Concept for the study group Bioanalytics

The main purpose of this group is besides „listing“ of methods and „applications“ a help at deciding what to use for what and warn about pitfalls. It might be a help for those using analytics in fields like life science, environment and food analysis and not being a really well-trained as Analysts. Tools should be provided.

1. General

1.1 Find classification according to

1.1.1 Analytical Method

1.1.2 Targeted Molecule

1.1.3 Application

1.2 Key list for literature search (fundamental books, review articles….)

- Analytical Chemistry: A Modern Approach to Analytical Science, 2nd Edition, Robert Kellner (Editor), Jean-Michel Mermet (Editor), Matthias Otto (Editor), Miguel Valcárcel (Editor), H. Michael Widmer (Editor) ISBN: 978-3-527-30590-2, 1209 pages, Wiley-VCH, Weinheim, 2004
- Principles of instrumental analysis, Douglas A. Skoog, F. James Holler, Stanley R. Crouch. Belmont, CA : Thomson, Brooks/Cole, 1039 pages, ISBN 978-0-495-01201-6, Thomson, 2007.1998.
- Chemical Instrumentation: A Systematic Approach, 3rd Edition, Howard A. Strobel, William R. Heineman, Wiley, ISBN: 978-0-471-61223-0, 1248 pages, 1989
- Bioanalytics, (Analytical Methods and Concepts in Biochemistry and Molecular Biology), Lottspeich / Engels, 2018. 1000 S. Hardcover, Wiley-VCH ISBN 978-3-527-33919-8
- A. Manz, P. S. Dittrich, N. Pamme, D. Iossifidis. Bioanalytical Chemistry, 2nd ed., Imperial College Press, Londom, 2015
- G. Evans. A Handbook of Bioanalysis and Drug Metabolism, CRC Press, Boca Raton (2004).
- Electrochemical Sensors in Bioanalysis, R.I. Stefan, J.F. van Staden and H.Y. Aboul-Enein, Marcel Dekker Inc., New York, USA, 2001.

1.3 Decision tree combining method, application, problem

1.3.1 Tutorial using analytics (adds and odds of methods)

During EuCheMS in Liverpool it has been a session “**ABCs of Analytics”** leading to a review where developments are described, and advice on using instrumentation and evaluation procedures in the field of life sciences, environment and food analysis is given. First, young scientists receive information about publishing in analytical journals; at the end, there will be a panel discussion about challenges and solutions in analytics.

In TrAC Trends in Analytical Chemistry frequently tutorials are published on analytical methods.

1.3.2 Short courses organized at conferences such as EUROANALYSIS,-EuChemS– for the training of new researchers in the field

1.3.3 Tutorials are given at the meeting of Analytical Divisions of European Chemical Societies

1.4 Classification of Bioanalytics

1.4.1 Definition

Analytical methods in biosciences like biochemistry, biology, biotechnology, molecular biology, molecular genetics, medicine, pharmacy as well in environmental sciences. Methods are used to get information on macromolecules biomolecules related to their

- identification
- characterization:

- structure,
  - E. Buxbaum. Fundamentals of Protein Structure and Function, Springer International Publishing (2015).
- dynamics,
  - A. Liwo (Ed.), Computational Methods to Study the Structure and Dynamics of Biomolecules and Biomolecular Processes: From Bioinformatics to Molecular Quantum Mechanics, Springer Series on Bio- and Neuro systems, Vol 8, Springer Nature Switzerland AG, Cham 2019
- effects and impact

- quantification and monitoring

1.4.2 Bioinformatics

Especially in live sciences, big data are produced using imaging techniques, high-throughput, arrays, dynamic measurements. Treatment of big data with artificial intelligence or by machine learning algorithms is an upcoming field.

- J. Pevsner. Bioinformatics and Functional Genomics, 3rd ed., John Wiley and Sons Ltd, Chichester, UK (2015).
- R. Meier et all, Bioinformatics can boost metabolomics research, Journal of Biotechnology, 261, 2017, 137-141
- K. Lan et all, A Survey of Data Mining and Deep Learning in Bioinformatics, Journal of Medical Systems (2018) 42: 139
- Ch. Satyanarayana et all, Computational Intelligence and Big Data Analytics (Applications in Bioinformatics) in SpringerBriefs in Applied Sciences and Technology: Forensic and Medical Bioinformatics, A. Kumar et all (Eds.), Springer Nature, Singapore, 2019

2 Analytical methods/techniques

IUPAC recommendations have been published in 2018 concerning the terminology of methods of bioanalytical chemistry. Terms related to samples, enzymatic and immuno-analytical methods, methods for –omics and to the interaction of biomolecules are defined.

- J. Labuda et al., Terminology of bioanalytical methods (IUPAC Recommendations 2018), Pure Appl. Chem. 2018; 90(7): 1121–1198

2.1 Separation sciences (LC, GC, SFC, CE, CEC)

2.1.1 Liquid Chromatography

Principle

The term “chromatography” originated from the Greek words meaning “color” and “to write”. Chromatographic techniques have been developed over the past century and have major input in many areas of science. Chromatography is a technique for the analysis of complex mixtures. The term chromatography means color writing and denotes a method by which the sample to be analysed is poured into a vertical glass tube containing an adsorbent, the various components of the substance moving through the adsorbent at different rates of speed, according to their degree of attraction to it, and producing bands of color at different levels of the adsorption column. The term has been extended to include other methods utilizing the same principle, although no colors are produced in the column.

Similar to this theory, the acronym of high performance liquid chromatography (HPLC), given by Prof. Csaba Horváth in his 1970 Pittcon paper, originally indicated that high pressure was used to generate the flow required for liquid chromatography in packed columns to separate the sample (that contains a single analyte and/or mixture), exploiting its breakdown between two phases.

The mobile phase of chromatography refers to the fluid that carries the mixture of substances in the sample through the adsorptive material. The stationary or adsorbent phase refers to either a solid, porous, surface-active material in small-particle form or a thin film of liquid coated on a solid support or column wall that takes up the particles of the substance passing through it. For example, kaolin, alumina, silica, and activated charcoal, etc. have been used as adsorbing substances or stationary phases.

The sample components are separated based on differences in their distribution between the stationary and mobile phases. Classification of chromatographic techniques is done accordingly with the type of stationary phase, the nature of the adsorptive force, the nature of the mobile phase, or the method by which the mobile phase is introduced. There are two well-known types of separation modes available on liquid chromatography: reversed phase and normal phase. Apart from these techniques, a rather new separation mode named hydrophilic interaction liquid chromatography (HILIC) has been used since the 1970s, later improved by Alpert in the 1990s

The technique is a valuable tool for bioanalytics, e.g. chromatography is used to detect and identify in body fluids sugars, amino acids, interleukins, etc.

Some other types of chromatography summarized the following: Paper chromatography is a form of chromatography in which a sheet of blotting paper, usually filter paper, is substituted for the adsorption column. After separation of the components as a consequence of their differential migratory velocities, they are stained to make the chromatogram visible. In the clinical laboratory, paper chromatography is employed to detect and identify sugars and amino acids.

Thin-layer chromatography in which the stationary phase is a thin layer of an adsorbent such as silica gel coated on a flat plate is similar to paper chromatography. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. The goal of TLC TLC can be used to ensure the purity of a compound.is to obtain well defined, well-separated spots. TLC is very simple to use and inexpensive. Therefore, the

length of separation is limited compared to other chromatographic techniques. Also, the detection limit is a lot higher.

Affinity chromatography is based on a highly specific biologic interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand. Any of these substances, covalently linked to an insoluble support or immobilized in a gel, may serve as the sorbent allowing the interacting substance to be isolated from relatively impure samples; often a 1000-fold purification can be achieved in one step.

Exclusion chromatography is the one in which the stationary phase is a gel having a closely controlled pore size. Molecules are separated based on molecular size and shape, smaller molecules being temporarily retained in the pores.

Ion exchange chromatography that utilizing ion exchange resins, which are coupled with either cations or anions that will exchange with other cations or anions in the material passed through their meshwork.

HPLC is the most preferable technique for the identification, quantification, and separation of components in a sample. High pressure is used for pushing mobile phase solvents through the column. It is an ideal way for the identification, separation, and quantification of active pharmaceutical ingredients: proteins, amino acids, hydrocarbons, nucleic acids, pesticides, carbohydrates, antibiotics, anticancer, antivirals, and steroids, as well as the countless other inorganic and organic substances. A wide range of detectors that meet different requirements are commercially available and can be used for the detection such as UV-Vis, PDA, Refractive Index, Conductivity, Light Scattering, Charged Aerosol, Fluorescence, Electrochemical and also Mass Spectrometry.

Chances and pitfalls

- Reliable qualitative and quantitative analysis
- Suitable for complex samples analysis
- High selectivity
- A wide range of detectors can be used for a different types of samples

Applications

- Clinical analysis;
- Pharmaceutical analysis;
- Environmental analysis
- Food analysis
- Bioanalytical analysis
- Polymer analysis
- Natural products

Literature

- Chromatography and its applications, Ed S. Dhanarasu, InTech, Croatia, 2012.
- A Handbook of Chromatography, Ed M. Braga Scholar's Press Verlag Omniscriptam, Deutschland, Germany, 2017
- Liquid Chromatography 2nd Edition, Fundamentals and Instrumentation, Salvatore Fanali Paul R. Haddad Colin Poole Marja-Liisa Riekkola Eds., Elsevier, 2017. ISBN: 9780128053935

- The HPLC expert: possibilities and limitations of modern high performance liquid chromatography, S. Kromidas, Wiley 2016, ISBN: 9783527336818
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- Software-assisted method development in high performance liquid chromatography, S. Fekete, I. Molnar, World Scientific, 2019. ISBN: 9781786345462
- Therapeutic Drug Monitoring and Toxicology by Liquid Chromatography, S. H.Y. Wong, CRC Press, 2017. ISBN: 9780824772468.
- Ultra-High Performance Liquid Chromatography and Its Applications, Q.A. Xu, Wiley, 2013. ISBN: 9780470938420
- Hydrophillic Interaction Liquid Chromatography (Hilic) and Advanced Applications, P.G. Wang, W.He, Taylor&Francis, 2011. ISBN: 9781439807538
- Practical High-Performance Liquid Chromatography, V.R. Meyer, Wiley, 2010. ISBN: 9780470682173
- Introduction to Modern Liquid Chromatography, L. R. Snyder, J. J. Kirkland, J. W. Dolan, Wiley,2010. ISBN: 9780470167540
- Modern Size-Exclusion Liquid Chromatography: Practice of Gel Permeation and Gel Filtration Chromatography, A. Striegel, W. W. Yau, J. J. Kirkland, D.D. Bly, Wiley, 2009. ISBN: 9780471201724
- Protein Liquid Chromatography, M. Kastner, Elsevier, 1999. ISBN: 9780444502117.
- High performance liquid chromatography, W.J., Lough, I.W., Wainer, Wiley, 1996.
- Liquid Chromatography of Oligomers, C.V., Uglea, CRC Press, 1996. ISBN: 9780824797201
- Carbohydrate analysis: high performance liquid chromatography and capillary electrophoresis, Z. El Rassi., Elsevier, 1994. ISBN: 9780444899811
- Gumustas, M., Kurbanoglu, S., Uslu, B. et al. UPLC versus HPLC on Drug Analysis: Advantageous, Applications and Their Validation Parameters. Chromatographia 76, 1365–1427 (2013).

2.1.2. Gas Chromatography

Principle

Gas chromatography (GC) is the premier technique for the separation and analysis of volatile compounds. It has been used to analyze gases, liquids, and solids, with the latter usually dissolved in volatile solvents. Both organic and inorganic materials can be analyzed, and molecular weights can range from 2 to over 1000Da.

GC is a form of chromatography in which an inert gas is a moving phase. In GC, the sample is vaporized and carried by the mobile gas phase (the carrier gas) through the column. In most analyses, samples partition (equilibrate) into and out of the stationary liquid phase, based on their solubilities in the stationary phase at the given temperature. The components of the sample (called solutes or analytes) separate from one another based on their relative vapour pressures and affinities for the stationary phase. Volatile components of the sample are separated in the column and measured by a detector. GC detectors can be Flame ionization detector (FID), Thermal conductivity detector (TCD), Electron capture detector (ECD), Flame thermionic detector (FTD), Flame photometric detector (FPD),Mass Spectrometer, etc. The method has been applied in the clinical laboratory to separate and quantify steroids, barbiturates, and lipids, etc.

Chances and pitfalls

- Due to its high efficiency, GC allows the separation of the components of complex mixtures in a reasonable time.
- Accurate quantitation (usually sharp reproducible peaks are obtained)
- Mature technique with many applications notes available for users.
- Multiple detectors with high sensitivity (ppb) are available, which can also be used in series with a mass spectrometer since MS is a non-destructive technique.
- Limited to thermally stable and volatile compounds.
- Most GC detectors are destructive, except for MS.

Applications

- Pharmaceutical
- Food and beverages
- Environmental
- Petrochemicals
- Polymers
- Gas purity analysis
- Clinical
- Natural products
-

Literature

- Gas Chromatography, Colin F. Poole Ed. Elsevier, 2021, ISBN: 9780128206751
- Basic gas chromatography, H.M. McNair, J. M. Miller, N.H. Snow, Wiley, 2019, ISBN: 9781119450757
- A Practical Guide to Gas Analysis by Gas Chromatography, J.Swinley, P. de Coning, Elsevier, 2019. ISBN: 9780128188880
- Gas Chromatography: Analysis, Methods and Practices, V. Warren, Nova Science Publishers, 2017. ISBN:9781536120134
- Practical Gas Chromatography: A Comprehensive Reference, K.Dettmer-Wilde, W.Engewald, Wiley, 2014. ISBN: 9783642546396
- Comprehensive two-dimensional gas chromatography, Volume 55 (Comprehensive Analytical Chemistry), L. Ramos, Elsevier, 2009. ISBN: 9780444532374
- Static Headspace-Gas Chromatography: Theory and Practice, Second Edition, B. Kolb, L.S. Ettre, Wiley, 2006. ISBN: 9780471749448
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Congress for LC and GC based separations

- International Symposium on the Separation of Proteins, Peptides and Polynucleotides.
- Pittcon Conference & Expo
- International Symposium on High-Performance Liquid Phase Separations and Related Techniques

- International Symposium on Preparative and Industrial Chromatography and Allied Techniques
- Internation Symposium on Pharmaceutical and Biomedical Analysis
- Euroanalysis
- International Symposium on Chromatography
- HPLC conference series
- Bioassays: Scientific Approaches & Regulatory Strategies
- Analytical Technologies Europe: Symposium on the Practical Applications, including CE, LC and MS in the Biopharmaceutical Industry

2.1.3. Supercritical Fluid Chromatography

Principle

SFC is a mixture of GC and HPLC and in some cases is superior to GC or HPLC. Supercritical CO_2, the same matrix used to selectively extract compounds from the products, can be used as a mobile phase in a chromatography system. SFC is similar to HPLC except for the mobile phase, as noted above, is supercritical CO_2. Due to the supercritical fluid, the columns can contain more theoretical plates than conventional HPLC systems. On the other hand, van Deemter curve for SFC is different from both GC and HPLC in that a minimum plate height exists over a very broad range of linear velocities. This technique can be used in many areas like in HPLC, but it is most commonly used in the separation and purification of chiral compounds in the pharma industry.

Congress

- SFC/SFE conference series by green chemistry group.

Chances and pitfalls

- It significantly reduces the amount of hazardous waste that often requires specific disposal methods.
- The advantages of SFC over liquid chromatography (LC) include higher efficiency separations and faster speed of analysis.
- The advantage that SFC has over gas chromatography (GC) is that SFC can efficiently separate thermally labile compounds
- The disadvantage of SFC is that the polarity of the mobile phase is limited but nowadays it is trying to be improved.
- Highly polar solutes are not soluble in supercritical fluids

Applications

- Pharmaceutical
- Environmental
- Natural products

Literature

- Supercritical Fluid Chromatography, Colin F. Poole Ed. Elsevier, 2017, ISBN: 9780128092071
- Advances in Chromatography, E. Grushka, N. Grinberg, 2009, CRC Press, ISBN: 9780429139741

- Modern Supercritical Fluid Chromatography: Carbon Dioxide Containing Mobile Phases, L. M. Miller, J. D. Pinkston, L. T. Taylor, Wiley, 2020, ISBN: 9781118948392
- Practical Supercritical Fluid Chromatography and Extraction, R. Bonnett; C. Caudell2017, CRC Press. ISBN: 9789057024092
- Supercritical Fluid Chromatography: Advances and Applications in Pharmaceutical Analysis, G.K. Webster, CRC Press, 2014. ISBN:9789814463010
- Supercritical Fluid Chromatography with Packed Columns, K. Anton, C. Berger, CRC Press, 1997. ISBN: 9780824700133
- Lesellier, E., West, C., The many faces of packed column supercritical fluid chromatography - A critical review, Journal of Chromatography A, 2015. 1382, pp. 2-46.
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2.1.4. Capillary Electrophoresis and Capillary Electrochromatography

Principle

Capillary electrophoresis is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage. The electrophoretic mobility is dependent upon the charge of the molecule, the viscosity, and the atom's radius. The rate at which the particle moves is directly proportional to the applied electric field--the greater the field strength, the faster the mobility. Neutral species are not affected, only ions move with the electric field. If two ions are the same size, the one with the greater charge will move the fastest. For ions of the same charge, the smaller particle has less friction and an overall faster migration rate. Capillary electrophoresis is used most predominately because it gives faster results and provides high-resolution separation. It is a useful technique because there is a large range of detection methods available.

There are six types of capillary electroseparation available: capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MEKC), capillary electrochromatography (CEC), capillary isoelectric focusing (CIEF), and capillary isotachophoresis (CITP).

Capillary Zone Electrophoresis (CZE), also known as free solution capillary electrophoresis, is the most commonly used technique of the six methods. A mixture in a solution can be separated into its components quickly and easily. The separation is based on the differences in electrophoretic mobility, which is directed proportional to the charge on the molecule, and inversely proportional to the viscosity of the solvent and radius of the atom. The velocity at which the ion moves is directly proportional to the electrophoretic mobility and the magnitude of the electric field.

The fused silica capillaries have silanol groups that become ionized in the buffer. The negatively charged SiO- ions attract positively charged cations, which form two layers—a stationary and diffuse cation layer. In the presence of an applied electric field, the diffuse layer migrates towards the negatively charged cathode creating an electrophoretic flow (\mu_{ep}) that drags bulk solvent along with it. Anions in solution are attracted to the positively charged anode, but get swept to the cathode as well. Cations with the largest charge-to-mass ratios separate first, followed by cations with reduced ratios, neutral species, anions with smaller charge-to-mass ratios, and finally anions with greater ratios. The electroosmotic velocity can be adjusted by altering pH, the viscosity of the solvent, ionic strength, voltage, and the dielectric constant of the buffer.

In Capillary Electrochromatography (CEC), the separation mechanism is a packed column similar to chromatography. The mobile liquid passes over the silica wall and the particles. An electroosmosis flow occurs because of the charges on the stationary surface. CEC is similar to CZE in that they both have a plug-type flow compared to the pumped parabolic flow that increases band broadening.

Congress

- International Symposium on Microscale Separations and Bioanalysis (MSB Series)
- International Symposium on Capillary Chromatography (ISCC)
- International Conference on Miniaturized Systems for Chemistry and Life Sciences.
- CE Pharm 2021: CE in the Biotechnology & Pharmaceutical Industries

Chances and pitfalls

- CE has a flat flow, compared to the pumped parabolic flow of the HPLC. The flat flow results in narrower peaks and better resolution (Figure \PageIndex{4}).
- CE has a greater peak capacity when compared to HPLC—CE uses millions of theoretical plates.
- HPLC is more thoroughly developed and has many mobile and stationary phases that can be implemented.
- HPLC has more complex instrumentation, while CE is simpler for the operator.
- HPLC has such a wide variety of column lengths and packing, whereas CE is limited to thin capillaries.
- Both techniques use similar modes of detection.
- Can be used complementary to one another**.**

Applications

- Pharmaceutical
- Food and beverages
- Environmental
- Petrochemicals
- Polymers
- Gas purity analysis
- Clinical
- Inorganic compounds
- Natural products

Literature

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Separation of the Enantiomers of Chiral Compounds

Principle

Up to now, it is well known that stereochemistry, especially chirality is a field of ever-growing importance in most areas of biology, toxicology, agriculture, clinical, different branches of chemistry, environmental, pharmacology.

Since the enantiomers of chiral compounds may have distinct profiles in terms of pharmacological, pharmacogenetic, pharmacokinetic profiles. Furthermore, absorption, distribution, metabolism, excretion (ADME) properties as well as the toxicity and molecular mechanism of action stages of the drug research and development are affected by chiral molecular recognition. Because of that reason, the scientist should develop effective methods for the separation, recognition, and discrimination of the enantiomers.

Differences between the racemates and the single enantiomers from the viewpoint of therapeutic, pharmacokinetic and especially the toxicological properties demonstrate a strong need for the development of new methods for enantioselective production of the chiral compounds, as well as the enantioselective analytical methods to detect and quantify even minor enantiomeric impurity in the presence of another enantiomer in large excess.

Chiral resolution methods can be divided sub-units such as kinetic resolution (chemical and enzyme-mediated), membrane-based separation and also as an instrumental technique, chiral chromatography included liquid chromatography (LC), supercritical fluid chromatography (SFC), gas chromatography (GC), capillary electrochromatography (CEC), simulated moving bed chromatography (SMBC), etc.

Gas chromatography: GC offers higher peak efficiency when compared with other chromatographic methods. However, the drawback of the method is the applicability of only volatile and thermostable compounds. Chiral GC analysis has been used especially for fragrances, flavours, essential oils, etc. Another improvement for GC based enantiomer separation is “multidimensional GC”. In this technique, one of the two ways can be preferred for the analysis. In one case heart-cutting, 2D-GC and another case comprehensive 2D GC (GCxGC) in which a chiral stationary phase is in the first dimension or the second can be used for the chiral analysis.

High-performance liquid chromatography: HPLC is preferred both for academia and industry for the separation of the enantiomers in the analytical as well as the preparative scale. direct HPLC separation of the enantiomers have some advantages such as this technique allowed to separate nonvolatile and thermolabile compounds, both enantiomers can be obtained pure single enantiomers even if the chiral selector has not had enough purity, it has the flexibility from the viewpoint of adjusting the mobile phase, and it is suitable for the analytical, preparative and industrial scale of separations. There are hundreds of chiral stationary phases (CSP) are available in the literature for the liquid chromatography based enantioseparation techniques the most popular classes are following: Brush type (Pirkle) selectors, cyclofructans and cyclodextrin derivatives, proteins, macrocyclic antibiotics, cinchona alkaloids, synthetic chiral polymers, polysaccharides (cellulose and amylose based).

Supercritical fluid chromatography: From the selectivity point of view, it is more likely to HPLC. On the other hand, it has peak efficiency somewhat like GC. Like in the HPLC, it is possible to work with nonvolatile and thermolabile substances, applicable to the preparative scale. Furthermore, the columns and detectors used in HPLC can be used in SFC.

Capillary electrophoresis: Several benefits come with the CE when comparing with the other techniques. Initially, while a very low amount of chiral selector is added to the system, expensive chiral columns are not required. In contrary to other techniques, no need for long waiting times for the equilibration of the system (switching from one chiral selector to the other is performed by changing the vial). In this technique combination of chiral selectors are easier than the others. It provides very high plate numbers. Both the analytes and the solvents consumed less than the other techniques. It comes with the ease of adjusting the enantioseparation factor. Even a very low thermodynamic selectivity of recognition is available, it can be transformed into a high resolution between the

enantiomers. However, the use of CE is not an easy task, and skilled users needed to perform this technique especially for achieving necessary precision and accuracy. Lastly, CE cannot be used for preparative purposes.

Capillary electrochromatography: This technique bridged between chromatography and electrokinetic migration principles. Three different modes can be performed in this system related to support material such as polymeric materials, silica particles, etc.: CEC with open tubular (OT) capillary, CEC with packed (p) capillary, and CEC with monolithic capillary. Like all discussed methodologies variations of the CSP's have been employed for the separation of the enantiomers in CEC: CD derivatives, polysaccharide derivatives, and quinine derivatives are the most preferred chiral selectors. CEC needs highly qualified users more than the CE.

Applications

- biology, toxicology, agriculture, clinical, different branches of chemistry, environmental, pharmacology, etc.

Literature

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2.2 Spectrometry

2.2.1 Mass Spectrometry

Bioanalytical mass spectrometry (MS) is based on the principle of the separation and counting of ions of ions generated from biomolecules in an ionization source. Ions are usually produced by the principle of electrospray, separated in a mass analyzer by their mass-to-charge ratio (m/z), and detected and counted by their respective m/z and abundance. MS is known for its good selectivity, sensitivity, the possibility of de novo identification of molecules at very low level on the basis of the accurate mass of the parent ion and the generated fragments. MS is a kay technique for the large-scale analysis -omics: proteomics and metabolomics (Section 4.1)

Mass spectrometers are classed according to mass resolution (the capacity to separate two ions with similar mass) and mass accuracy (the difference between the real and measured mass). The most popular mass analyzers include quadrupole (resolution 3000), time of flight (resolution 30,000), Orbitrap (300,000) and FT Ion Cyclotron Resonance (FTICR) (3,000,000). Mass accuracy is another characteristics of HR MS. It varies from a single ppm level (TOF, Orbitrap) to 0.1 ppm level for FT ICR.

Mass spectrometers in bioanalysis are often use in a tandem mode, separated by a collision/reaction cell where the parent ions isolated by the first mass analyzer are fragmented by collision induced dissociation (CID) and fragments are separated and detected by the second mass analyzer. The typical configurations include: triple quadrupole (QqQ) MS, QqTOF MS, Q-Orbitrap MS and Q- FT ICR MS.

Bioanalytical mass spectrometry is seldom used as a standalone technique (it is sometimes the case of FT ICR) but coupled with gas or liquid chromatography resulted in hyphenated techniques (section xx). Advancements are underway for in-situ mass spectrometric characterization in microfluidic devices and sensitive methods of miniature probe sampling of biological systems, such as single-cell metabolomics.

Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI MSI) is performed by recording MS spectra in thin layers of biological and clinical specimens point by point over a large area

on the sample surface and reconstructing artificial images correlating the relative intensities of one or a set of molecules with the original positions of the sampling.

Advanced LC-MS/MS approaches are fundamental or the comprehensive characterizations of proteomes, lipidomes, and metabolomes for a variety of biological samples.

Literature

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2.3 Spectroscopy

2.3.1 Colorimetric

Colorimetric sensors are an important part of optical sensors that demonstrate distinguishable color change (400–800 nm range) upon reaction with the analyte. These sensors can provide both qualitative and quantitative information. The quantification of the analyte is possible by the visual comparison of the sensor with a color gradient scale/color wheel in an instrument-free analysis. For a more precise analysis, UV-Vis spectrophotometers can be used and the concentration of the analyte may be calculated using the Beer-Lambert law. A digital image of the optical sensor can also be taken and analyzed with the help of a special application.

Colorimetric sensors are based on a concept of associative/dissociative interactions, which take place between different molecules resulting in the emergence of an optical signal. Chemical interactions involved in the functionality of chemical sensors should be selective but reversible, so that the sensor can be regenerated and reused. These interactions include metal coordination via ion-dipole interactions (for alkaline metal ions) and covalent bond formation (for transition metal complexes) as well as a range of supramolecular interactions (ion-ion interactions, hydrogen and halogen bonding, hydrophobic interactions, π-π and H-π interactions, and cation-π and anion-π interactions). There are two main approaches to the design of colorimetric chemical sensors: sensing based on direct binding of an analyte and sensing based on the competitive binding of an analyte (displacement assays).

Colorimetric arrays (available in solid or liquid form) use multiple sensors to provide a colorimetric signature (a pattern) of a molecule for unique substance identification. Aditionally, multiple analytes can be interrogated in a single test.

In recent years, extensive research has been performed on the use of metal nanoparticles as colorimetric sensors for the selective and sensitive recognition of molecules. The mechanisms involved are based on aggregation of NPs, decomposition of NPs, catalysis of nanozymes and ligand-receptor interaction.

Limited selectivity and sensitivity are the major drawbacks of colorimetric sensors, as it is difficult to determine individual components of a mixture, which significantly limits their applications.

Colorimetric sensors are mainly used for environmental safety applications to measure the concentration of inorganic species, such as heavy metals (arsenic, mercury, zinc, nickel, cadmium etc.), but they can also be used to test drugs, biomolecules or pesticides. They are appreciated for being fast, economical and simple to use.

Literature

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2.3.2 Raman

Raman spectroscopy is the study of **inelastic light scattering** of photons by molecules, providing an insight into their chemical structure (in particular their vibrational structure). It can be used to rapidly provide chemical and/or structural information of a sample, whether solid, liquid, gas, gel, slurry or powder.

Raman analysis provides numerous **advantages**, such as: testing of both organic and inorganic materials in aqueous or non-aqueous media, high specificity (fingerprint-type spectra), short analysis times and low sample requirements (both liquid or solid, < 1 µm in diameter). Moreover, it is non-destructive and allows multiplex detection. Usually, no sample preparation or pretreatment is needed and samples can be analyzed directly or through a glass or a polymer packaging. Additionally, laser light and Raman scattered light can be transmitted by optical fibers over long distances for remote analysis and a relatively wide spectral region (4000 cm^{-1} to 50 cm^{-1}) can be covered by a single recording.

Raman analysis can quickly and easily provide key spectroscopic information regarding qualitative or quantitative data. Even so, the Raman effect is very weak (i.e. there is an inelastic scattering for every ten million elastically scattered photons), which leads to **low sensitivity** (the major disadvantage of this method), making it difficult to measure low concentrations of a substance. Another drawback is represented by the **fluorescence** of impurities or of the sample itself which can cover the Raman spectrum, as some compounds fluoresce when irradiated by the laser beam. Furthermore, sample **heating** through the intense laser radiation may destroy the sample or cover the Raman spectrum, thus minimal laser power should be used to avoid this issue.

Although the acquired spectra are very rich in structural information and extensive spectral databases are available, Raman analysis is more suitable for applications where analytes are major components of the sample (e.g. study of pharmaceuticals, raw materials, excipients, etc. – from drug discovery, drug development to quality control) and less/not suitable for trace analysis (clinical analysis, food and environmental safety).

2.3.3 SERS

In order to benefit from the Raman advantages and exceed the low-sensitivity impediment, surface-enhanced Raman scattering (SERS) can be considered. The SERS effect denotes a strong increase (by factors up to 10^8 or even larger, enabling single-molecule SERS in some cases) in the Raman signal of a molecule with the help of a special substrate, typically represented by metallic nanostructures.

The enhancement of the Raman signal by the metal substrate is explained mainly by an **electromagnetic mechanism** cumulated or not with a **chemical** one. At a certain resonant frequency, the interaction of electromagnetic radiation with metal nanostructures leads to collective oscillations of the conduction electrons. One of the main consequences of this resonant plasmon excitation is the strong enhancement of the electromagnetic near fields at the metal surface. It is this resonantly enhanced near field that is the main contributor to the Raman signal enhancement in SERS. Since near fields decay exponentially away from the surface, it has been established that it is critical for the molecule to be **in close proximity of the metallic surface** (furthermost 10 nm) to increase the Raman signal. The enhancement is maximized for molecules in direct contact with the surface, and it decreases with the increase in distance between the substrate and the analyte. By matching the surface plasmon resonances (localized or propagative) to the excitation laser, which can be done by material, size, and shape adjustments, the SERS efficiency of a substrate can be maximized at the desired wavelength

The SERS enhancement based on the electromagnetic mechanism applies to all molecules and leads to enhancement factors (EF) as high as 10^6–10^8. The chemical mechanism, on the other hand, is based on charge transfer interactions that take place between molecules and the metal surface. This requires the molecule to be chemically adsorbed on the surface of the metal substrate, making this mechanism analyte-dependent and site-specific. Molecules can be adsorbed on the surface either through physisorption (van der Waals forces) or chemisorption (chemical bonds such as covalent or electrostatic interactions).

As it provides all the advantages of conventional Raman analysis, but with an outstanding boost in sensitivity, SERS is a **highly appreciated tool for bioanalytics**. Since this is a complex and versatile technique, some considerations have to be taken into account in the method selection process and experimental planning.

The SERS spectra of an analyte is comparable with its normal Raman spectra. While mostly they look similar, there are often differences in the number of vibrational modes present. Some bands not found in the conventional Raman spectrum may be present in the SERS spectrum, while other modes may disappear. This can be explained by different molecular orientation profiles or interaction modes with the substrate, by contaminants from the metallic substrate and by different selection rules. Moreover, SERS signals acquired from analytes at low concentrations can fluctuate due to changes in SERS substrate conditions, adsorption-desorption of analyte and/or photobleaching. Hence, a **database** with SERS spectra is hard to create.

For identification and quantification, **univariate** regression models which rely on a specific, identifiable unique peak of the target analyte are most commonly used. A simple calibration curve can be generated by plotting the intensity or area of the Raman band against the analyte's concentration. This model is challenged when real samples with complex matrix effect are analyzed. Therefore, more advances chemometric approaches (e.g. multivariate regression analysis) are needed and use the whole SERS spectra rather than specific bands for quantification.

For maximum signal amplification and thus low limits of detection, both an **efficient substrate** and a **high affinity** toward the analyte are needed. **SERS substrates** are typically represented by noble metal nanostructures, like **Au, Ag, or Cu**. They come in a large variety of structures included in multiple

categories such as liquid-phase metallic colloids and their aggregates/assemblies, and solid substrates consisting of nanoparticle films/sheets, or patterned surfaces fabricated by electrochemical techniques or different variants of nano-lithography. SERS substrates are commercially available or they can be synthesized in the lab. In the second scenario, one must choose the most suitable substrate for a certain bioanalytical application and be aware of the inter-lab reproducibility problems that almost always occur due to differences in water quality, impurities in substances used (different suppliers or different batches), temperature, etc. Nanostructures for sensor fabrication must be prepared in a way that ensures signal reproducibility. Also, the nanoparticles must be stable against aggregation and, in the case of *in vivo* applications, they ought to be biocompatible.

Adsorption of a particular molecule on the metallic SERS-active substrate is guided by its size, charge, water solubility, etc. For example, molecules that contain **thiol** functionalities are efficiently bound on gold surfaces, making them easy to detect, while hydrophobic molecules (e.g. persistent organic pollutants) do not have affinity for noble metals and various strategies to bring them close to the surface are needed, making the detection process more challenging.

Apart from efficient substrates and high affinity towards the analytes, the **intrinsic Raman properties** (cross-section) of the targeted molecule is also important for trace analysis, since not all molecules are good SERS probes. However, detecting weak SERS-active species may be overcome by their labelling or other chemical modifications.

SERS analysis of **complex samples** including environmental (natural or waste waters) or biological (serum, plasma, urine, tears), would most probably require some form of sample processing, in order to control as much as possible the matrix effect. These samples may require sedimentation and/or filtration prior to analysis to ensure the separation of their larger constituents that may provide vibrational interference and fluorescence contamination. Moreover, solid samples (food, soil, etc.) would require an extraction step prior to analysis.

Improper control of reproducibility is currently the main **disadvantage** of SERS analysis. It is strongly dependent on the quality/uniformity of the SERS substrates and the experimental conditions – nanometric changes in molecular position, orientation, and nanoparticle geometry should result in very large changes of the scattering response. Consequently, the results obtained by SERS alone should be validated by another analytical technique. Additional improvements in reproducibility may be achieved by collecting spectral data from multiple points from a larger area of the solid SERS substrate instead of a single laser spot.

Applications – detection, identification and/or quantification of a variety of analytes:

- **pharmaceuticals** and **drugs** from pharmaceutical formulations or body fluids
- **pollutants**
- food **contaminants** and **additives**
- bacteria (Bacillus anthracis), viruses or yeasts for **biomedical** or **forensic** purposes
- nucleic acids
- proteins
- lipids
- intracellular investigation of cells
- biomarkers (glucose sensing), etc.

Literature

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2.3.4 Fluorescence

Fluorescence measurements allow very low LODs. Fluorescence excitation wavelengths should be at higher wavelengths to avoid photodegradation and scattering. Some biomolecules show self-fluorescence (bioluminescence). Fluorescent markers are preferable which have a large Stokes shift. Mostly the fluorescence intensity is measured using the concentration intensity relationship. Wavelength-ratiometric probes provide a quantitative determination of many analytes. However, The fluorescence lifetime results in an absolute measure independent of concentration in contrast to steady state intensity, which is relative (photobleaching). It allows getting dynamic information on changes in the nano environment by viscosity, pH, polarity, or solvation and molecular interactions.

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- Fluorescence Spectroscopy, New Methods and Applications, Otto S. Wolfbeis (Ed.) (1993), Springer-Verlag, Heidelberg
- J. Zhou et al. (2019) Two-component ratiometric sensor for Cu2+ detection on paper-based device, Anal. Bioanal. Chem. 412

2.3.5 Direct optical methods

Measurement of the product of refractive index times the physical thickness of a layer

- Refractometric
  surface plasmon resonance, grating coupler, Mach-Zehnder, prism coupler.
- Reflectometric
  ellipsometry, reflectometric interference spectroscopy

- Details in Handbook of Spectroscopy (Editors: G. Gauglitz, D.S. Moore), 2nd Completely Revised and Enlarged Edition, Vol. 3, “Direct Optical Detection in Bioanalytics” (G. Gauglitz, N.J. Goddard), Wiley-VCH 2014

2.4 Mass sensitive methods

The gravimetric chemical sensors respond to modifications of the ambient chemical composition through changes in their mass.

- Chemical and Biochemical Sensors, 1. Fundamentals, Nicolae Barsan, Günter Gauglitz, Alexandru Oprea. Edwin Ostertag, Günther Proll, Karsten Rebner, Klaus Schierbaum, Frank Schleifenbaum, Udo Weimar, Ullmann's Encyclopedia of Industrial Chemistry, Chapter 2.5, Wiley online Library, https://doi.org/10.1002/14356007.b06_121.pub2

2.4.1 QMB sensors

The first piezoelectric devices were produced as small wafers from piezoelectric single crystals, provided with a pair of electrodes on opposite faces. The mechanical oscillation type and the resonance frequency depend on the thickness and cut direction of the wafer (Sauerbrey equation). Viscosity has to be considered especially in bioanalytics.

2.4.2 SAW sensors

Surface acoustic wave sensors are piezoelectric substrates that produce from an electrically modulated signal a mechanical (acoustic) wave that is transformed back into an electrical signal. Changes at the sensor surface or in the volume influence amplitude, phase, frequency, or time-delay between the input and output electrical signals and correlate to sensing effects.

2.4.3 Cantilevers based sensors

Cantilevers are gravimetric devices as a beam or a plate able to elastically bend under mechanical stress which can be optically detected. Its modulation of vibrational frequency correlates to sensing.

2.5 Thermal Conductivity and Calorimetry

The operation principle of a thermal conductivity sensor uses two metal coils, typically made of platinum, or thermistors of small sizes in two different compartments called sampling and reference tubes. The coils or thermistors are arranged in a Wheatstone bridge circuit and are self-heated by passing an electric current through them. Their resistances depend on the adjusted temperature, which is given by the temperature-coefficient of the wire or thermistor material. In contrast to thermal conductivity sensors, in calorimetric sensors chemical reactions with the analyte come into play. The by far most important category of calorimetric sensors is the catalytic bead sensor, also called pellistor.

- Chemical and Biochemical Sensors, 1. Fundamentals, Nicolae Barsan, Günter Gauglitz, Alexandru Oprea. Edwin Ostertag, Günther Proll, Karsten Rebner, Klaus Schierbaum, Frank Schleifenbaum, Udo Weimar, Ullmann's Encyclopedia of Industrial Chemistry, Chapter 2.4, Wiley online Library, https://doi.org/10.1002/14356007.b06_121.pub2

2.6 Electrochemistry

2.6.1 Potentiometric methods

Potentiometric sensors are electrochemical cells operating in open circuit configuration, whose potential is modulated by chemical information. The detected analyte has to be electrically charged. The sensor readout is a voltage measuring device with high input impedance (electrometer or suitable integrated circuits). They follow the Nernst equation. There exist Potentiometric enzyme sensors, Solid State Potentiometric Sensors.

2.6.2 Amperometric and Voltammetric methods

The amperometric measurements are in principle current measurements made at a given value of the potential. The voltammetric measurements use single or multiple voltage sequences to generate a time dependent current in the electrochemical cell. Conductometric and Impedimetric
Electrolytes are ionic conductors and, in the case of some solid state materials, also electronic conductors. When a voltage below the range over which electrochemical processes occur is applied between two electrodes immersed in the electrolyte, a current starts to flow, usually proportional to voltage; the proportionality constant is the sample conductance (Ohm's law). The specific conductance (conductivity) is the product of ion mobility, concentration, and charge. Impedance spectroscopy (EIS) investigates the interface at the electrode. EIS uses the frequency sweep of a small a.c. voltage modulated over a d.c. bias to extract the equivalent circuit configuration. The d.c. bias sets the electrochemical properties of the interfaces. The circuit configuration is obtained from the plots of the imaginary and real part of the impedance (Cole–Cole or Nyquist diagram). The apparatus size and

complicated data interpretation hinder the use of EIS operation principle for real sensors, but laboratory setups are utilized for sensing purposes.

- Electrochemical Sensors in Bioanalysis, R.I. Stefan, J.F. van Staden and H.Y. Aboul-Enein, Marcel Dekker Inc., New York, USA, 2001.
- Chemical and Biochemical Sensors, 1. Fundamentals, Nicolae Barsan, Günter Gauglitz, Alexandru Oprea. Edwin Ostertag, Günther Proll, Karsten Rebner, Klaus Schierbaum, Frank Schleifenbaum, Udo Weimar, Ullmann's Encyclopedia of Industrial Chemistry, Chapter 2.1, Wiley online Library, https://doi.org/10.1002/14356007.b06_121.pub2

2.7 Hyphenated techniques

Hyphenated techniques combine chromatography, and spectroscopy or mass spectrometry to exploit the advantages of both. Chromatography produces pure or nearly pure fractions of chemical components in a mixture. Spectroscopy (spectrometry) produces selective information for identification using standards or library spectra. The hyphenated technique is developed by the physical coupling of both techniques.

Hyphenated techniques have found numerous applications in bioanalysis. Techniques such as, e.g., GC-MS, LC-MS, LC-FTIR, LC-NMR, CE-MS, etc. have been massively used for the crude extracts or fractions from various natural sources, isolation and on-line detection of natural products, chemotaxonomic studies, chemical fingerprinting, quality control of herbal products, and metabolomic studies.

2.7.1. LC-MS

2.7.3. LC-MS/MS

2.7.4. GC-MS

Literature:

- Patel, K. N., Patel, J. K., Patel, M. P., Rajput, G. C., & Patel, H. A. (2010). Introduction to hyphenated techniques and their applications in pharmacy. Pharmaceutical methods, 1(1), 2–13. https://doi.org/10.4103/2229-4708.72222

2.8 Biosensors

2.8.1 Principle

Biosensors are devices capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element that is in contact with a transduction element. Such a biosensor contains:

- a transduction element (electrochemical, optical, thermos, micro-balanced, surface-acoustic wave …). It transfers an effect offered by a biomolecular interaction process to an electronic signal;
- recognition elements (realizing biomolecular interaction processes between antigene/antibodies, analyte/biomimetics, proteins/proteins, peptide libraries), is responsible for selectivity; forming the sensitive layer, which inserts an effect to the transduction element;
- a shielding layer to suppress or avoid nonspecific interaction (especially in complex matrices such as blood or milk) and acting as an anchor layer for the recognition elements
- electronic read-out and data processing unit

There exist some definitions of a sensor, the most appropriate is considered the Cambridge definition: "Chemical sensors are miniaturized devices which can deliver real-time and online information on the presence of specific compounds or ions in even complex samples" (K. Cammann, E. A. Guibault, H. Hall H, R. Kellner, O.S. Wolfbeis, The Cambridge Definition of Chemical Sensors, In: Proceedings of the Cambridge Workshop on Chemical Sensors and Biosensors (1996) Cambridge University Press, New York),

However, a biosensor might have problems being real-time (reversible) and be used online. Therefore Thevenot modified it for electrochemical biosensors. Real-time is possible, but reversible has to be substituted by regeneration. Further information Nagl/Wolfbeis.

- D. R. THEÂVENOT et al. Electrochemical Biosensors: Recommended Definitions and Classification (Technical Report), Pure Appl. Chem., Vol. 71, No. 12, pp. 2333-2348, 1999
- D. Thevenot, K.Toth, R. Durst, G. Wilson. Electrochemical biosensors: recommended definitions and classification. Biosensors and Bioelectronics, Elsevier, 2001, 16, pp.121 - 131.
- S. Nagl, O. S. Wolfbeis, Classification of Chemical Sensors and Biosensors Based on Fluorescence and Phosphorescence, The final version of this book chapter was published in 2008 in Standardization and Quality Assurance in Fluorescence Measurements I, Springer Series on Fluorescence Volume 5, 2008, pp 325-346 and is available online at http://link.springer.com/chapter/10.1007%2F4243_2008_022

2.8.2 Biosensor Literature

A large number of reviews on principles, transduction methods, recognition elements, read-out possibilities and data evaluation are available

- Biosensing for the 21st century (Series Editor: T. Scheper; Volume Editors: R. Rennebert, F. Lisdat; Advances in Chemical Engineering/Biotechnologies 109; Springer Heidelberg, 2008
- Biosensors for Environmental Monitoring (Editors: U. Bilitewski, A.P.F. Turner); Harwood academic publishers, 2000
- Biosensors and Biodetection Methods and Protocols (Editors: A. Rasooly, K.E. Herold), Methods in Molecular Biology 503; Springer Protocols; Methods and Protocols, Vol. 1, Optical-Based Detectors, Humana Press 2017
- Handbook of Biophotonics (Editors: J. Popp, V.V. Tuchin, A. Chiou, S.H. Heinemann), Vol. 3: Photonics in Pharmaceutics, Bioanalysis and Environmental Research; Wiley-VCH 2012
- Handbook of Spectroscopy (Editors: G. Gauglitz, D.S. Moore), 2nd Completely Revised and Enlarged Edition, Vol. 3, "Direct Optical Detection in Bioanalytics" (G. Gauglitz, N.J. Goddard), Wiley-VCH 2014
- Handbook of Spectroscopy (Editors: G. Gauglitz, D.S. Moore), 2nd Completely Revised and Enlarged Edition, Vol. 3, "Immunoassays" (G. Proll, M. Ehni), Wiley-VCH 2014

2.8.3 Congresses

- Biosensors; international congress, every other year on a different continent in a different country;
- European Biosensor Symposium; every other year in a different European country;
- Workshop on Biosensors & Bioanalytical Microtechniques in Environment, Food & Clinical Analysis; every other year in different countries;

2.8.4 Chances and pitfalls

Experienced handling of detection methods, recognition elements, and evaluation of data is essential to avoid the following pitfalls

- Avoiding nonspecific interaction;
- Suitable detection element
- selecting the best assay type (direct, competitive, binding inhibition, Sandwich);
- determination of limits of detection and limits of quantification because of sigmoid calibration curve;
- evaluation of binding curves (equilibrium, association and dissociation rate constants)
  - G. Gauglitz, (2018) Analytical evaluation of sensor measurements, Anal. Bioanal. Chem. 410, 5-13

2.8.5 Applications

Biosensors are used for many analytical problems, e.g. trace analysis in case of environmental problems, diagnostics, and biomolecular interaction analysis

- imaging of DNA strands;
- protein/protein interactions;
- peptide libraries for biomarker identification
- point-of-care diagnostics
- high throughput screening
- water quality

2.9 Stochastic sensors

2.9.1 Principle

Stochastic sensors are new tools developed for bioanalysis, based on a very simple principle: channel conductivity. When a molecule is entering the channel, it blocked it, and the current is getting to zero value until the molecule is entering the channel (this time is called toff and is used for qualitative analysis), the time spent in the channel is called ton and it is used the quantitative measurements. Such a sensor contains:

- a membrane/active site of the sensor containing channels or pores.

2.9.2 Literature

- Pattern recognition of 8-hydroxy-2’-deoxyguanosine in biological fluids, Anal Bioanal Chem, RI Stefan-van Staden et al., January 2018, Volume 410, Issue 1, pp 115–121
- Engineering of protein nanopores for sequencing, chemical or protein sensing and disease diagnosis, S Wang et al., Current Opinion in Biotechnology, Volume 51, June 2018, Pages 80-89

- Stochastic sensors inspired by biology, H. Bayley & P.S. Cremer, Nature, 413, pages 226–230 (13 September 2001).
- Stochastic sensors, J. Schmidt, Journal of Materials Chemistry, 2005,15, 831-840.
- Stochastic nanopore sensors for the detection of terrorist agents: Current status and challenges, A. Liu et al., Anal.Chim.Acta, 675, Issue 2, 24 August 2010, Pages 106-115

2.9.3 Chances and pitfalls

- Reliable qualitative and quantitative analyses can be performed
- Suitable for multianalyte detection
- High selectivity
- Capable to perform analysis from very complex matrices because both quantitative and qualitative analysis is not influenced by the nature and complexity of the matrix from where the analytes are determined;

2.9.4 Applications

Stochastic sensors are used for trace and ultra trace analysis in:

- Environmental analysis;
- Clinical analysis, including point-of-care diagnostics
- High throughput screening
- Pharmaceutical analysis
- Food analysis

3 Type of molecules to be determined

- Proteins
- Peptides
- Amino acids
- DNA/RNA
- Oligonucleotides
- Carbohydrates
- Lipids
-

4 Special Applications

✓ **Screening of biological samples for early diagnosis**

✓ **-omics**

A number of fields referred to with a term -omics have appeared to study biological molecular machinery at a systems level. They include genomics, proteomics, lipidomics, and others.

✓ **Metabolomics**

The terms “metabolomics” refers to a comprehensive analysis of metabolites in a cell, tissue or organism and its studies in a wide range of conditions, including health and disease. The analysis for metabolomes is currenly achieved by nuclear magnetic resonance (NMR) and mass spectrometry (MS).

The major strengths of NMR are precise quantitation and superior compound identification but the technique suffers from a relatively low sensitivity (metabolites must exceed 1µM). In contrast to that, MS, coupled to either gas chromatography (GC) or liquid chromatography (LC), can routinely detect hundreds of compounds in the femtomolar to attomolar range, in a single sample and run. Advancements of MS have been accompanied by those of databases/libraries, having enabled metabolomics to emerge as its own field of study, and to complement genomics and proteomics.

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2. Biosensors and Bioelectronics
3. Bioanalysis
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6. Journal of Bioanalysis & Biomedicine
7. Journal of Pharmaceutical and Biomedical Analysis
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9. Journal of Analytical Toxicology
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