

Synthesis and Analysis of Chemical Components in Nanoscale

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Abstract

The interest for chemistry performed in nanoscale is growing rapidly, particularly due to the potential of combinatorial approaches for optimizing reactions as well as development of new materials. Also in biochemistry, there is a need for greatly enhanced throughput, which can be obtained with parallel chemistry.

In this paper, a number of strategies for miniaturized chemistry and analysis are outlined including the use of microfluidic dispensers, robotized sample handling as well as integrated detection and analysis. Critical criteria are discussed, *e.g.* solvent evaporation, anomalies in fluid transport, as well as application examples such as nanotitration and PCR amplification.

Keywords: nanochemistry, microfluidics, nano-robotics, combinatorial chemistry

1. Introduction

Only a decade ago, the use of microchips was considered unapproachable by the majority of chemists, however the situation has changed drastically. Microchip issues are now a major part of analytical conferences and the number of people active in this area is growing tremendously. However, it is not always evident in which way conversions to the microchip world brings crucial advantages. Too often, particularly in chip-based separation systems, it seems that resolution is sacrificed for speed of analysis which can severely limit the scope of application. We are of the opinion that more attention should be given to scale-related contributions of forces in selective mass transport. However, of the many obvious advantages, which have been pointed out extensively, perhaps two issues deserve to be highlighted in particular. First, the use of miniaturized chip based systems allows massive parallel chemistry to be performed, at comparatively low cost. This opens the door to accelerated development of new materials, including drugs, polymers, catalysts etc, using combinatorial approaches [1], and it also opens the possibility to design high throughput platforms *e.g.* drug screening, medical diagnostics or proteomics. The second important issue is related to situations where the amount of sample is minute. With miniaturized systems, high sample concentrations can be maintained due to the reduced volumes. This improves reaction kinetics and detectability (if concentration dependent detection methods are used). Chemical reactions in miniaturized formats will therefore become more and more important.

2. Reactor format and platform

Two basic principles for miniaturized reactors can be employed. In the first principle, the sample and reagents are kept in a static environment, such as a nanovial or a capillary tube. The other principle uses a dynamic environment, *i.e.* the sample is transported through a capillary tube or a channel, while reagents can be added in advance or downstream *e.g.* from fluidic side streams, depending on the desired reaction conditions. A further sub-division can be made into an open or closed mode [2]. Both principles and modes have particular advantages and disadvantages. The use of open nanoliter or picoliter sized vials has the benefit, that individual vials can be addressed any time (before, during or after a reaction) in any sequence by microdispensers such as capillary tubes, piezo-electric dispensers, micro electro-mechanical dispensers, electrostatic dispensers or micro separation columns. This degree of freedom in time and space includes the entire matrix of vials. However, functional sample delivery and detection units have to be integrated in the form of a mechanical precision platform. A typical platform, which is used in our laboratory is shown schematically in **Figure 1**.

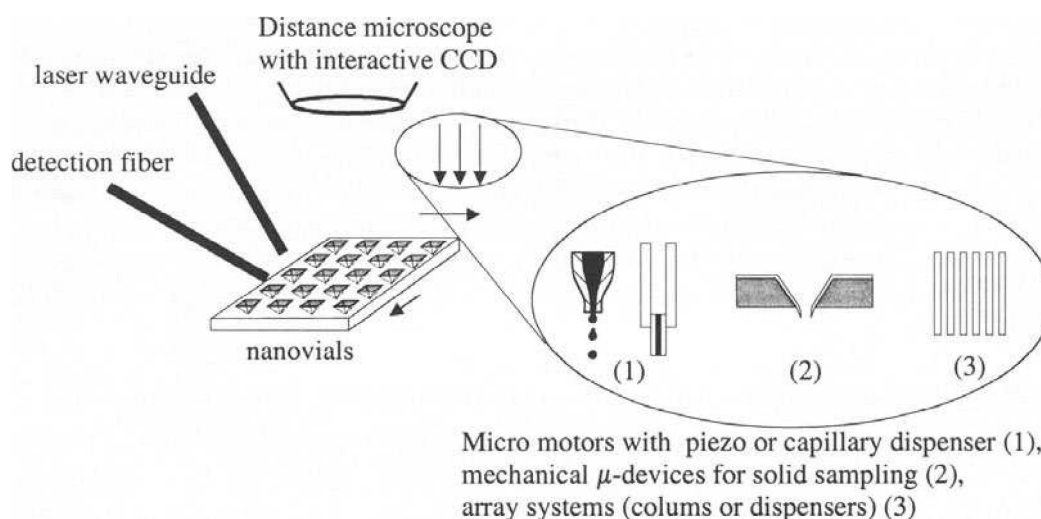


Figure 1: Robotics platform for nanoscale chemistry in open vials.

Basically, the platform, of which the technical details have recently been described [2,3], consists of a X-Y translation table with a resolution of $1\ \mu\text{m}$ in both directions over a distance of $20 \times 20\ \text{cm}$. Onto this table, a holder for *e.g.* a silicon wafer, containing anisotropically etched microvials, or glass, quartz or polymer substrates containing microcontainers, can be mounted. The vials are observed by a distance microscope, including a CCD camera and an image analysis system. In this way, morphological changes as well as changes in colour or light intensity can be monitored simultaneously in a large number of vials. Alternatively, specific areas or vials can be illuminated by a laser beam *via* an optical fiber, while an adjacent fiber can collect light from *e.g.* fluorescence or luminescence. The platform further includes a capillary electrophoretic column (not shown in the figure) for direct sampling of reaction fluid from the microvials. Several precision micromotors, mounted in the Z-direction are also part of the platform. Different dispensing tools like piezo-

electric dispensers, microcapillaries, flow-through tubes, devices for sampling of solids etc. can be mounted on these micromotors. Also parallel arrays of tubes or electrophoretic columns (with a spacing corresponding to the pitch of the nanovials) can be mounted and utilized in this way. The entire system is controlled by software (which includes a temperature control of the vial plate and pressure control for solvent delivery), and all units can be linked to a time sequence as well as operated in an interactive mode. It is therefore possible to pre-program unattended sequential chemical operations, while the computer can make decisions (e.g. incremental increase of reagent addition, keeping fluid volumes constant etc) based on data obtained from the detection devices. Further facilities, like an on-line fiber optic UV detector for micro-preparative CE, or capillary HPLC (sample “cleanup”) have recently been added.

3. Solvent evaporation – the volatile liquid lid concept

The described platform has the advantage of an extreme flexibility, in contrast to closed integrated monolithic microsystems, which are usually designed for one dedicated application. However, the use of open nanoliter or sub-nanoliter sized vials has an important drawback. Due to the increased surface area/volume, solvent evaporation becomes a fundamental problem. A nanoliter of water brought onto a surface at room temperature will evaporate within seconds. One method to deal with this problem is to increase the humidity in the surrounding atmosphere, but the beneficial effect is limited and becomes difficult or impossible to be utilized at elevated temperatures [3]. Another way to address the problem is to continuously add solvent to the sample, to compensate for the evaporation. One way to supply the solvent is *via* capillary tubing [4]. Another technique is to utilize a piezo-electric dispenser, which can generate very small droplets (e.g. 10 pL). By interactive surveillance with the described CCD-monitoring unit, fluid levels in vials of low-nanoliter volumes or less can be kept constant.

We have utilized this principle in miniaturized-titrations, where nanoliter samples were dispensed on a flat surface by one piezo-electric pipette, while, in consecutive order, a second piezo-electric pipette delivered low picoliter volumes of titrant [5]. By correctly timing of the fluid delivery, control of the volume of the reaction mixture could be maintained. It should be possible to use this principle for numerous other applications, e.g. controlled microcrystallization from nano-droplets, controlled sequential concentration and dilution during reactions etc. Also, detection can be integrated if the reaction is carried out on the flat top of an optical fiber (Figure 2).

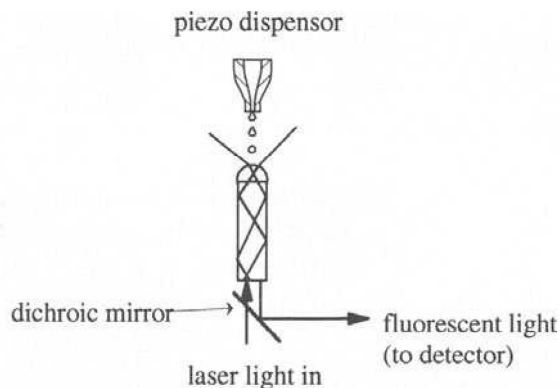
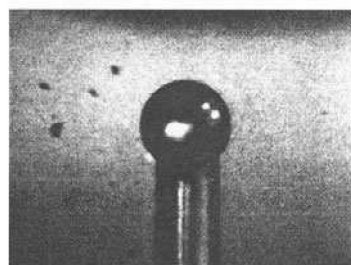


Figure 2: Photograph (left side picture) and schematic (right side picture) of the liquid lens reactor on an optical fiber.

The hydrophobic clad of the fiber acts as a surface tension barrier for the droplet, and therefore even large droplets will keep steady positions on the fiber end. Moreover, the droplet itself acts as a liquid lens and is therefore an efficient light collector, enhancing the numerical aperture of the fiber. The optical fiber will transmit light (e.g. fluorescent light), by total internal reflexion, to a suitable detector away from the reactor. Light from an array or a matrix of fibers can simply be imaged by a CCD camera. Different optical schemes, including back scattering designs, using e.g. a dichroic mirror, are possible.

However, the continuous solvent compensation principle is difficult to accomplish simultaneously for a large number of microreactions. At present, it would not be feasible to feed solvent into e.g. 100,000 vials simultaneously, although the use of only a few piezo-electric devices, combined with droplet routing, exploiting electrostatic charging would be an interesting alternative, but we have not yet investigated this concept.

A way to prevent evaporation from open vials is to cover them with a non volatile, non miscible oil, as is common practice in PCR. However, such liquids are difficult to remove from the nanoliter volumes of sample, and will disturb subsequent analysis and/or material recovery. We have therefore applied a different principle, where the covering liquid is volatile, but immiscible with the reaction matrix [6]. Suitable liquids in connection with water-based reactions are hydrocarbons like e.g. hexane. In order to prevent evaporation of the thin film of hexane, a continuous addition of this solvent is performed in an overflow mode (**Figure 3**). After completion of the reaction, the flow of hexane is discontinued, and consequently, the remaining hexane and thereafter the water evaporates, leaving an uncontaminated dry sample, ready for analysis by e.g. mass spectrometry.

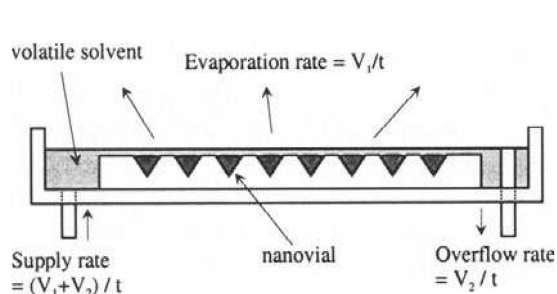


Figure 3: Principle of the volatile liquid lid.

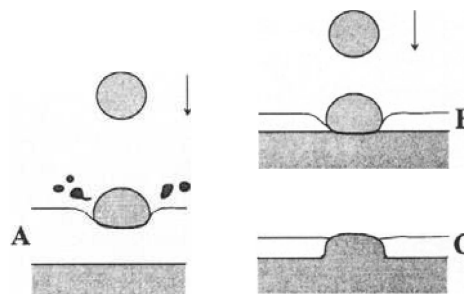


Figure 4: Impact of a droplet ejected from a piezo-electric dispenser onto samples with a solvent layer of different thickness.

A particular feature of the volatile solvent barrier is the possibility to utilize piezo-electric dispensers to “shoot” through the solvent layer. This is possible due to the low viscosity of the volatile solvent. However, it is important to optimize the hydrodynamics of the system. The thickness of the layer, as well as the size and the velocity of the droplet are critical parameters. If the solvent layer is too thick (**Figure 4A**), the droplet will not penetrate through the layer. Instead it will bounce off and partly disintegrate into smaller droplets. In an optimized setup, the front edge of the droplet penetrates through the layer (**Figure 4B**), where it coalesces with the liquid sample underneath. Apart from the action of the velocity vector,

this creates an extra cohesive drag to the droplet, which then completely merges with the sample (**Figure 4C**). In this way, a non-contact mode dispensing of sample and reagents into very small volumes can be accomplished, while solvent evaporation is effectively prevented. In fact, a “closed system” is obtained, while retaining the full flexibility of an open system.

The volatile liquid lid concept has been used for several applications, *e.g.* tryptic digest of proteins. We also found that it was possible to carry out successful PCR reactions in volumes down to 50 nl, using octane as a covering fluid. Water loss was experienced when smaller sample volumes (*e.g.* 15 nl) were used, when the sample was kept at 95 °C after only a few minutes. We are presently investigating the limiting factors which affect the loss of water from these small volumes. **Figure 5** shows a photograph of a setup of the robotics for chip-based nano-PCR.

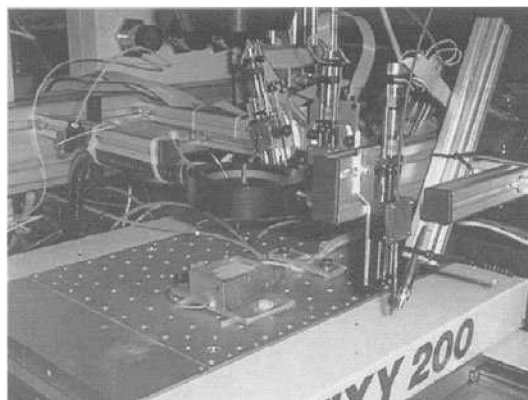


Figure 5: Photograph of the nano-robotic set-up for open vial chip-based PCR.

4. Flow-based reactors

Microfluidic systems also offer exciting possibilities for nanoscale reactions. The simplest version of a fluidic reactor is the transport of a pre-mixed sample through a channel, which has the desired reaction conditions *e.g.* an elevated temperature zone and/or zones with surface bonded substrates like enzymes, ligands, catalysts etc. Such microreactors can be stacked, and utilized in a continuous mode, which is a concept of great promise for production of bulk chemicals [7]. For analytical purposes, a single channel or an array of channels with *different* samples are employed in order to increase the diagnostic or combinatorial throughput. An elegant example of a microflow reactor was presented by Manz *et al.* [8], who realized flow PCR in a chip-based channel. In a more general system, it is of course possible to carry out several different reactions along the flow trajectory, by adding reagents *via* downstream side channels.

One of the attractive features of flow reactors is the possibility to create a serial sequence of reaction mixtures, separated by small zones to enhance the component number capacity. However, in this context, some fundamental phenomena must be taken into account. The well-known Taylor dispersion in pressure driven fluid flow will cause a lagging as well as a dilution of the sample. For small sample zones, this can lead to reaction problems as well as severe cross contamination. Also in electrically driven flows (where Taylor dispersion is irrelevant) problems can occur such as electrophoretic separation of reagents, apart from the

electrostatic and ionic conditions needed to obtain flow, which are limiting the degree of system freedom.

Reducing carry-over is of major importance to enhance the sample number capacity of flow reactors. One way is to create sample plugs, interspaced by air (or gas). However, the wetting properties of the surface are of utmost importance. A sample plug, passing a wetted surface will leave a thin film behind the backside-meniscus of the plug. This film is picked up by the next plug, and results in a severe cross contamination. The thickness of the leaving film (**Figure 6A**) is related to the internal friction within the liquid as well as the velocity and wetting angle, and has been quantified by Concus [9] according to:

$$d_f = C d \left(\frac{v\eta}{\gamma} \right)^{2/3}$$

where d_f is the film thickness, d is the inner diameter of the channel, v is the velocity, η is the viscosity of the fluid and γ is the contact angle of the liquid (C is a constant).

Transport fluids in non-wetted surfaces will theoretically not lead to cross contamination, but contact angle hysteresis, micro roughness as well as chemical affinity can offset this rule. Also, there is a critical speed, at which the sample integrity can not be maintained. This critical speed is reached, when the cohesive forces within the fluid are offset by the friction with the surface. Typically, the backside meniscus disintegrates in a wavelike appearance, leaving very small droplets behind (the situations are schematically depicted in **Figure 6**), leading to cross contamination.

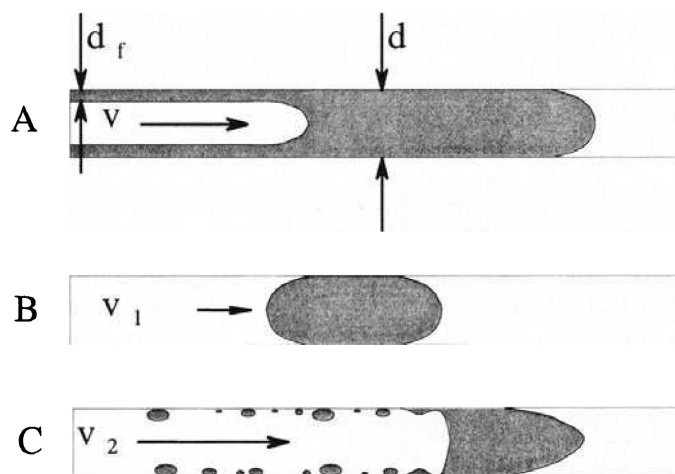


Figure 6: Transport of a droplet in a channel. A: wetting conditions B: Ideal transport under non-wetting conditions C: Non-wetting conditions where the velocity v_2 exceeds a critical limit.

A concept which should have one of the best potentials for high throughput sequential flow reactions is the use of systems with two immiscible fluids. A droplet of water in a continuous flow of a hydrocarbon in a hydrophobized channel will keep well separated from an adjacent water droplet, even at high velocities. Instead of disintegration into smaller droplets, the water droplet detaches from the surface while the hydrocarbon creates a layer between the channel surface and the droplet (**Figure 7**).

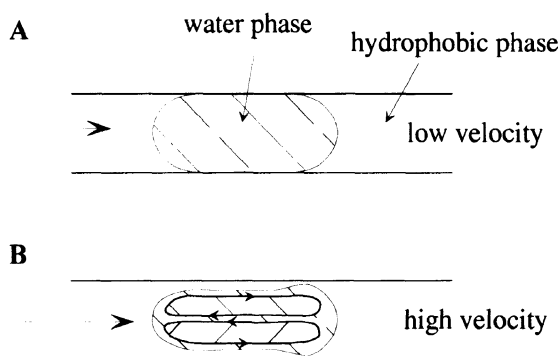


Figure 7: A: Transport of a water droplet in a hydrophobic solvent at low velocity. B: Same as in A, but at high velocity.

Thus, there is no more surface contact of the water (and no more risk for surface interactions). An additional benefit of this situation is that the friction between the hydrocarbon and the water leads to an intrasegmental movement within the droplet, which means that an effective mixing action is obtained. The principles of this phenomenon have been described in detail [10].

We have carried out preliminary experiments with a flow based PCR reaction, where the reaction mixture is interspaced with a hydrocarbon, as described above. Amplification was successful, and the results were fully comparable with the results of the commercial standard procedure. It seems that this mode of operation could hold the potential to carry out a massive number of PCR reactions in nanoliter or even sub-nanoliter plugs of solution. This would have fundamental impacts on throughput bottle-necks in molecular biology.

Acknowledgements

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