

Nanostructures in Flow Analysis

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Abstract

Review of current trends in miniaturization of instrumentation for flow analysis based on 84 literature references is presented, including design and applications of microfluidics, and use of nanostructures for detection purposes and design of nanofluidics.

Keywords flow analysis, miniaturization, microfluidics, nanostructures, nanofluidics

1. Introduction

Progress in science and technology obviously affects evolution and improvement of all devices for personal use and needed for everyday life, as well as highly specialized devices and instruments, including instrumentation for chemical analysis. The progress in this area has a driving force in increasing demand of modern society for quicker and better chemical characterization of materials. This increasing demand for analytical determinations results from necessity to analyze increasing number of various samples, as well as from the need of design such analytical instruments and methods, which can be employed directly by end-user without need of use of service of specialized analytical laboratories. This increasing demand includes also necessity of improvement of quality of analytical determinations. Depending of the area of application it may be need to shorten time of analysis, minimization of amount of sample needed for analysis, need to achieve a lower limits of detection or better selectivity (resolution) in multicomponent determinations, or obtaining better precision and/or accuracy of determination.

One of the directions in the progress of analytical instrumentation is taking place already more than 60 years development of flow analysis and dedicated to such measurements instrumentation, including its miniaturization employing progress in material science, micromachining, electronics and informatics.

2. Microsystems in flow analysis

First laboratory systems for flow analysis, based on concept of using air-segmentation of flowing stream in order to limit dispersion of sample components in the system, have revolutionized clinical analytical laboratories in the beginning of 1960-ties, and later were also introduced to environmental, agriculture and industrial laboratories. Their evolution during resulted in both laboratory-made and commercially available large analyzers, of which a good example can be computer controlled fully mechanized multicomponent clinical analyzer SMACX from Technicon®, performing for the same sample determination of several tens of assays [1,2]. It has be also mentioned, however progressing integration of multimodule analyzers, or even design of compact submersible capsule for underwater environmental monitoring. Further steps in miniaturization of measuring devices for flow analysis concern

instrumentation for flow-injection analysis (FIA), for instance design of integrated microconduits with miniature detectors and large part of hydraulic part for transport of solutions [3,4]. This trend includes replacement of typical peristaltic pumps with much smaller syringe or piezoelectric one [5], or incorporation of various parts of systems (flow-through reactors, detectors *e.g.* [6]) into a rotary valve for sample injection, which was named lab-on-valve [7]. See examples of such devices in Fig.1. All these mentioned miniaturized FIA systems have dimensions counted in centimeters and diameters of channels being fraction of millimeters.

A particularly significant step in miniaturization of instrumentation for flow analysis was in beginning of 1990-ties development of first flow microsystems described as microfluidics [8,9]. The diameters of channels were scaled down to tens of micrometer, and therefore, the same systems can be very successfully used also for capillary electrophoresis, and to give same examples, employed for determination of amino acids with fluorescence detection in meteorite samples [10], or measuring activity of enzymes for clinical and pharmaceutical needs [11]. Such microfluidics are usually fabricated in format of thin plate of with and length in few centimeters, with pattern of microchannels, the possibility of performing various different microunit operations (see Fig.2), and sometimes with miniature flow detector. For FIA measurements they were employed with various methods of detection, *e.g.* with amperometric one for immunochemical assays [12], with potentiometric for determination of copper [13], fluorimetric for determination of pH [14], employing chemiluminescence detection for enzymatic determination of glucose [15], and spectroelectrochemical with thermal lens spectroscopy for determination of o-tolidine [16], or Co(II) [8], where different microunit operations can be combined in a dedicated microsystem (Fig.3). The microfluidic system was also designed *e.g.* for sample processing in flow conditions for MALDI mass spectroscopy [17] (Fig.4), and for carrying both FIA and capillary electrophoretic determinations with amperometric detection [18]. In the latter one (Fig.5) the protocol of analysis involves repetitive rapid flow injection (screening) assays to provide a timely warning and alarm, and switching to the electrophoretic separation when harmful analytes are detected.

Various configurations of microfluidic devices are already commercially available from several manufacturers (*e.g.* [19]), however to date, they are built on a highly specialized way to a particular applications. So, generally the require to be interface

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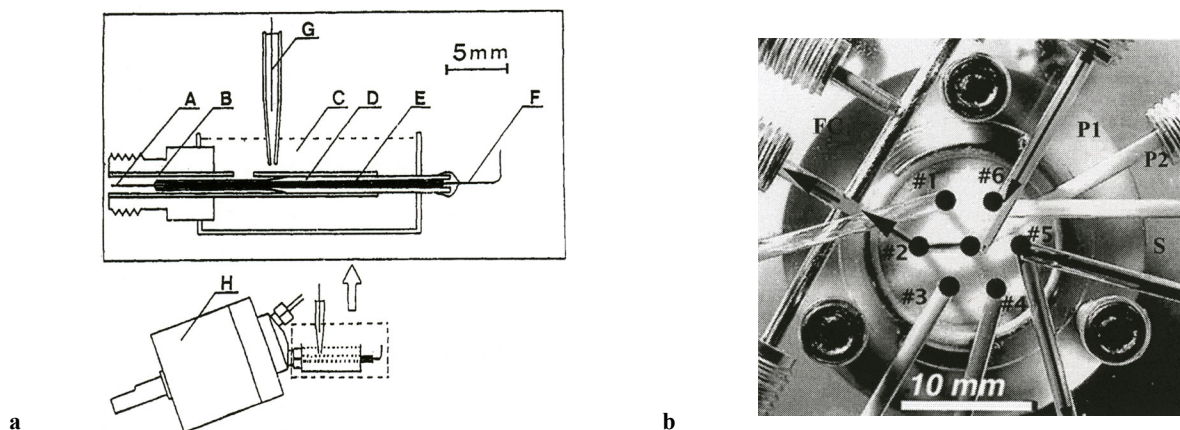


Fig. 1. The injection valves for FIA systems with incorporated different elements: a – valve with carbon fiber electrochemical detector [6]: A-carbon fiber working electrode, B-Teflon tubing, C-electrolyte solution, D-glass pipet, E-mercury, F-metal contact, G-reference electrode, H-injection valve; b – lab-on-valve design with incorporated flow cell (FC) using two optical fibers facing each other (1.5 mm light path) [7].

with the required control electronic, reagent supply, detectors, programming, and they are designed to perform only a limited set of operations, such as liquid transport, separation, or sensing. Further developments of microfluidics towards a Micro Total Analytical Systems require the definition of architectural and performance concepts for assembling microfluidics devices into networks. This requires a hierarchical integrated microfluidic design approach, which was described to facilitate scalable designs for many different applications [20], and a very intensive work in this area is in progress. In being developed fully integrated Lab-on-a-chip devices also sensors and detectors are combined with microfluidic channels, pumps, mixers, separators and valves, to provide much better functionality. The example of such a monolithic integrated flow injection systems that implements Berthelot's reaction for determination of ammonia is shown in Fig.6 [21]. The complete system contains four piezoelectrically driven micropumps, reaction chamber, fluidic channels and optical detection cell are integrated on a 10-cm diameter silicon plate.

As a particular microscale approach in this field of miniaturization, the concept of digital microfluidics can be also mentioned that arose in the late 1990s and involves the manipulation of discrete volumes of liquids on a surface [22] (see schematic illustrations of its principle in Fig.7). Manipulation of droplets can occur through electrowetting, dielectrophoresis, thermocapillary transport, and surface acoustic transport. In contrary to continuous-flow microfluidics discussed above, digital microfluidic architecture is under software-driven electronic control, eliminating the need of mechanical tubes, pumps, and valves.

3. Nanotechnology in chemical analysis

It is difficult to indicate one particular discovery in history of science, which has initiated at the end of 20th century very rapid development of nanotechnology. Equally difficult is to define such terms as nanoscience or nanotechnology. Very generally nanoscience and nanotechnology refer to the world as it works on the nanometer scale, from one nanometer to several hundred

nanometers [23]. In another attempt one can find interpretation that nanoscience is a discipline dealing with objects and systems, in which at least one dimension is of 1-100 nm magnitude [24]. And one more definition of nanotechnology, very general but it seems to be very convincing, that it is creation and utilization of materials, devices and systems by the control of matter on the nanometer scale [25].

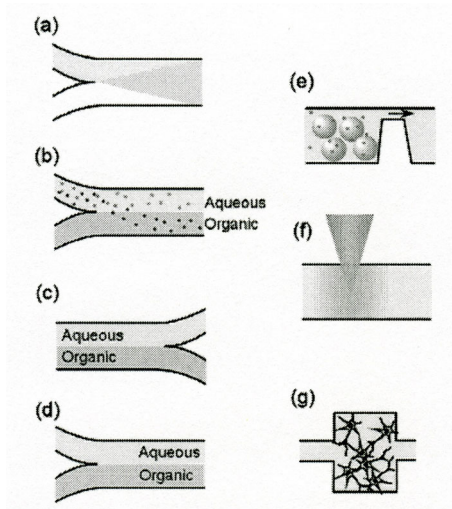


Fig. 2. Schematic illustration of performing various microfluidic operations in microfluidics [8]: a-mixing and reaction, b-solvent extraction, c-phase separation, d-two-phase formation, e-solid-phase extraction, f-heating, g-cell culture.

Very often in the literature as the beginning of nanotechnology era is indicated the famous lecture of Nobel laureate in physics R. Feynman in 1959 in California Institute of Technology, entitled "There is plenty of room at the bottom". Based on examples of natural biological systems in nanometer scale, and extrapolation of known laws of physics, a vision of artificial fabrication of

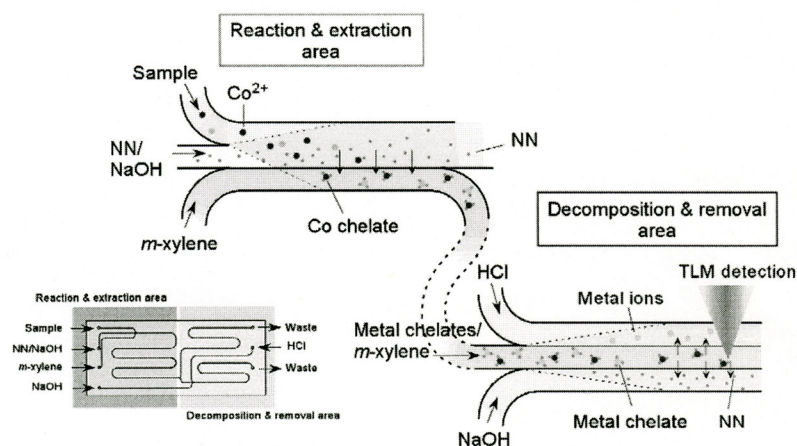


Fig. 3. Schematic illustration of combining different micronit operations in microfluidics for determination of Co(II) with thermal lens detection [8].

such objects in nanoscale was given. Such various objects, designed and investigated in recent 20 years, exhibit interesting physical properties based on quantum phenomena, and unusual optical and electromagnetic properties of material different than larger objects of the same material. Source of inspiration in nanotechnology is nature, and in particular biology. Cell as basic entity of life contains numerous sophisticated nanomachines [24]. One of the most common natural nanostructures of 3-9 nm thickness are bilayer lipid membranes (BLM), already utilized for analytical purposes [26]. Their structure in blood cells was discovered in 1920-ties, and since 1960-ties they are fabricated artificially and employed for various purposes [27].

A fundamental feature of nanostructures is a self-assembling in particular, favorable conditions. So, they include for instance self-assembled monolayers (SAM) formed by thiols on metallic surfaces [24], which are commonly employed analytically. Both

BLMs and SAMs are widely used in electroanalysis in design of chemical and biochemical sensors.

A marked impetus in development of nanotechnology was given by discovery in 1991 of carbon nanotubes (CNTs) [28]. The graphene sheets of hexagonally formed carbon atoms are rolled in single or multi-wall tubes of internal diameters from several to few tens nanometers, and length even up to several ten micrometers. This discover has opened new chapter in nanotechnology and eruption on very different applications due to their unusual properties. From synthetic point of view, for instance, the method was reported how in controlled system one can regulate the growth of length of single wall CNTs [29], or how to obtain a multibranching CNTs [30]. Carbon nanotubes can be self-assembled from various organic compounds, e.g. porphyrins [31].

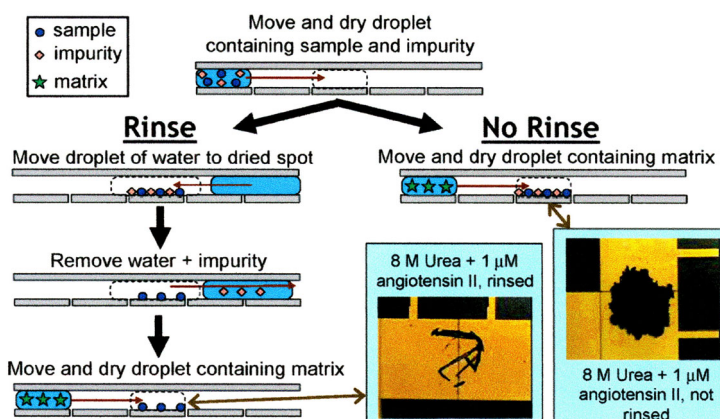


Fig. 4. Schematic of functioning of the electrowetting-on-dielectric driven sample purification in digital microfluidic system for MALDI-mass spectrometry for proteomics analyses and micrographs of dried spots [17]. The rinsed sample is primarily composed of the MALDI matrix 2,5-dihydroxybenzoic acid (DHB) crystals, while the not-rinsed sample (right) is dominated by urea.

The range of different applications of nanotechnology is rapidly growing in recent years. In chemistry it can be, for instance, proteomics [32], or synthesis and separation of chiral compounds [33]. Among carbon nanostructures the largest number of applications has been reported for CNTs, and this number rapidly increases [34]. Large number of applications one can find in electroanalysis [35,36], and for design of sensors and biosensors [37-39]. The first chromatographic applications of CNTs were in mentioned above proteomics [32], and they were also used for preconcentration of trace analytes, *e.g.* [40,41].

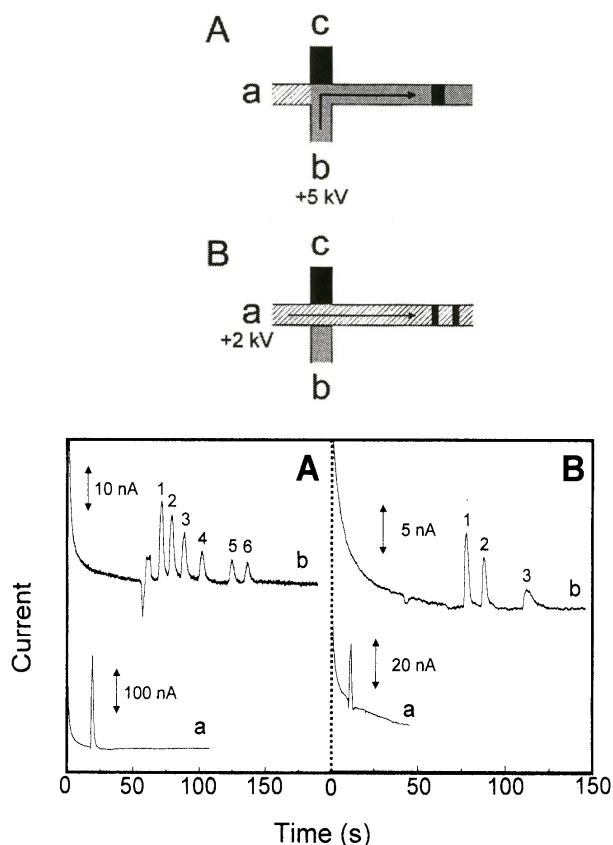


Fig. 5. Single-channel microfluidics for fast flow-injection screening and detailed capillary electrophoretic identification of nitroaromatic explosives or organophosphate nerve agents [18]. In upper part: schematic of the total (A) and individual (B) on-chip assays, a-run buffer with surfactant, b-run buffer without surfactant, c-sample. In lower part: a-flow-injection determinations of total assays, b-electropherogram of individual analytes in analysis of mixture of nitroaromatic explosives (A) and nerve agents (B).

4. Flow-through detectors employing nanostructures

Already in many papers on flow analysis one can find applications of nanostructures, especially dealing with flow-injection methods. Numerous of them concern use of SAMs on metal surfaces for design of electrochemical sensors. For instance, flow-injection determination of p-aminophenyl phosphate was developed with an alkaline phosphatase immobilized on gold nanoelectrode ensemble, which was fabricated by electroless gold deposition onto the nanopores (50 nm) of polycarbonate membranes [42]. Gold nanoelectrodes

were modified with SAM of 2-aminoethanethiol for immobilization of enzyme. Another interesting example is design of portable sequential injection system for determination of lead with voltammetric detection [43]. The sensor working in wall-jet flow-through cell was fabricated from a carbon paste electrode modified with acetamidophosphonic acid SAM on mesoporous silica. The SAM of mercaptopropionic acid was employed in design of enzymatic biosensor with immobilized horseradish peroxidase for FIA determination of hydrogen peroxide [44].

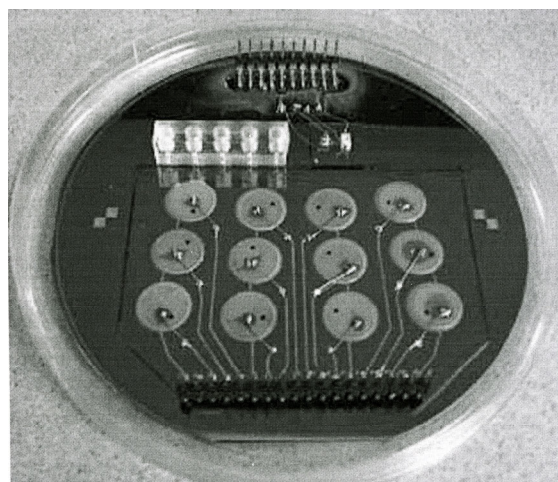


Fig. 6. The integrated microfluidic flow injection system (lab-on-a-chip) for spectrophotometric determination of ammonia [21]. Photograph of the system integrated on a 10-cm diameter silicon plate. The 12 gray circular elements are piezoelectric disks; three of such discs form one peristaltic micropump.

Especially worth to present here is the role of such SAM nanostructures in flow measurements with optical detection based on surface plasmon resonance (SPR) [45]. In this method a small change in refractive index or dielectric constant in the vicinity of the surface of the gold film on the SPR sensor chip, caused by interactions between the biomolecules, can be detected with a high sensitivity. For this purpose immobilization of biomolecules on the gold surface usually is carried out by formation of SAM, and measurements are made during the flow of solution through SPR detector. The analytical literature contains numerous examples of such flow measurements, *e.g.* determination of mono- and disaccharides based on interaction with boronic acid immobilized using SAM [46], or numerous immunochemical determinations with the use of antibodies immobilized on gold layer via SAM, employed for determination of pesticides [47], bovine serum albumin [48], or *Vibrio cholerae* O1 bacteria [49]. Fig.8 shows schematic diagram of a flow detector with SAM for binding of appropriate antibodies on gold layer for plasmon surface resonance and typical recordings of SPR signal. SAMs were also employed for coating of a gold electrodes of a piezoelectric crystal for determination of heavy metal ions in FIA system [50]. The detection was based on binding of metal ions by bipyridinium receptor, which forms SAM due to covalent bond with gold electrodes. In another interesting application of SAM in FIA systems, lead(II) was preconcentrated on gold electrode modified with SAM formed of mercapto-derivative of 15-crown-5 [51]. Detection was based on electrochemical release

of analyte to FIA system with subsequent detection by ICP-AES.

The analytical literature provides also examples of application in FIA systems of detectors with artificially formed BLMs. In many electrochemical applications BLMs were formed on various supports with different modifications of their chemical composition. The BLMs formed on glass microfibre filter were used for flow-injection monitoring of the triazine herbicides [52], while BLMs with incorporated DNA were used for flow-injection analysis of mixtures of hydrazine compounds, based on different time of appearance of the transient response for different hydrazine compounds [53]. In another works BLM modified with ionophore nonactin was used for flow monitoring of ammonia in gas phase [54], and modified with DNA in determination of aflatoxin M1 [55]. All these determinations were carried out with amperometric detection, similarly to determinations with enzymatic biosensors employing enzymes incorporated into BLMs [56,57].

Dynamics of self-assembling of lipid bilayers was utilized in photolithographically fabricated microfluidic microchannel device to detect intermolecular interactions when molecules carried out by the self-spreading lipid bilayer collide with each other in the microchannel [58].

A wide application in flow-through detectors, especially electrochemical ones, find carbon nanotubes. Most often they are employed for surface modification of conventional working electrodes in flow-injection amperometry, or for effective immobilization of biomolecules. As example of first case, the flow-injection determination of galactose with working glassy carbon electrode modified with single-wall CNTs [59]. In FIA amperometric detection of organophosphate pesticides as working electrode a biosensor with acetylcholinesterase immobilized on CNTs [60]. The self-assembling glucose oxidase on CNTs was employed for flow-injection amperometric

determination of glucose [61], while amperometric biosensor fabricated through electrostatic assembly of bienzyme/polyelectrolyte hybrid layers on CNTs was reported for determination of choline using choline oxidase and horseradish peroxidase [62]. For FIA amperometry of glucose also much more complex CNT-based biosensor was described in the literature [63]. The glucose oxidase was adsorptive immobilized on silane composite layer, containing CNTs and conjugate of albumin with ferrocenecarboxylic acid that served as mediator in electrode process. A similar system was also reported for FIA determination of hydrogen peroxide with immobilized horseradish peroxidase [64].

In flow-injection measurements in gas phase and piezoelectric detection the determination of simple aliphatic alcohols was reported with the cadmium arachidate prepared by the Langmuir-Blodgett technique, which has been used as multilayered buffer material to promote the adhesion of the single-wall CNTs [65].

Instead of CNT a very favorable can be also use of carbon nanofiber for modification of working electrode, as they produce more edge sites on the outer wall which can facilitate the electron transfer of electroactive analytes and provide excellent catalytic activity. This was shown for highly sensitive flow injection detection of hydrogen peroxide using a carbon nanofiber-modified glassy carbon electrode [66].

The detectors for flow measurements have been also developed using a gold nanoparticles. Numerous such applications were reported for SPR measurements [67], and *e.g.* in flow-injection voltammetric determination of dopamine using gold nanoparticles were immobilized on an amine-terminated SAM on a polycrystalline gold electrode [68] and FIA voltammetric determination of As(III) was reported with glassy carbon electrode with surface deposited gold nanoparticles [69].

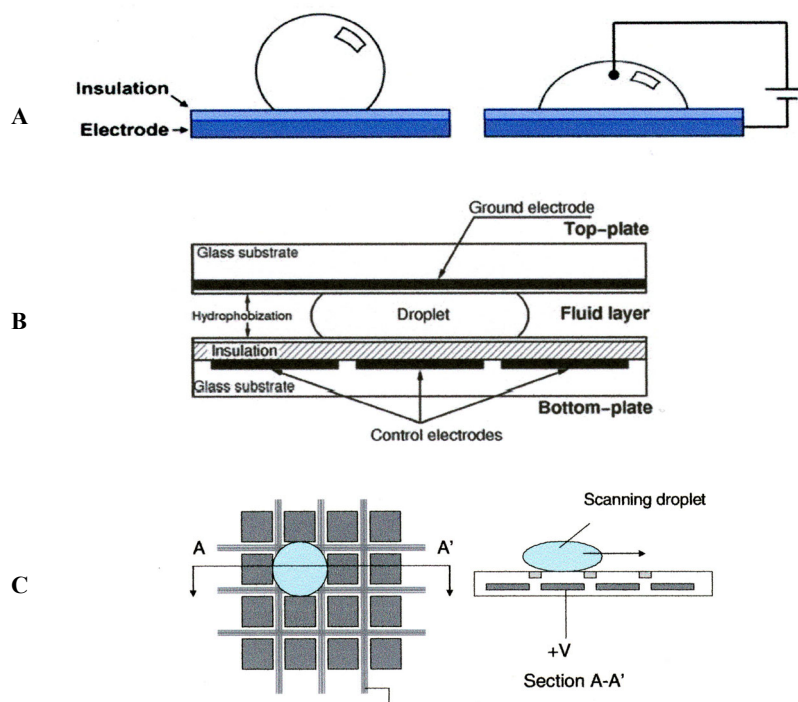


Fig. 7. Illustration of principle of design of digital microfluidics [22]. A - electrowetting on dielectric, where application of an voltage results in lowering the interfacial tension, B - droplets are sandwiched between two parallel plates, where the bottom one is the chip surface housing the addressable electrode array, C- co-planar actuation array for droplet scanning for transport of droplets.

Also silica nanotubes have been used for biosensing for flow-injection measurements [70]. A synthetic enzyme-loading nanotube membrane was based on an anodic alumina oxide film that has cylindrical pores with nanoscopic diameters and silica nanotubeds were synthesized within the pores through sol-gel chemistry. This membrane was used as enzyme reactor for FIA determination of glucose.

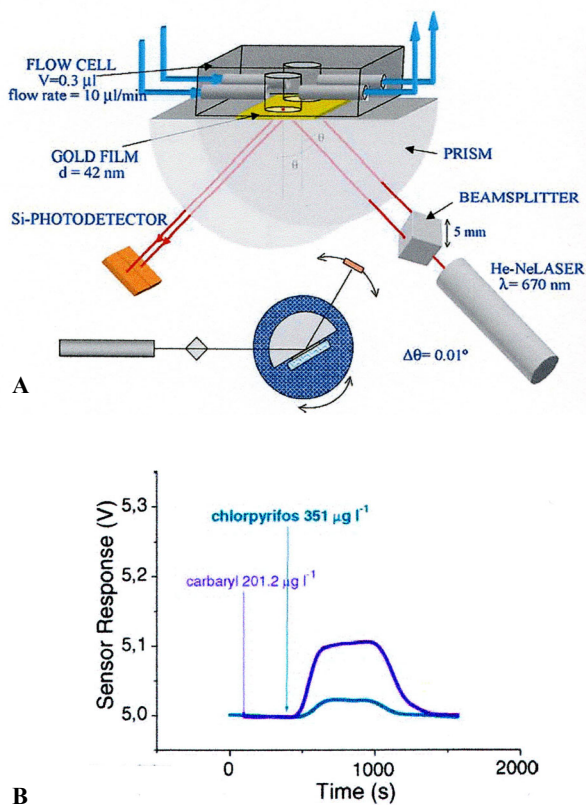


Fig. 8. Schematic diagram of SPR flow-through detection system with sensing layer obtained by SAM on the gold film (A), and example of sensor response for two pesticides using appropriate antibodies immobilized by a carbodiimide coupling to SAM (B) [47].

5. Nanofluidics

At present time, when flow analysis in microfluidics is already very advanced, and some systems – especially those for electrophoretic applications – are commercially available an increasing attention is being focused on design of nano-flow systems [71]. Nanometer dimension concerns first of all inner diameters of channels transporting solution and further miniaturization of detection devices. Papers published in few recent years to much extent are devoted to theoretical prediction and description of phenomena and interactions, which take place in this scale, but there are also reports on various technical approaches to build and utilize such systems.

Many forces are generally distinguished that play a role on the nanoscale. In the movement of analyte molecules in the submicrometers there essential changes of physical and chemical interactions compared to micrometer scale. Many interactions

which are marginal in microfluidics play an essential role in nanofluidics. The two major forces acting in the nanometer range between surfaces are electrostatic forces and van der Waals forces, the latter one can act within the distance below 2 nm. Another forces that has been observed are solvation forces that represent attachment of layers of solvent molecules to the surface, and extends several layers into the solution. They may contribute to certain resistance of flow in nanochannels, as result of resistance of shifting of these layers relative each to other. Steric repulsive forces are attributed to entropic stabilization exerted by freely fluctuating polymer brushes attached to the surface. In this repulsion a significant contribution may originate from hydration energy, when polymer chains are well hydrated.

A very strong force acting in nanoscale is the capillary force, which originates from the adhesion between the liquid and the solid surface molecules. Another important phenomena observed in nanofluidics is that liquid may slip past the surface decreasing the fluid resistance. In some case a considerable can be dielectrophoretic forces, which are generated in rapidly changing high electrical field gradients and resulting from differences in dielectric properties between analyte molecules and medium. A detailed discussion of these phenomena one can find in recent review [71].

The forces acting in nanoscale may affect also ionic equilibria and kinetic properties [72]. In nanochannels with charged wall surfaces, the extension of the electrical double layer, which affects the permselectivity of such structures, results in the electrostatic exclusion of co-ions and enrichment in counter-ions. The electric double layer may also induce certain selectivity of transport, which was already investigated in case of porous membranes for biotechnology. The comparison of experimental results with theoretical predictions justifies that electrostatic forces are the governing forces in nanofluidics [72].

In nanoscale channels the pressure-driven flow and resulting electroosmotic backflow exhibit coupled dispersion effect when hydraulic diameter is on the order of the electrical double layer thickness. In recently developed model it was shown that streaming potential in nanochannels decreases the effective diffusion coefficient of the solute, while increasing the dispersion coefficient as traditionally defined [73].

The fabrication of nanochannels from different materials and in different shapes is a subject of nanotechnology being developed in numerous research groups, e.g. [74,75].

One of the most commonly known, so far, nanofluidic systems employed in analytical research are nanoporous materials, such as stationary phases in gel chromatography. Three dimensional nanosystems can be also formed as multimembrane structure of nanocapillary membranes, where functioning is controlled by applied potential [76] (Fig.9). Each membrane plays in the system different role, i.e. in each a different physico-chemical operation or chemical reaction can be carried out. Nanometer size pores are randomly placed in polycarbonate track etched membranes, produced by exposing thin sheets of polycarbonate to collimated nuclear radiation and subsequent chemical etching. Such systems are example of evolution of flow microsystems to nanosystems without need of use nanofluidic fabrication facilities. They have been already applied with fluorimetric detection for determination of various analytes, e.g. to follow derivatization of glycine with o-phthalaldehyde or 2-mercaptoethanol and also calcium binding to calcium-labeled dextrans with the presence of complexone EGTA [77]. Such systems can be well suited to screening of combinatorial

chemistry products. In another work of the same group lead determination has been reported based on lead-specific DNAzyme in nanocapillary interconnected microfluidic device [78]. The determination was based on the use of dual-labeled cleavable substrate DNA whose 5' and 3' ends are labeled with a fluorophore and a quencher, respectively, and an enzyme strand whose 3' end is labeled with a quencher. Initially, the fluorescence of fluorophore is quenched because of the close proximity of the quencher but in the presence of lead(II) the substrate DNA is cleaved, resulting in release of fragment and a concomitant increase of fluorescence. In all these systems a hybrid three-dimensional nanofluidic/microfluidic devices using molecular gates were employed. The net flow direction inside the microdevices is principally controlled by the magnitude of the electrical and physical flow impedance of the nanoporous membrane relative to that of the microchannels and the surface chemical functionalities which determine the polarity of the excess charge in the nanochannels.

A nanofluidics with so called entropic traps can be employed for separation in flow conditions, as alternative to chromatographic or capillary electrophoretic separations. In the system fabricated for separation of long DNA molecules, the channel comprised narrow constrictions and wider regions that caused size-dependent trapping DNA at the onset of the constriction, and such process creates electrophoretic mobility differences, thus enabling efficient separation [79]. Another method of DNA separation based on flow in nanopores was employed by the use of α -hemolysin toxin as a suitable channels because it self-assembles in artificially lipid bilayers and separated molecules can be driven by an applied electric field

[80], and also by incorporation an antibody-based molecular recognition element onto the nanopore surface [81]. The wall of nanopores of polycarbonate membrane was electroless plated of gold and antibody was immobilized via gold-thiol chemistry as thiolated fragment of antigen. This can be incorporated into microfluidic devices, and was demonstrated for selective capture and release of human insulin.

As another way to construct nanofluidics the of GaN nanotubes integrated into metal-oxide solution filed effect transistor was reported, in such a way that gate voltage affects the electrostatic potential of solution inside nanotubes and modulate ionic conductance [82]. These nanofluidic devices have been demonstrated to be useful for single molecules sensing of DNA with detection by charge effect or by geometry effect.

It seems that an open area of possible application in design of nanofluidics is the use of molecular nanomechanics, a new class of motors made from nanoscale building blocs that derive on-board or off-board power from in-situ chemical reactions [83]. One can imagine that as result of further development such a micro- and nano machines they can be utilized in design of nanofluidics *e.g.* for transport of mass, for improvement of certain modes of detection by enhancing mass transport to the detector, or by activation of a movement of nanoparticles in the flow system, which can serve as moveable separation systems. Some example directing towards this vision might be a molecular nanomachines that can convert energy inputs into controlled motion on a surface and transport of nano-cargo (material or information) from one place to another on the surface [84].

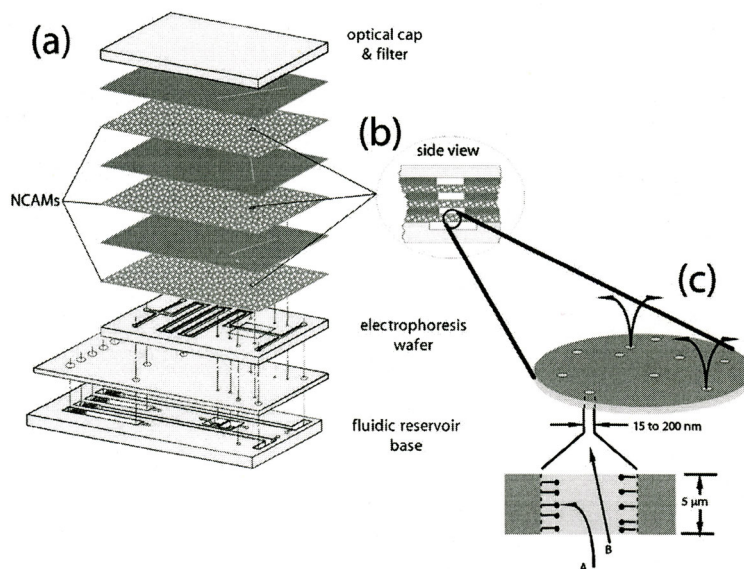


Fig. 9 (a) Exploded schematic view of a multi-level vertically integrated nanocapillary-based microfluidics displaying a fluid input layer (bottom), a pre-separation (electrophoresis) layer and 3 microfluidic layers (dark gray) separated by nanocapillary array membrane switching layers (NCAMs) [76]. (b) Side view of the vertically stacked microfluidic channels separated by NCAMs. (c) Schematic diagram of a single NCAM with exploded view of an individual nanopore with chemically derivatized interior.

6. Conclusions

Miniaturization of analytical instruments is, similarly to many other devices in various areas of contemporary life, a strong development trend in analytical chemistry. Very generally it leads to lowering the cost of instrumentation and potentially more common use of these miniaturized devices. It is also very important in flow analysis. In final designs and mass production, based on new materials and micro- and nano-machining, it is much more difficult, than experimenting with laboratory-made, simple flow systems in academic research laboratories. Then, although they are much more difficult technologically, one can hope, that microfluidics and perhaps in further futures nanofluidics, as lab-on-a-chip devices integrating all steps of analytical procedure, will be in some cases more competitive to other instrumentation and will be wider used routinely. It seems that their chance are applications in such cases, where *e.g.* too complex matrix of analyzed sample is a limited factor for use of various discrete analyzers or direct sensing by chemical or biochemical sensors. As particular field of applications can be expected health-care devices for personal care as well as portable, miniaturized instruments for environmental monitoring or field devices for agriculture applications.

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