff base ligands can be easily separated on the reversed phase system, as it is presented on Fig. 2. At room temperature *nappn* and *napen* complexes are unseparated. Decrease of temperature significantly increases selectivity of the separation do not influencing system performance (Fig. 3). It was found that *salpn* complex gives very broad peak. This effect decreases with the increase of temperature what suggest kinetic origin.

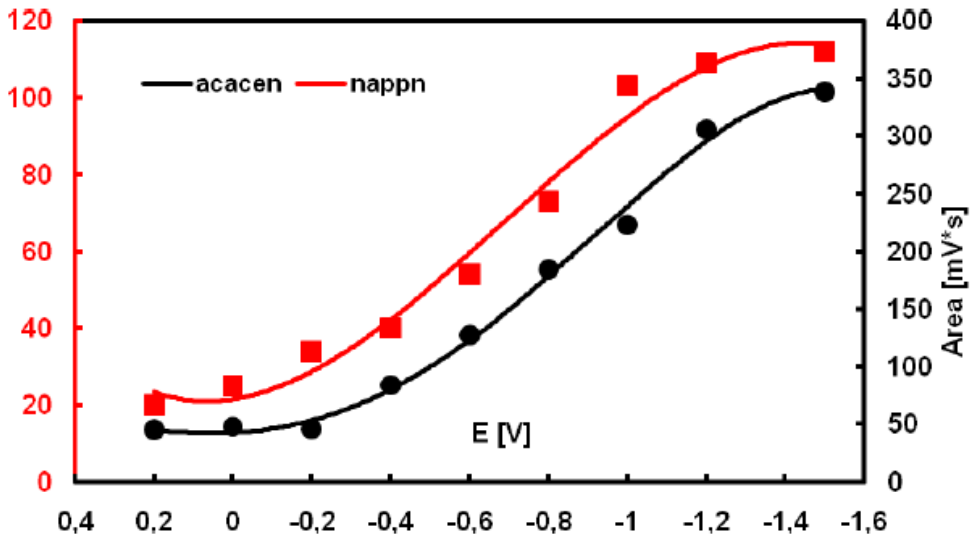

Fig. 4. Hydrodynamic voltamograms of nickel complexes with Schiff base ligands. Chromatographic conditions as on Fig. 1.

As it was mentioned above nickel complexes with Schiff base ligands can be easily oxidized/reduced. Therefore we have used amperometric detector to the analysis. Because investigated complexes are not stable in water solutions therefore pure methanol has been used as a mobile phase. Platinum electrode has been used as a working electrode, because it is more suitable for non-aqueous solvents. It turned out that good detection conditions were obtained at negative potentials (reduction current). Hydrodynamic voltammograms of these complexes are shown on Fig. 4.

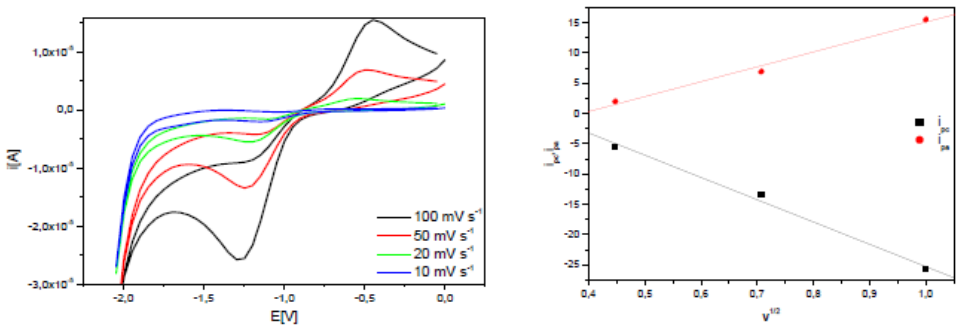

Fig. 5. Voltamograms of [Ni(acacen)]·0.5 H_2O in 10 mM ACN/TBAP (left). Scan rates – 10, 20, 50 and 100 mV·s^{-1}, potential range - 0.0 ÷ 2.0 V. Influence of the square root from the scan rate on cathodic (ipc) and anodic (ipa) currents (right).

The cyclic voltametric measurements were performed to obtain optimal condition of the electrochemical detection. An examples of the obtained results are presented on Fig. 5. Different shapes of voltammograms were obtained in both cases. It can be explained by the fact that in the electrochemical detection additional convectional current is measured, from the other side lack of the capacity current is observed. Both, oxidation and reduction, processes are irreversible (lack of the reversible peaks on Fig. 5).

However, linear dependence between cathodic and anodic currents and the square root from the scan rates suggests quasi-reversible process.

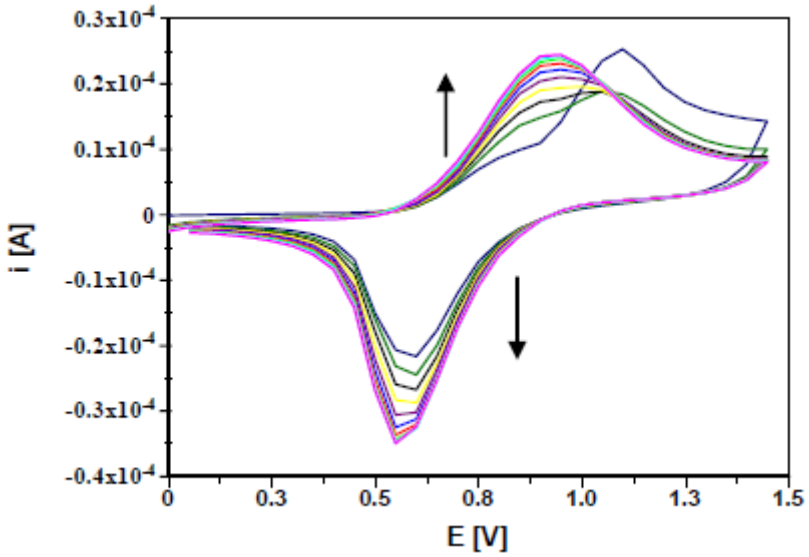

Fig. 6. Voltamograms of [Ni(salpn)] in 10 mM CH_2Cl_2/TMAP. Scan rate – 100 $mV \cdot s^{-1}$, potential range - 0.0 ÷ 1.4 V.

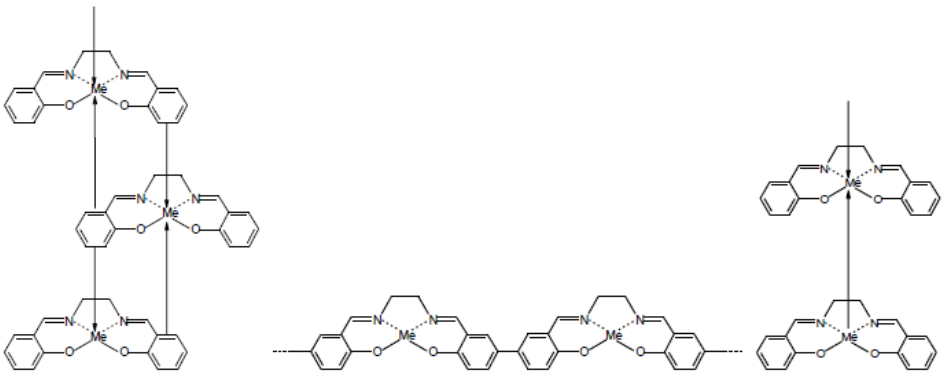

Fig. 7. Proposed structures of electropolymers obtained from the nickel complexes with Schiff base ligands [14-17].

On Fig. 6 cyclic voltamograms of [Ni(salpn)] in dichloromethane are presented. It turned out that in this case (solvents characterized by small donor number) conducting polymers have been obtained (increase of reduction/oxidation currents in the successes cycles) [15-17]. Possible structures of the obtained polymers are presented on Fig. 7.

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HPLC TOTAL ANTIOXIDANT POTENTIAL ASSAY BASED ON HYDROXYL RADICALS REACTION WITH P-HYDROXYBENZOIC ACID

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ABSTRACT

Free radicals adversely modify biologically active molecules as well as whole cells and are implicated in various degenerative diseases and aging. Their mediated process have been implicated in the pathogenesis of several diseases. It is widely believed that these modifications are preventable by exogenous antioxidants. There is a need for a method to assess and compare strength of particular antioxidants in order to select these of the highest potential for further development as drugs. However, it turned out that frequently more information (e.g. synergetic effects) is obtained measuring total antioxidant potential (TAP) of biological samples than concentration of particular antioxidants separately.

In the literature we can find many methods describing the hydroxyl radicals analysis using HPLC. They are based on reaction of these radicals with the spin trap reagents. Products of these reaction can be monitored using electrochemical as well as fluorescence detection. Previously we have showed that these assays can be also applied to the TAP measurements. In this case hydroxyl radicals were generated in the Fenton reaction. They were detected using: (i) *p*-hydroxybenzoic acid (*p*-HBA) with the electrochemical

detection or (ii) terephthalic acid (TFA) with the fluorescence detection. In the presentation TAP measurements using *p*-HBA with fluorescence detection will be discussed. The elaborated
assay will be tested on alcoholic beverages as well as on pure compounds, such as indoles, flavones and triazines.

INTRODUCTION

Oxygen is necessary to live. However, it is also toxic for living organisms [1, 2]. It is used as electron acceptor. During its reduction free radicals and, more widely, the so called reactive oxygen species (ROS) are created. Free radicals are chemical individuals (atoms, ions, molecules or their fragments) having unpaired electrons. It gives them paramagnetic properties. They are present in every living cell [3, 4]. However, their concentration in tissues change widely after interactions with chemical compounds, radiation, sickness, stress, hypermetabolism or aging. In living organisms free radicals are generated in physiological as well as pathological conditions mainly on the inner surface of the mitochondrial membrane. In physiological they do not accumulate in tissue because they are scavenged by the local antyoxydative mechanisms. Free radicals react nearly with all cell compounds. Among the biologically active compounds we can enumerate lipids, proteins and DNA. These reactions can cause degeneration or even death of the cells. The most exposed, on the free radicals attack, cell organelles are mitochondria. The most reactive radical is hydroxyl radical [6]. It can interact with number of organic compounds through addition, free radical substitution or electron transfer. One of the most damaging effects of hydroxyl radicals is the chain peroxidation of polyunsaturated lipids located in cellular and intracellular membranes. It is estimated that a single hydroxyl radical may damage up to 700 lipid molecules.

Hydroxyl radicals are very reactive and short-lived. Therefore they are difficult to the direct analysis. However, they can be chromatographically analyzed after spin trapping. Benzene ring of an aromatic compound (spin trap) is attacked by hydroxyl radical, and hydroxylated products of this reaction are separated and detected. As spin traps, endogenous phenylalanine, or exogenous aspirin can be used [5]. The aspirin-based assay is the most popular. In the biological environment aspirin (*o*-acetylsalicylic acid) is quickly hydrolyzed to salicylic acid which reacts with hydroxyl radicals giving three main products: 2,3- and 2,5-dihydroxybenzoic acids (DHBA) and *o*-catechol (with the yields of 49, 40 and 11%, respectively). These derivatives may be separated using reversed phase HPLC with photometric detection. More sensitive is the electrochemical detection [5, 6].

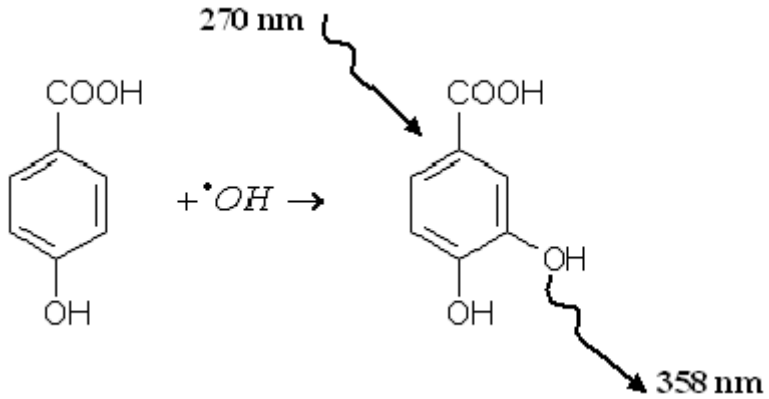
It turned out that salicylic acid is not optimal as a spin trap for hydroxyl radicals. Detection limit is increased because two main products are formed, and aspirin and its derivatives present in food may contaminate biological samples. To avoid these problems the

use of *p*-hydroxybenzoic acid have been proposed, which upon the reaction with hydroxyl radicals is converted to one main product, 3,4-dihydroxybenzoic acid [7-9].

Because of the interaction between free radicals and antioxidants in pathology changes of antioxidants concentration are observed. Complementary the antioxidants concentrations can be measured. It turned out that estimation of total antioxidant potential (TAP) is frequently much more useful then separate analyzing all of them. Cooperation between different antioxidants gives sometime better protection than separate compounds. As an example of synergism we can mention glutathione, which regenerate ascorbate or ascorbic acid regenerating α-tocopherol. Both these

magnitudes, *i.e.* oxidative stress and total antioxidant potential are correlated each other and even at times identified.

In the presentation we would like to show application of the *p*HBA acid to the estimation of the total antioxidant potential. With hydroxyl radicals it gives one product of the reaction. Instead of the electrochemical detection the fluorometric one has been used.



EXPERIMENTAL

Instrumentation

Measurements were performed by means of a chromatograph comprising a Interface Box, 4 channel Degasser K-5004, Solvent Organizer K-1500, Dynamic Mixing Chamber, HPLC Pump K-1001, Diode Array UV Detector K-2600, Eurochrom 2000 chromatographic data acquisition and analysis software (all from Knauer GmbH, Berlin, Germany), Basic+ marathon Autosampler (Spark Holland B.V., Emmen, The Netherlands), Jet-Stream Plus Column Thermostat (Industrial Electronics, Langenzersdorf, Austria) and fluorometric

detector (Shimadzu, Tokyo, Japan). Samples were separated on a Hypresil RP-18 5 m, 250x3 mm I.D. (E. Merck, Darmstadt, Germany) column.

Reagents

p-Hydoxybenzoic acid and phosphate buffered saline (PBS) tablets were obtained from Sigma (St. Louis, MO, USA). All other reagents (Sigma, St. Louis, USA; Fluka, Buchs, Switzerland; and POCh, Gliwice, Poland) were of analytical-reagent grade and were used without further purification. Water was passed through Millipore (Bedford, USA) Milli-RO4 and Milli-Q water purification systems. Mobile phases were filtered through a 0.22-μm membrane filter (Millipore, Bedford, USA).

Procedures

Chromatographic experiments were performed with a flow rate 0.7 ml/min. Column was stabilized at 20 ºC by passage of mobile phase for 1 h prior to the chromatographic measurements. Phosphate buffer (pH 6.6) has been used as mobile phase. 10 mmol L^{-1} stock solutions of the analyzed compounds were prepared in Milli-Q water and diluted to the required concentration before use. 20 μL samples were injected using autosampler. Fluorescence detector working at excitation and emission wavelengths equal 270 nm and 358 nm, respectively, has been used. Every sample was injected six times and the average was taken for further elaboration.

Hydroxyl radicals are generated by Fenton reaction and both the detector and the analyte scavenge the radicals. They were generated through Fenton reaction by 1 min incubation of 0.5 mM Fe^{2+} and 2 mM H_2O_2 in 50 mM phosphate buffer (pH 7.4) in the presence of 1 mM *p*-HBA and analyzed sample at 37 ºC. Product of reaction of *p*-HBA with hydroxyl radicals, namely 3,4-dihydroxybenzoic acid (DHBA) was detected fluorometrically. Sample added to the reaction mixture decreased its peak because competition reaction with radicals. If the analyte "performs better" than the detector, generation of the DHBA is decreased. This assay enables to compare the

hydroxyl radical scavenging performance of various substances, measured as a decrease of peak height of DHBA.

RESULTS AND DISCUSSION

It was found that TAP is, in general, non-linear function of the antioxidant concentration. However, for the small concentration linear dependencies were obtained. Therefore all experiments were performed at small antioxidants (samples) concentrations.

The elaborated assay has been applied to the TAP estimation of pure compounds and different kinds of wines (Fig. 1a.) and strong alcohols (Fig. 2b.). It turned out that TAP values were strongly dependent on the ethanol concentration in the sample. The results are compared with the previously obtained with terephthalic acid used as a detector. It turned out that higher responses were obtained in this second case. Different antioxidant series were obtained with both assays. However, changes are statistically un-significant (Fig. 2.).

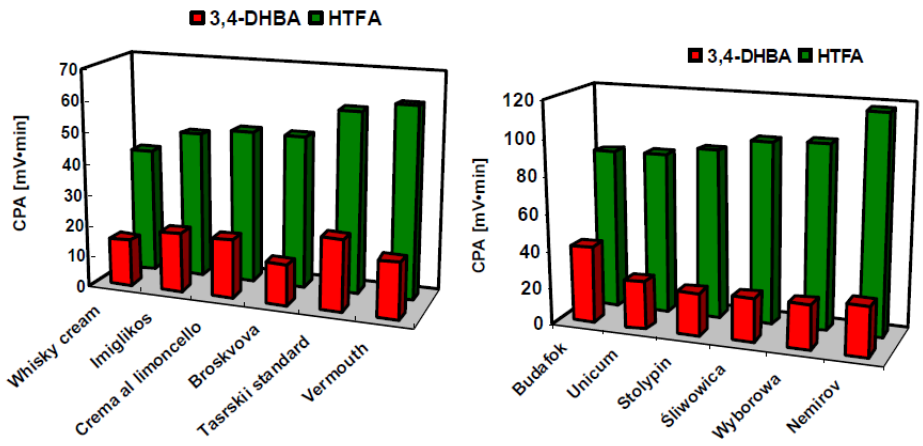


Fig. 1. TAP values of the different alcohols obtained using pHBA and TFA as detectors.

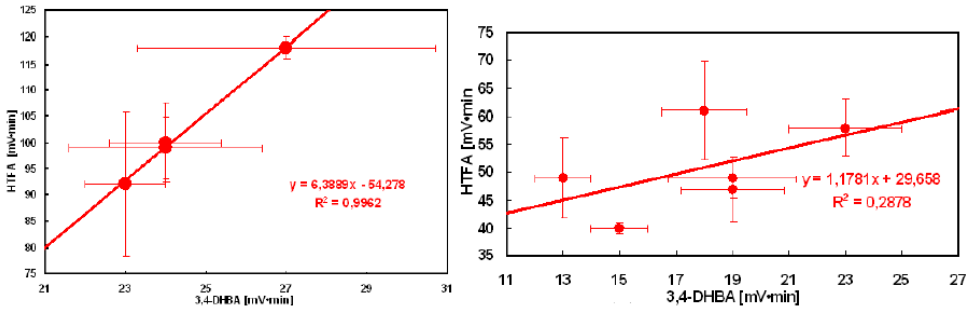


Fig. 2. Comparisons of TAP values of the different alcohols obtained using pHBA and TFA as detectors.

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