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ROLE OF MICROFLUIDICS IN DISCOVERING NEW MARKETABLE SUBSTANCES – A SURVEY

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Abstract: *Paper surveys developments in combinatorial chemistry and related search for new medicaments and biomedical procedures, characterised by simultaneous testing of many substances (and their mixtures). Attention is focused on advantages offered in this field by small-scale no-moving-part microfluidics. Typical problems and their solution are then demonstrated on author's development of a microfluidic unit for parallel tests of catalyst having potential to improve the effectiveness of Fischer-Tropsch synthesis of liquid fuels from biomass*

Keywords: ***Combinatorial chemistry, pressure-driven microfluoidics***

1. Introduction

There is, unfortunately, no theory capable of predicting properties of substances from knowledge of their molecular structure. As a result, the properties have to be evaluated empirically. This is done by synthesising the substances one by one and then measuring and evaluating their mechanical, optical, bactericidal and other properties. The process is slow and expensive. Depending on the complexity of the synthesis and the laboriousness of the testing, an experienced investigator can study and evaluate between 10^1 and 10^3 new materials per year. In some areas the discovery and evaluation is even much slower. For example the process of putting on market a newly discovered pharmaceutical product takes, on average, 12 years, usually starting from either (**a**) just the serendipity of the inventor, or (**b**) identification of active component in a natural product — and finishing by clinical tests. The final cost, typically $ ~500 million per single new drug, includes the expenses spent on typically ~ 1000 discarded unsuccessful attempts. What makes any progress with new drugs and new materials difficult in general is the huge number of possible combinations and permutations to be evaluated. There are ~ 10^{200} possible and synthesizable simple molecule configurations (considering only those with relative molecular mass less than 850). It is estimated that of them something like ~ 10^{40} compounds possess marketable properties. This number is beyond any hope of being investigated completely, especially if the search is not systematic. Moreover, a single substance itself need not be the final target of the testing. Catalysts and enzymes are typical examples of empirically tested substances that have their properties fully evinced only in mixtures consisting of several different components (some catalytic substances are inefficient when used alone), with the activity strongly dependent on their mixing ratio. In addition, the catalytic action depends on reaction temperature, being really effective only inside a certain temperature "window" - so that the testing has to include a wide range of temperature conditions.

2. Combinatorial testing

Testing suitable candidate substances at random one by one is so hopelessly slow that already in the last century methods were sought of accelerating the progress. A step forward was to systematically synthesize and test many related substances simultaneously, each tested sample

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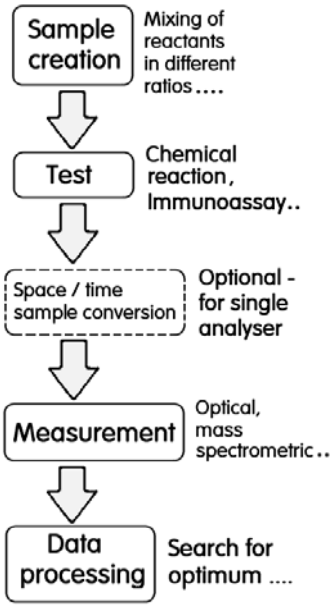


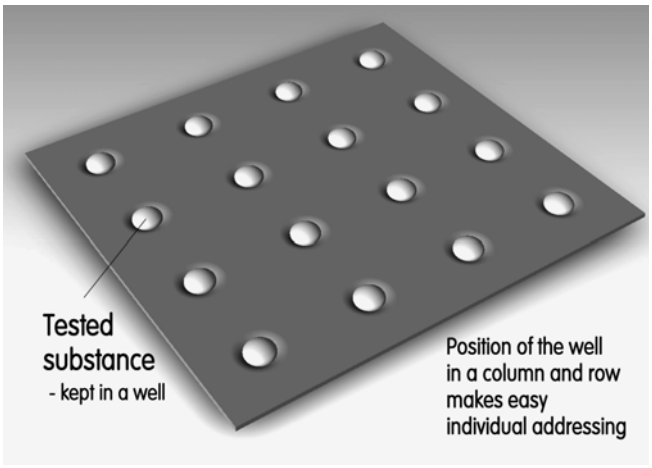


Fig. 1 (Left) The basic steps in combinatorial testing. Until recently performed manually and recently by mini-robotic systems, many of these steps may be profitably performed by microfluidics.

Fig. 2 (Right) An example of a (very small) titre plate. Small samples of different substances are held during the tests in separate wells on the plate top surface. Typical number of wells in a titre plate is currently much larger, sometimes more than 1000.

differing by a small amount from its predecessor. Of course, the larger is the ensemble of tested compounds (and perhaps of their combinations), the higher is the probability that a new substance of significant commercial value is found. An advantage of parallel testing are identical test conditions - all candidate catalyst mixtures are tested over exactly the same time inside the same oven heated to the same temperature.

Because of the typically large number of variables that may (and have to be) varied, the search for new useful substances is called ***combinatorial chemistry***. Steps followed in a typical search are listed in Fig. 1. Note in particular the last item. It may seem trivial but in fact the decision about the direction of each new testing has to be taken on the basis of previous results. This data processing often involves highly sophisticated mathematical optimisation methods (e.g., Goldberg 1989, Gobbi and Poppinger 1998), which may substantially improve the effectiveness of the search.

Until recently, all the steps presented in Fig. 1 were done manually. A characteristic basic device that has been used to accelerate the parallel testing procedures is the titre-plate or titre-tray, the principle of which is shown (in the very minimal case for mere 16 parallel tests) in Fig.2. The tested candidate substances are put into wells (or sometimes directly synthesised in these wells) made on the plate top surface. There they undergo simultaneously the test reaction and subsequently their properties are measured. The number of parallel tests (i.e. the number of the wells on a plate) has been gradually increasing so that present-day there are typically 96 – 1536 wells – the more tests made simultaneously, the better. Significant progress is also made in adopting the methods of microchemistry so that only a tiny amount of the substance is sufficient for the testing purposes, minimising the requirements on the titre-plate size.

Keeping the samples in a well is, of course, easily manageable in testing liquid and solid substances (the latter are usually pulverised). This is not a significant limitation; after all, most tested reactions as well as practically all biological assays take place in liquid phase. In principle it is possible – but obviously much less convenient – to test also volatile and gaseous

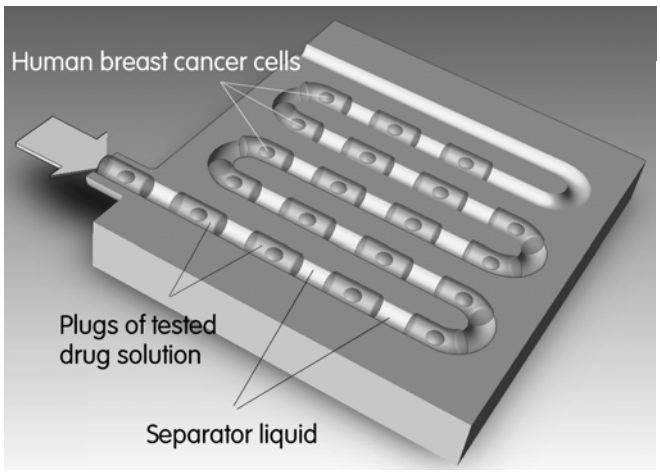
substances, which requires replacing the wells by vials having more or less sophisticated lids or caps that close the entrance openings between those instances (like insertion of a pipette for taking a sample) at which an access is needed.

Many chemical reactions are accompanied by measurable changes in colour, optical density, or fluorescence (the latter may be induced by laser irradiation). If optical response is missing, then many reactions may be more or less easily adapted (perhaps by addition of suitable chemically neutral colour changing reagents) to produce such an optical effect. In such cases, the advantage of the titre plates is the fact that the measurement step listed in Fig. 1 may be done at once for all the tested samples simply by taking a photograph of the whole plate. This simplifies the measurements significantly (processing the images may be done off-line) and makes easier the question of addressing and labelling the individual sample locations. In specialised facilities, instead of using a standard camera, the records are taken in a titre-plate holder provided with a an array of ccd chips at the well locations. It is then possible to supply into the test-processing computer directly the electric signals from these chips.

An important part of the discovery is storage of the results. The results of the tests and sometimes the sample themselves are saved in what is called a ***library*** of results. The complete titre plates may be stored with the samples in position (as long, of course, the samples are not volatile and do not deteriorate in time). This may be somewhat less convenient than storage of numerical data, but has the undeniable advantage of availability of the synthesised substances for later use — for checks or perhaps for testing a different property, one that was not investigated when the library was set up.

3. Robotics or fluidics (microfluidics) in the test facility

Handling the samples and placing the reagents into the wells manually becomes a tedious task whenever the number of the wells is of an order higher than ~ $10^1 - 10^2$. The tedious manual operations may easily lead to errors and mistakes. To ease the task, special handling tools were developed and are already standard – such as the multiple-exit syringes with an array of exits placed at distances equal to the pitch of the wells in the plate. Nevertheless even this does not help much. The inserted fluid need not be the same in all wells. Manual handling is also too slow. There are incessant requests for increasing the throughput and these have led to



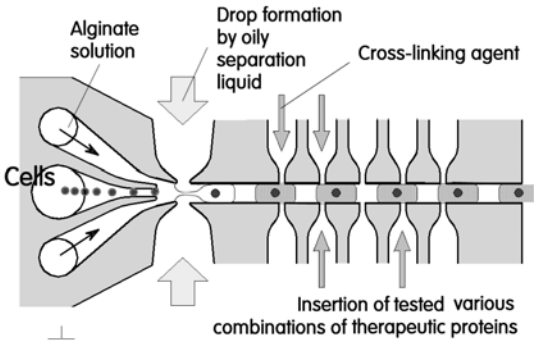


Fig. 3 (Left) Fluidic transport of samples in liquid carrier plugs (here for pharmaceutical testing of cancer cells)). The layout mimics the titre-plate layout, obtaining the advantage of simultaneous photography of laser-induced fluorescence. It is not necessary to insert and remove the tested cells individually.

Fig. 4 (Right) An example of a fluidic injector generating the carrier liquid plugs shown in Fig. 3. The liquid contains various combinations of tested therapeutic proteins, Trivedi et al. (2009).

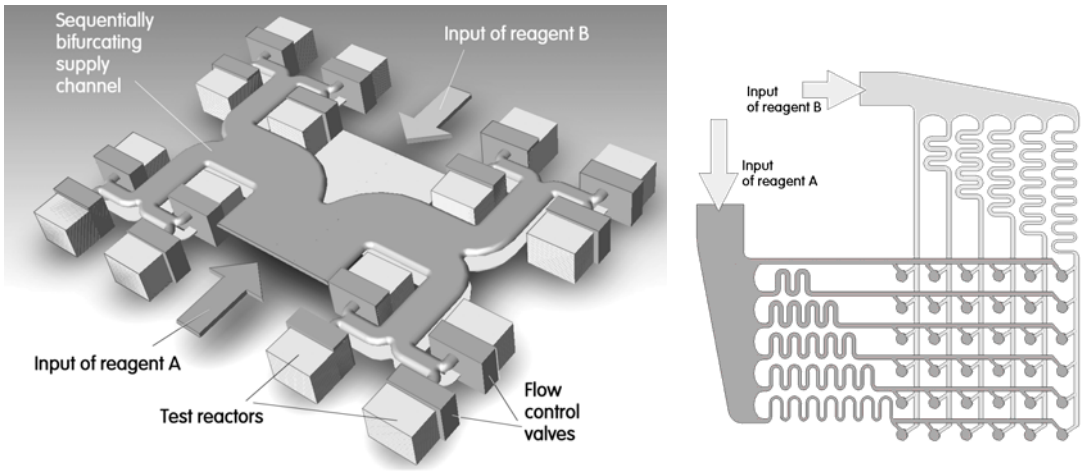


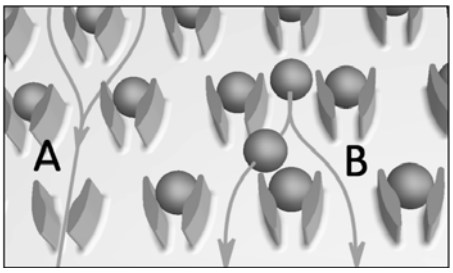
Fig. 5 (Left) Independently accessed test reactors distributed in a two-dimensional array. The non-negligible advantage over the single-path principle shown in Fig, 3 (where all samples have to move through the same passage) is avoidance of cross-contamination between the tested liquids, which may be an important aspect. The entry into the (schematically represented) test reactors is controlled by (also shown in a schematic way) flow control valves. Note that the principle of progressive bifurcation (Tesař, 2011) results in a large surface area occupied by the flow distribution channels (especially if etched into constant depth by isotropic etching).

Fig. 6 (Right) The matrix layout of sample liquid insertion (at different mixing ratios) with passive control of the flow rate by fluidic resistors. Less expensive and easier to manufacture than the case from Fig. 5, the generated mixing percentages are much less precisely defined than with active valves.

introduction of specialised ***robots.*** A typical component of such a dedicated robot is an arm that may be sequentionally moved into the wells to input there the reagents used in synthesising the samples – while later this arm may serve for taking a sample and carrying it into a central analyser. Some more sophisticated robotic arms can even unscrew the vial stoppers to enter the gaseous or volatile sample stored inside. Robots can increase the productivity significantly, to 10^4 - 10^5 tested substances per day. The throughput is limited by the reaction rate of the test reactions and by the inertia of the moved robotic arms and other parts of the robot, which puts constraints on the speed of movements. Of course, introducing the robots increases the initial capital cost of the laboratory and with the current trends towards the microchemical processes (some authors even speak about sub-millimetre distances between neighbour wells) robots may reach their limits of motion speed and positioning accuracy.

A solution has been found in using ***fluidics*** – or, more importantly, microfluidics with its extreme achievable volumetric density of devices. Fluidics can accelerate the processes – in particular the insertion of the reagents into the individually addressable positions (Figs. 3, 5, 6). Initial attempts at applying fluidics remained strongly influenced by the idea of the titre plate, with its typical two-dimensional (Figs. 3, 5, 6, 7, 8) layout of test locations. Of course, this layout makes possible, as with the titre plate, a simultaneous measurements of all samples by a photographic record. Currently, however, microfluidics applied to the combinatorial testing tends to leave the titre plate idea. It prefers performing the test reactions in closed through-flow reactors and subsequent transporting the samples by a system of microchannels to the measuring instrument. The method of transporting by liquid flows is applied even if the objects of the testing are, e.g. living cells (Fig. 3). The cells may be transported either by a neutral carrier liquid or directly by the solutions of anti-cancer drugs involved in the testing.

The fluidic transport of the sample to the measuring instruments made possible a much wider range of detection and measurement methods (Mairhofer, Roppert and Ert, 2009) Measurements made in a single central location make possible using sophisticated and more



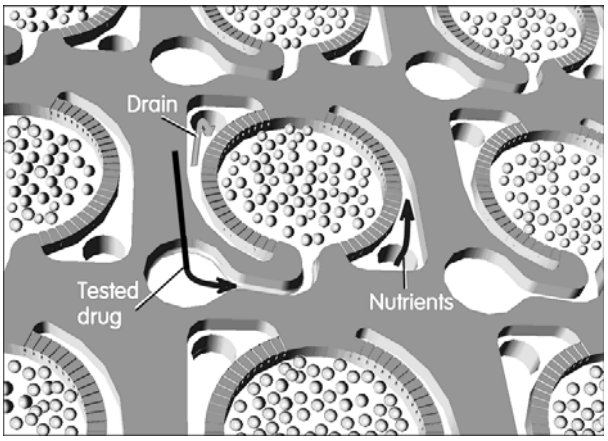


Fig. 7 (Left) Automatic positioning of individual living cells in the titre plate by the flow of culture medium. Flow A is focused into an empty position while streamlines like those in the case B avoid an already occupied location, carrying away with it also a not yet located cell. This is an interesting method of fluidic self-assembly – applicable if the tested cells are all identical.

Fig. 8 (Right) Testing of drug effect on a family of cells kept living in circular chambers supplied with nutrients. Flows of nutrients and tested drug enter the chambers distributed by the method of Fig. 5 or Fig. 6 – while metabolic products leave into the drain exit.

expensive testing instruments. Currently used methods, briefly mentioned, range from electrochemical measurements, magneto-resistive detection, acoustic and mass spectrometric, nuclear magnetic resonance, to chromatography. Particularly rapid progress in detection and measurement techniques is currently the field of biosensors for discovery of pharmaceuticals substances. Characteristic is the intensive development activity in DNA based pathogen detection. Since minimum detection levels vary between 10^5-10^6 target molecules, direct target probing using hybridization-based assays would be of limited sensitivity and the centralised detector is therefore usually integral with signal enhancement techniques, such as continuous-flow micro-polymerase chain reaction. This may be miniaturised to share with the DNA detector a single small chip (necessary zones of different temperatures for thermal cycling are established along a channel through which the sample is moved). Another powerful analytical tool attracting currently considerable development effort are immunological methods that rely on the specific affinities of protein-protein, protein-carbohydrate or protein-DNA interactions. Very promising for microscale measurements are surface plasmon resonance biosensors (Homola and Piliarik, 2006).

4. Fluidic control of flows in the test

Apart from the transport of samples, fluidics can also perform other useful tasks, such as the control of the essential item in the list of the steps in Fig. 1 - the test reaction. A typical example is the control of flow rate through a chemical micro-reactor by a valve at the reactor inlet or outlet in Fig. 9. An example of the valve for this purpose is described in Tesař (2002). Presented in Figs. 10, and 11 are illustrations from this paper. The opposing jet can generate virtually a variable plug (Fig. 11) that can block almost completely the outflow of the reaction products. This valve principle has a quite unique position. Typical principles used in fluidic valves use inertia of the fluid, accelerated onto the desired flowpath – or, to turn down the flow, use centrifugal action in rotating fluid. Both, however, fail at low Reynolds numbers (which may be usefully interpreted as the ratio of an acceleration force to viscous damping force acting on fluid). In chemical reactions the flow velocities may be rather slow (flow rates are limited by the kinetics of the chemical reaction) and the handled fluid may have quite high

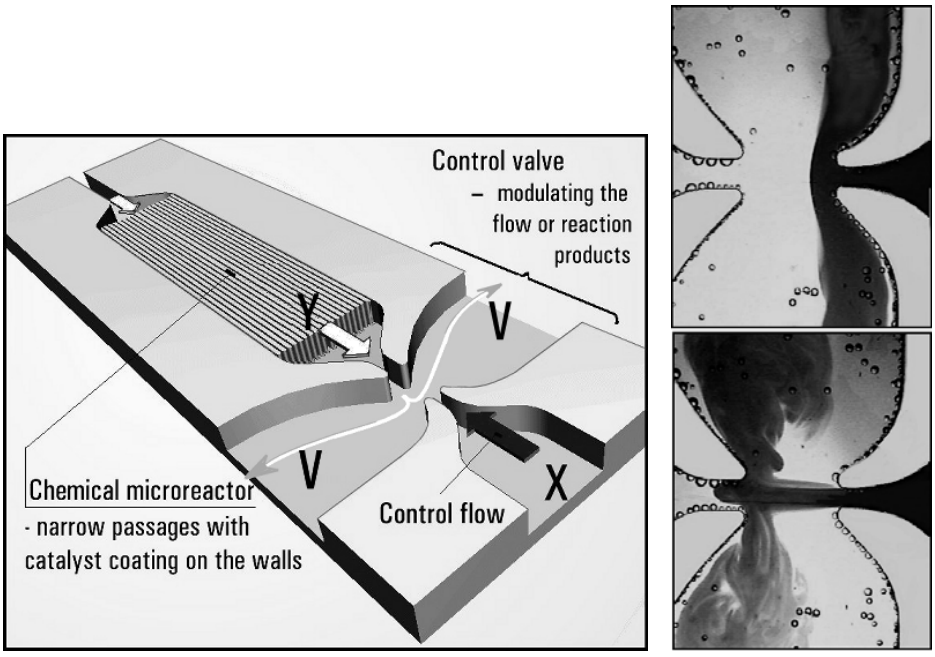


Fig. 9 (Left) En example of low Reynolds number fluidic flow control valve. The flow rate of reaction products at reactor exit Y into the discarding vents V is varied by the control flow from X.

Fig. 10 (Right top) Microscope pictures of the flowfield inside the colliding-flows valve in Fig. 9. In the case of small control flow in X shown here there is no obstacle to the flow of reaction products.

Fig. 11 (Right bottom) The same valve as above, with increased control flow in X effectively plugs the reactor exit.

viscosity (high viscosity values are a typical property of in many biological fluids). If this is associated with the always desirable small size, Reynolds number values may be really small. It is important that the no-moving-part flow control principle demonstrated here in Figs. 9, 10, and 11 can work effectively at very small Reynolds numbers where the fluid flow may have practically a character of creeping motions.

5. Combinatorial testing of catalysts and enzymes

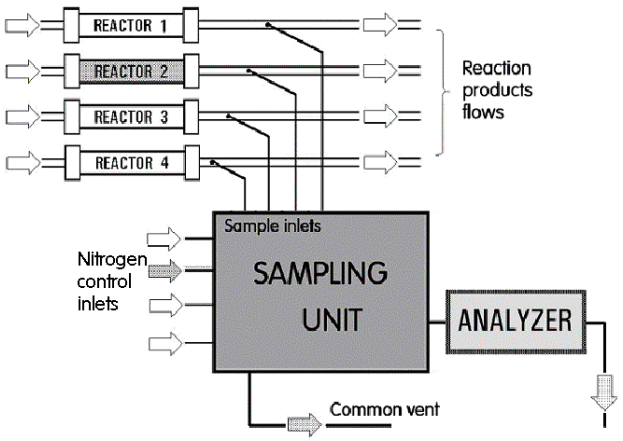
As was already mentioned, testing of catalysts has a particular position in combinatorial chemistry because the mechanism of catalyst action is still not well understood and empirical tests are practically a necessity. Moreover, a specific character of discovering efficient catalysts is caused by the necessity of their use in mixtures (and many also at increased temperatures). The resultant additional degrees of freedom require tests with a vast number of combinations of the prospective candidate substances at different mixing ratios.

Very similar – and in fact even less understood - are tests of enzymes. These behave practically as catalysts in organic chemistry and biochemistry — and are also typically used in multi-component mixtures. A particular example of such mixtures that are currently an object of intensive research are enzymes needed for plant cell wall destruction. This is the decisive step in generating biogas from biomass – and also the most expensive part of the production of ethanol from lignocellulosic biomass (e.g., Adney et al., 1991). The enzyme mixtures currently developed are based on naturally occurring fungal proteins. A recent result of such discovery activity was finding the optimum six-component enzymes mixture for biomass processing (Bharadwaj et al., 2010) – by investigations that characteristically used the high-throughput microfluidic techniques.

5.1 A particular case: Catalysts for Fischer-Tropsch synthesis

Chemical synthesis known as the Fischer–Tropsch process is a collection of reactions, concentrating on hydrogenation of carbon monoxide, that converts into liquid hydrocarbons a mixture of CO and H_2, known as "*synthetic gas*" or "*syngas*", . The process was developed in Germany (a country having no noticeable fossil liquid hydrocarbon sources) during WWI when it played an essential role in the war, being used to produce automobile synthetic fuel and petroleum-derived lubricants from coal (After WWI patented in the USA: Fischer and Tropsch, 1926). Synthetic fuels made by early form of the Fischer–Tropsch process were expensive and when the war was ouver they could not compete with the then cheap crude oil. It was, however, used in Germany again during WWII. Currently it is gaining renewed popularity in the USA due to the recently discovered large deposits of exploitable shoal gas in that country. Shoal gas may be converted (by reforming) into the syngas for the Fischer–Tropsch process. Another reason for current interest is the possibility of producing low-sulfur diesel fuel (Hu et al., 2012) from biogas. According to some analyses, fast multiplying unicellular plants (algae) may be rather economically grown for their biomass, with Fischer-Tropsch reaction converting it into automobile fuels. This would remove current dependence on the fossil crude oil from politically unstable regions. At any rate, gradual exhaustion of fossil hydrocarbon reserves is foreseen and the algae biomass process with conversion to liquid fuel is a promising possibility how to use effectively the energy from Sun.

An inconvenient aspect of the classical Fisher-Tropsch synthesis process was the necessity of working at high temperature and pressure levels. The two inventors themselves found the possibility of reducing the requirements by using suitable catalysts - which can also improve aspects like selectivity and yield of the synthesis. The catalysts used are heterogeneous, usually applied as a thin coating on reactor walls (or surfaces of small refractory bodies inserted into the reactor). Cobalt oxide was introduced as catalyst initially by Fisher and Tropsch themselves in 1913 – a step that made the reaction industrially practicable at 120 bar and 400 ^{o}C. Subsequent development of the process has later, during WW II, led to catalysts consisting of various mixtures of Rh, Cu, SiO_2, K_2O which made the synthesis possible at only 10 bar and 225 ^{o}C. Continuing decrease of these parameters is essential for economy of the process. Indeed, recent reports indicate that the progress with advanced catalyst mixtures has already led to a process demonstrably performed at essentially atmospheric pressure - although at high temperature and with some inconvenient steps (that may be removed by further progress).



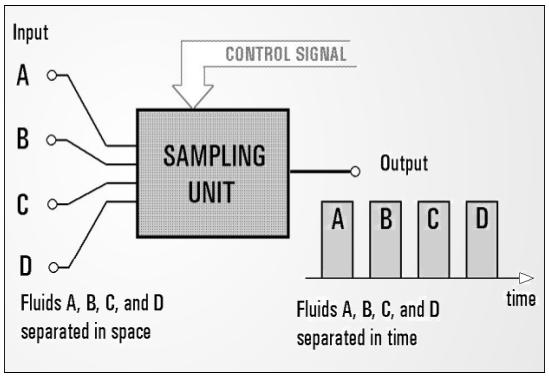


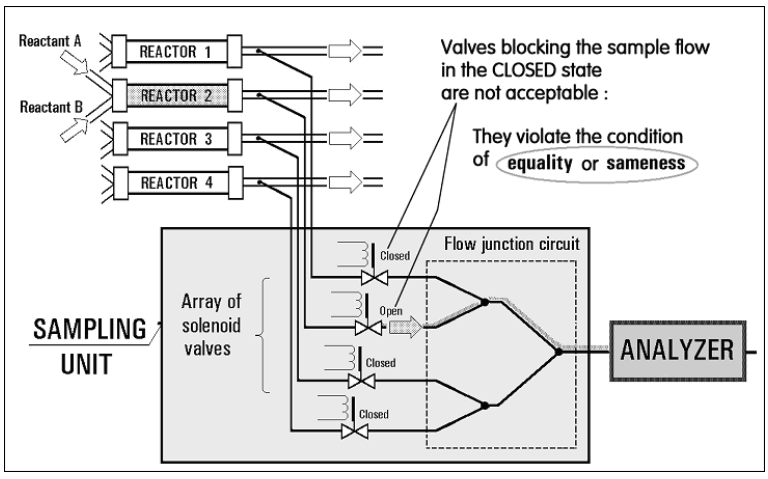
Fig. 12 (Left) The task solved by the present author: taking gas samples from an array of test reactors and delivering them, one by one, with high sample purity, into the IR spectrometer analyser. For high-temperature operation, the sampling unit consisted of an array of no-moving-part fluidic valves.

Fig. 13 (Right) What the sampling unit in Fig. 12 does is, in principle, a conversion of spatial distribution at its input into the temporal sequence of the samples at the unit exit.

7. Author's own experience – the task

Present author acquired a first-hand experience with designing a facility for high-throughput testing of catalytic mixtures at elevated temperature and pressure levels - which it may be useful to present here in more detail. The project in which the author participated was financed by a grant from the Institute of Applied Catalysis in London. The particular task was to develop catalyst mixtures improving economy of Fischer-Tropsch process of CO hydrogenation. The laboratory facility developed in the project was based on the idea of multichannel system of parallel reactors, quantitative on-line FT infra-red analysis of gas samples, and generic algorithm library searching technique of Wolf, Buyevskaya, and Baerns (2000). The catalysts were in the form of thin coatings on the surfaces of silica beads contained in the reactor cavities. They were mainly based on rhodium, cobalt, and manganese with alkali metal promoters. The metal concentrations were varied within close limits so that the optimum was evaluated from a library containing 16 000 tested catalysts.

The basic ideas of this combinatorial testing were described in Wilkin et al., 2002. It is a typical case of working with gases rather than liquids. Any vials or similar static gas-holding spaces were out of question. Instead, the tests involved full running of the hydrogenation reactions in the parallel reactors and using a sampling unit (Figs. 12, 13) to take a sample of the gas from the reactor outputs one by one in a fixed sequence and delivering it into the FT-IR analyser. The sampling unit with fluidic (and later microfluidic) valves was made integral with the reactors and author's task was to design - and test in laboratory - this core part of the facility. The whole assembly of the sampling unit and reactors was placed inside a specially built oven, capable of keeping within the reactors a temperature (adjusted in a programmed sequence) up to 400°C. While the target was identification of the optimum catalyst mixture that would allow performing the synthesis at low (if possible practically atmospheric) pressure, the design was requested to be able to handle even high-pressure tests up to 4 MPa (necessary for early classical catalysts). The core part of the facility was made as a stack of 0.25 mm thin stainless steel foils. The cavities for the gas flow inside them were made by photoetching (the operation was made by Microponents Inc., Birmingham, U.K.). Any leakage at the high pressure - in circumstances that did not allow for use of standard soft sealing materials - was prevented by externally pressing together all stainless steel plates in



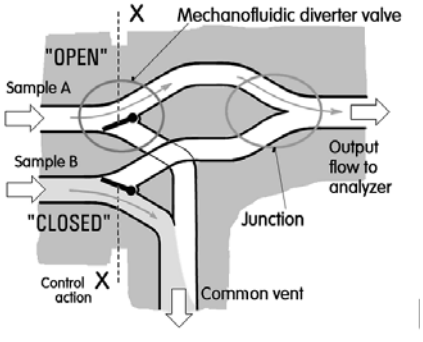


Fig. 14 (Left) The hypothetical sampling unit formed by an array of solenoid ON-OFF flow-blockage valves. This may seem to be the simplest approach – unfortunately not acceptable. Apart from the impossibility of exposing these valves to the high temperatures, opening a valve would change the pressure conditions in the corresponding reactor. What was needed were obviously diverter valves rather than turn-down ones.

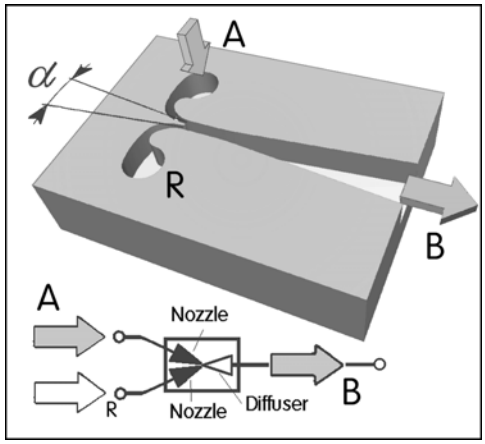
Fig. 15 (Right) Schematic representation of mechanical diverter valves in two neighbour flowpaths, one in the OPEN state (sample flow allowed to enter the analyser) and the other in the CLOSED state, leading the flow from the reactor into the vent.

the stack, the clamping force surpassing the stainless steel yield strength. The sampling unit was essentially an array of no-moving-part fluidic valves. They were made by etching in round-shaped stainless-steel foils. In each foils there were 16 flow control valves. In the final version of the sampling unit the valves were arranged radially (in earliest designs they were parallel). Number of foils forming the sampling unit stack was variable – though, due to the exploratory character and inevitable need for changes during the development, most testing was made with only a single 16-valve foil. The high operating temperature was one of the main reasons why the sample flow control could not be done simply by standard mechanical valves - e.g., solenoid valves as they are shown schematically in Fig. 14. The fluidic valves were chosen instead because no solenoid valves available on the market could withstand the 400°C temperature condition inside the oven. The fluidic valves were switched between two regimes – called OPEN and CLOSED state - by fluidic signals. These were flows of chemically neutral nitrogen, brought in from outside the oven. In contrast to taking just a small sample (as suggested in Figs. 12 and 14), later small size of the reactors led to all the reactant flow being brought into the analyser when the valve in the sampling unit was in OPEN state.

There were two essential (perhaps seemingly simple) requirements the design had to strictly observe and meet:

(1) ***Sameness – in particular keeping the same pressure in all reactors all the time***, irrespective whether the flowpath through a reactor led into the analyser or into the common vent outlet.

(2) ***Avoidance of any cross-contamination between different samples.***



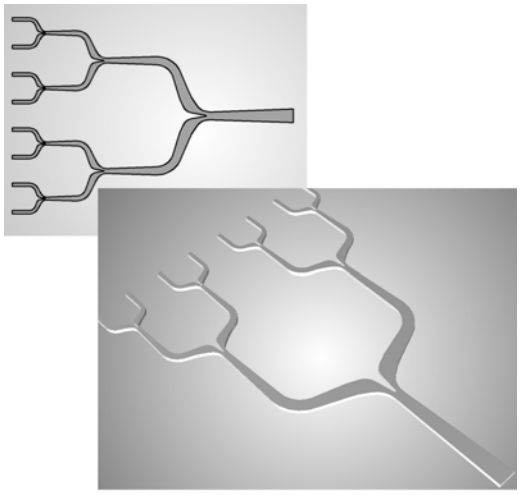
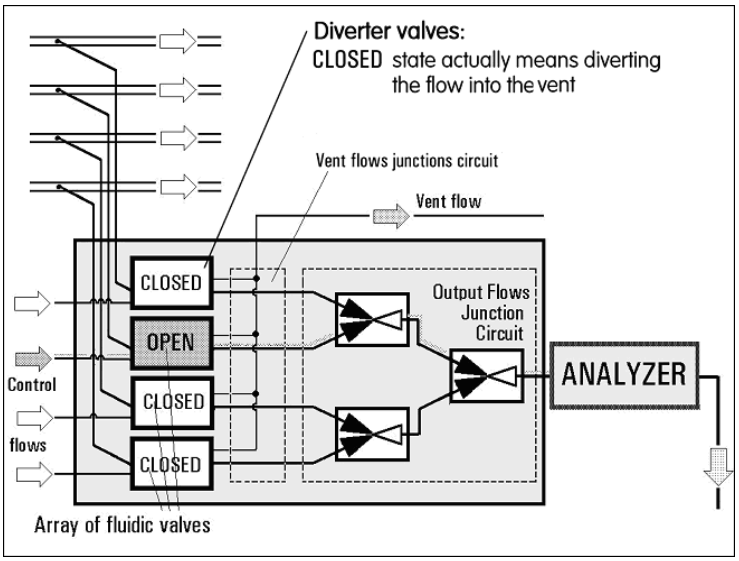


Fig. 16 (Left) Schematic representation and an example of a fluidic device in which the mutual jet-pumping action prevents a return into R of the fluid that flows from A into B.

Fig. 17 (Right) Junction circuit consisting of the devices shown in Fig. 16. Fluid entering any one of the inlets at left must pass only into the single outlet at right. It should not get into any one of the other inlets irrespective of what pressure levels (within reasonable limits) are present there. In this case this is achieved by the jet-pumping effect in the junctors (which are, in principle, symmetric jet pumps.

It may be not always recognized that the sampling unit actually consist of two essential constituent parts: apart from the array of the valves there is the less obvious but also important flow junction circuit (- shown at the right-hand part of the sampling unit in Fig. 14). If conventional mechanical valves were used as is the case in Figs. 14 and 15, the junction circuit may be rather trivial - just all the valve outlets are connected into the single exit into the analyser. The mechanical closure would eliminate any communication between the



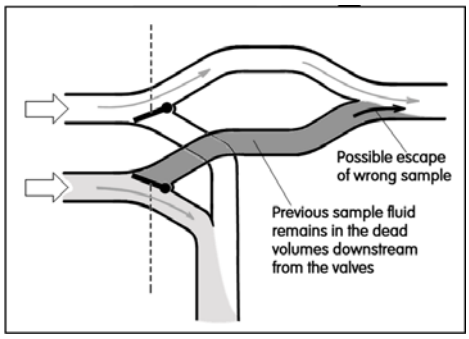


Fig. 18 (Left) Schematic representation of a sampling unit based on diverter valves with the passive junctor in the junction circuit. In the CLOSED state of the valve, the sample flow is dumped into the common vent. The junction circuit contains the mutual jet pumping devices according to Fig. 16. Their operation is based on dynamic action, which means that no proper functioning can be expected at very low Reynolds numbers.

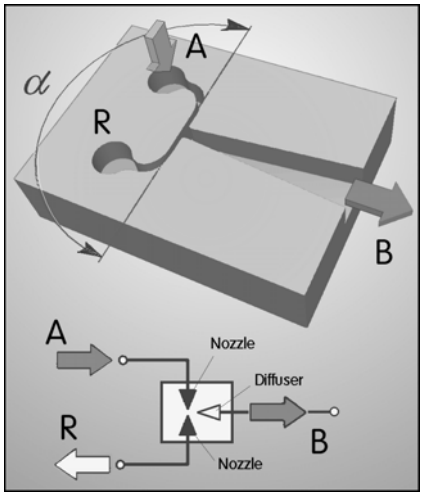
Fig. 19 (Right) Schematic representation of a part (showing only two valves) of the sampling unit with the diverter valves as shown in Fig. 15. The dark shaded area indicates the dead volume in which remains a previous sample that may escape and contaminate a sample from the neighbour test.

cavities containing different samples. The more elegant no-moving-part pure fluidic solution does not have such automatic prevention of the possible contamination between the different samples. Thus much more thought had to be given to the junction circuit and its operation. The manner in which the flowpaths from individual valves are interconnected to lead to the analyser – and similarly also to the vent – may influence significantly the observance of the no cross-contamination request. The possibility of the contamination – in fact quite improbable, nevertheless not absolutely eliminated – is due to the absence of the flowpath blockage in a fluidic jet-type. The reaction products from all CLOSED valves (Fig. 18) are discarded into the vent, where they are mixed. Obviously, it is absolutely necessary to prevent the return of this mixture from the vent into the valve which is temporarily in the OPEN state. This is the task of the vent-flows junction circuit. In Fig. 19 (showing for clarity only two valves) it may be noted that with the mechanical moving parts in the valves no such return flow is possible.

Another possibility of contamination is a contact with remains of previous samples that may be trapped inside various cavities of the sampling unit. The solution schematically represented in Fig. 18 also contains passive flow-interaction devices – in the output-flows junction circuit. The devices presented there correspond to those in Figs. 16 and 17 (although they may have different angles α than is shown in Fig. 16).

7.1 Sampling unit with high Re fluidics

Author's initial response to the requirements was designing the sampling unit using known (and more or less already proven) fluidic valves operating at rather large Reynolds-numbers. Similar operating regime was also considered for the passive devices forming the junction circuit. The first main requirement, that of the sameness, was met by the use of diverter-type valves. It should be noted that although the flow regimes in the valves are described as OPEN and CLOSED, what the valves perform is not a closure of the flowpath. Instead, the flow in the CLOSED state is led into the vent and the flow in the OPEN state is led into the analyser. The



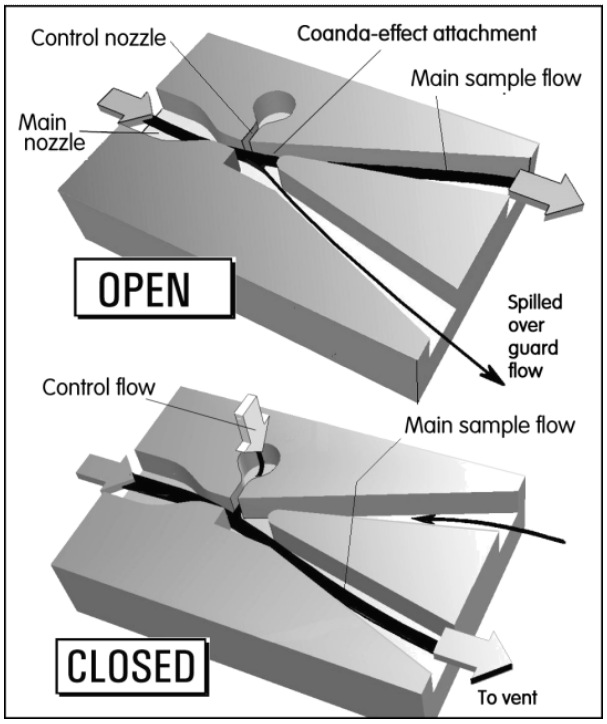
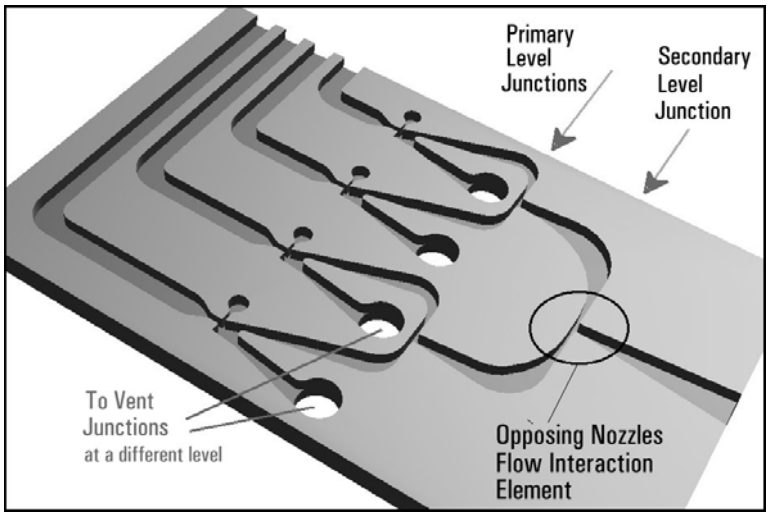


Fig. 20 (Left) Schematic representation and an example of a fluidic device related to the junctor shown in Fig. 16, differing in the large interaction angle α . The fluid flow from A into B here does not allow any flow in B being contaminated by the other fluid from R.

Fig. 21 (Right) An example of a monostable switching valve based on the Coanda-effect attachment of a jet to an inclined wall after leaving the main nozzle. These valves were the basic building blocks of the sampling unit, as shown in the next Fig. 22.

conditions inside the vent were adjusted so that the pressure inside the reactors is the same whether the sample flows passing through them go into the analyser or into the vent. The fluidic valves used in this initial design were of the monostable Coanda-effect jet-deflection type as shown in Fig. 21. There are actually two illustrations in Fig. 21, the one above showing the flows in the OPEN and the other below showing the CLOSED state. The valves were made by etching of the cavities into the stainless-steel foil. The top cover plate which is normally fixed to the top of the body is in these pictures removed so that it is possible to see the internal fluid flows. Valves are shown with their inlet for the gas sample at left. On the opposite right-hand side there are two outlets. In the OPEN state the top one of these outlets leads the sample into the analyser. In the CLOSED state the other outlet leads it into the vent. The flow of the gas sample leaves the main nozzle as a jet and enters the interaction cavity of the valve. The cavity is a space between two walls. One of the walls – the attachment wall – is placed nearer to the nozzle exit. The other wall, on the opposite side, is by a visible distance farther from the main nozzle exit (there is a step shown at this side of the main nozzle exit in Fig. 21). This makes attachment of flow to this side more difficult (if any attachment can occur at all). At one side of the valve – the side where there is the attachment wall - there is the control inlet leading into the narrow control nozzle, positioned between the main nozzle exit and the beginning of the attachment wall.

In the OPEN state (top part of Fig. 21) there is no control flow. The jet leaving the main nozzle attaches by the Coanda effect to the attachment wall (because, as mentioned above, an attachment on the opposite side is suppressed by the sidestep while the attachment wall is nearer to the jet). The sample flow is thus led into the analyser. Switching into the CLOSED



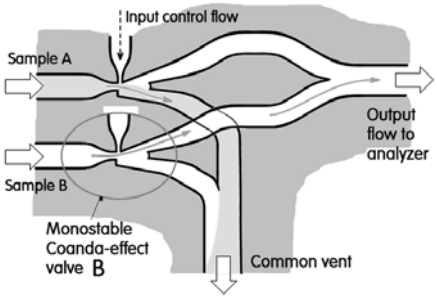


Fig. 22 (Left) An example of a part of integrated circuits of the high Re sampling unit, here with only four monostable valves as they were shown above in Fig. 21, together with the corresponding part of the junction circuit. Both were made in the same stainless steel plate in a single manufacturing operation (photoetching). Depending of the aspects of possible contaminations, the junction circuit may consist of either the devices from Fig. 16 or those shown in Fig. 20 (or something in-between, dependent on the choice of the angle α (Figs. 16 and 20). Shown here is the case α = 180 deg (Fig. 20).

Fig. 23 (Right) Schematic representation of a part (showing only two valves) of the sampling unit with the diverter valves as shown in Figs. 21 and 22. The control flow diverts the sample A into the vent. Absence of the control flow allows the sample B to flow into the analyser. The junctor devices are here only rudimentary.

state is achieved by the action of the small control flow of nitrogen fed into the control terminal. It displaces the jet from the attachment wall and pushes it to the opposite side of the interaction cavity. Even though this control flow is small, its momentum suffices for separating the flow from the attachment wall and thus for directing the sample into the other outlet, the one leading to the vent.

Characteristic features of the sampling unit designed for the high Reynolds number regime are apparent from Fig. 22, which shows a small part (only 4 valves) of the sampling unit. The valves there are arranged in parallel (according to the earlier line of though concerning the sampling unit layout – later in the development history the arrangement was changed to radial). Apart from the valves there is in Fig. 22 also the output-flows junction circuit (cf. Fig. 18) handling the flow into the analyser at progressive junction levels (in the four-valve case there are only two levels, a primary and secondary one).

The other basic requirement of the sampling unit design, elimination of the possibility of cross-contamination between the samples, was also met in the large Reynolds number version of the sampling unit. There are two possible contamination effects, as already mentioned above, and their suppression is made by small auxiliary flows: (a) The "guard" flow eliminating the possibility of return flow from the vent, and (b) cleaning return flow in the CLOSED regime eliminating the possible mixing with samples from "dead" regions downstream from the valves.

7.2 Protective flows

a) The idea of the "guard" flow was invented by Dr. Tippetts at the University of Sheffield. Its purpose is to prevent a return of the uncontrolled mixture of samples from the vent (Fig. 24) back into the valve in its OPEN state (from where it would go to the analyser, endangering accuracy of the analysis). There are two alternative possibilities how to obtain this flow – both alternatives may be used together to support one another. One of the approaches may use the

properties of the passive devices shown in Figs. 16 and 17 placed into the flowpaths from the OPEN state exits from the valves into the common vent. Another possibility how to generate this protective flow is to adjust geometry of the downstream flowpath cavities as well as the resistances of the channels leading to the analyser (the analyser itself has a minimal aerodynamic resistance) so that in the absence of the control flow not all sample fluid can get into the analyser. A small part of the sample is sacrificed and left to spill over onto the vent as the "guard" flow. Its flow direction is opposite to the possible flowpath of the unwelcome return flow from the vent into the OPEN state valve.

b) The cleaning return flow in the CLOSED regime is shown in Fig. 25. It removes into the vent the remaining previous sample, which in the analogous Fig. 19 (with mechanical rather than fluidic valves) remained trapped inside the unused channel (it is shown in Fig. 19 as the dark shaded area) between the CLOSED valve and the nearest downstream junctor device.

There are also here two alternative methods how to set up this removal of sample trapped inside the "dead" spaces (in Fig. 22 is shown a case of musing both methods simultaneously). Both are sacrificial methods. This means a small amount of the currently analysed gas sample is pushed in the direction opposite to the normal flow from the valve to the analyser. One of the methods is the pressure balancing method with passive devices of the type shown in Fig. 20 placed in the output-flows junction circuit, as seen in Fig. 22. To generate the cleaning return flow is the task of passive junctor elements with opposed nozzles (Figs. 20 and 22), i.e. with the angle $\alpha = 180$ deg (this is also as shown in Fig. 22). The other method is represented in Fig. 25. It uses the properties of the jet-deflection flow control valve. The contamination is eliminated by the return flows produced by the jet-pumping effect in the valve as shown in Fig. 21.

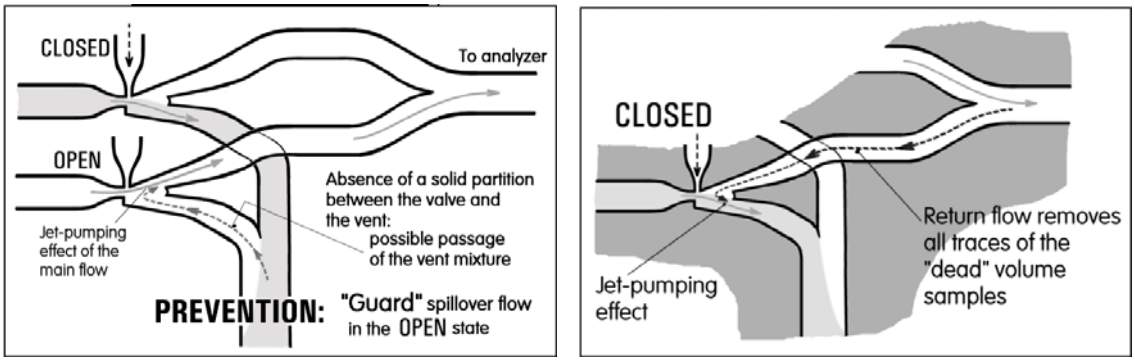


Fig. 24 (Left) With the no-moving-part fluidic valves in the sampling unit, there is no solid-wall separation between the sample flow into the analyzer and the incontrollable mixture of fluids in the common vent. Although the danger of a return flow from the vent is not high, in the interest of absolute sample purity it is advisable not to lose sight of this eventuality and to arrange generation in the OPEN state of small "guard" over-spilling flows protecting the sample.

Fig. 25 (Right) One of the advantages of fluidic valves in the sampling unit is the prevention of the "dead" volumes contamination by the return flow. To enhance this effect, it is desirable to make the junction circuit as consisting of the passive devices shown in Fig. 20.

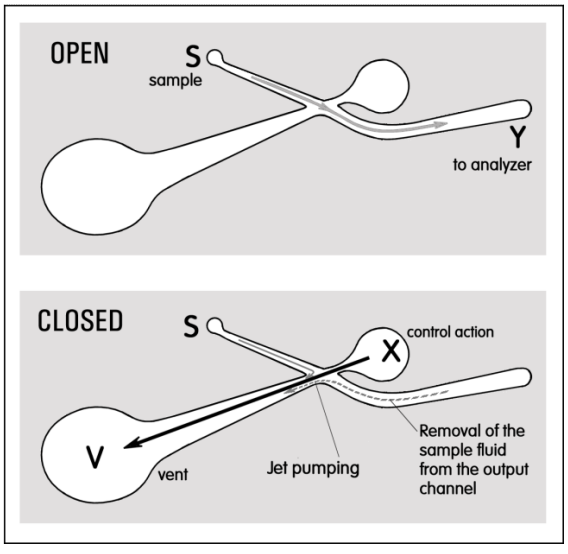
7.2 Low Re microfluidics

Designing devices with suitable properties and then securing the pressure balance the whole fluidic systems for proper functioning were by no means simple. After laborious development, the configuration corresponding to Fig. 22 was run successfully when tested with cold air flows. Then, however, the ideas about the whole facility underwent a considerable change. On one hand, opinions have changed concerning the number of

simultaneous tests. It became desirable to have in the test facility smaller test reactors - with much smaller reactant flows. This allows testing simultaneously more catalysts. Of course, this meant lower velocities in the fluidic valves. Also, as opposed to the favourable experience with cold air, there was a problem with high viscosity of syngas (containing a considerable proportion of large-viscosity hydrogen) especially at the high temperature levels (of course, gas kinematic viscosity increases with increasing temperatures). With these two factors and desirable small valve dimensions, it became obvious that it is necessary to work with smaller than originally envisaged Reynolds number. In fact, already in what is described here as "high Reynolds number" version of the sampling unit, the values were lower than those making possible optimum operation of jet-deflection valves.

The Coanda effect of jet attachment becomes very unreliable at Reynolds numbers less than about Re ~ 800 and ceases to exist at all at values no much lower. The changed opinions and requirements of the chemists (that were in charge of the overall project) indicated desirability of Re ~ 200 or even less (in the end the valves were actually operated at Reynolds number as low as Re ~ 30). These values, of course, are typical for laminar character of flows – with very small, nearly negligible jet pumping and also weak and unreliable jet attachment to a wall. It became obvious that the circumstances called for a wholly new look at the sampling unit. The unit was to be designed with different, microfluidic valves. The opportunity to enter the not yet fully investigated region of pressure-driven microfluidics (Tesař, 2007) also was challenging and the author therefore did not object to this new start.

To answer the challenge, it was decided to retain the basic philosophy of the flow-diverting valve design with the two sorts of small protective flows. Perhaps surprisingly, the new design has actually led to deserting the very basic idea of fluidics: the idea of an amplifier in which a rather weak control flow signal influences a powerful flow from an external supply.



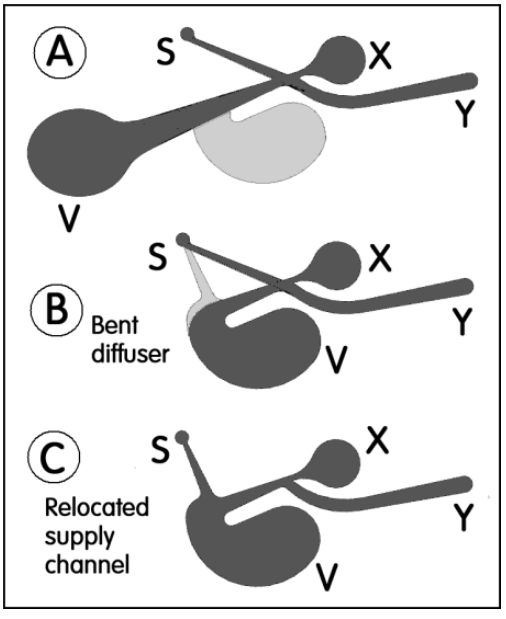
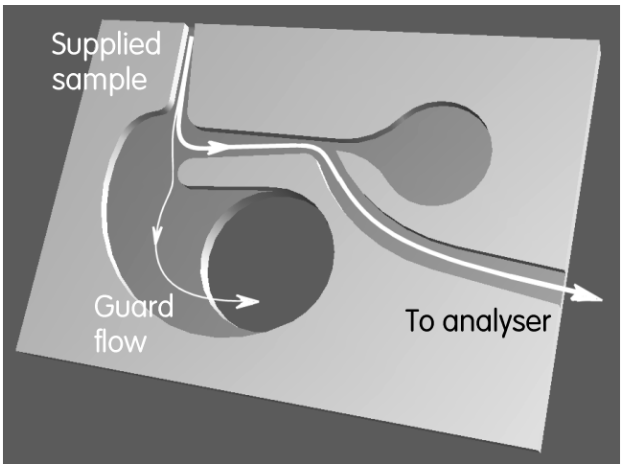


Fig. 26 (Left) The problem of very low Reynolds number valves. Shown here is one of the initial geometric variants of the low-Reynolds-number valve developed for the sampling unit. Instead of the insertion of a solid partition, in the CLOSED state the flow of the sample from S to the output Y is prevented by the powerful control jet from the control terminal X. The jet-pumping entrainment action of this control flow also performs the secondary task - the generation of the return flow from the downstream cavities, as shown in Fig. 24.

Fig. 27 (Right) Stages of development of valve configuration. The original valve geometry A corresponding to Fig. 26 progressed through stage B with the bent diffuser to the shape C with different position of the entrance from supply terminal.



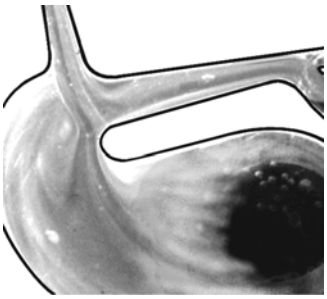


Fig. 28 (Left) The very low Reynolds number valve. Shown here by arrows in the valve configuration C from Fig. 27 is the sample flow in the OPEN state. Note that the relocated supply channel mouth (changed from shape B to C in Fig. 27) produced the desired "guard" flow, a small sacrificial flow into the vent which prevents the vent mixture from coming back into the valve cavities.

Fig. 29 (Right) Visualised flow of the sample in the OPEN state of the valve from Fig. 28. Visualisation of water flow in a scaled-up model with dye added to water. The image shows the existence of the "guard" flow into the vent.

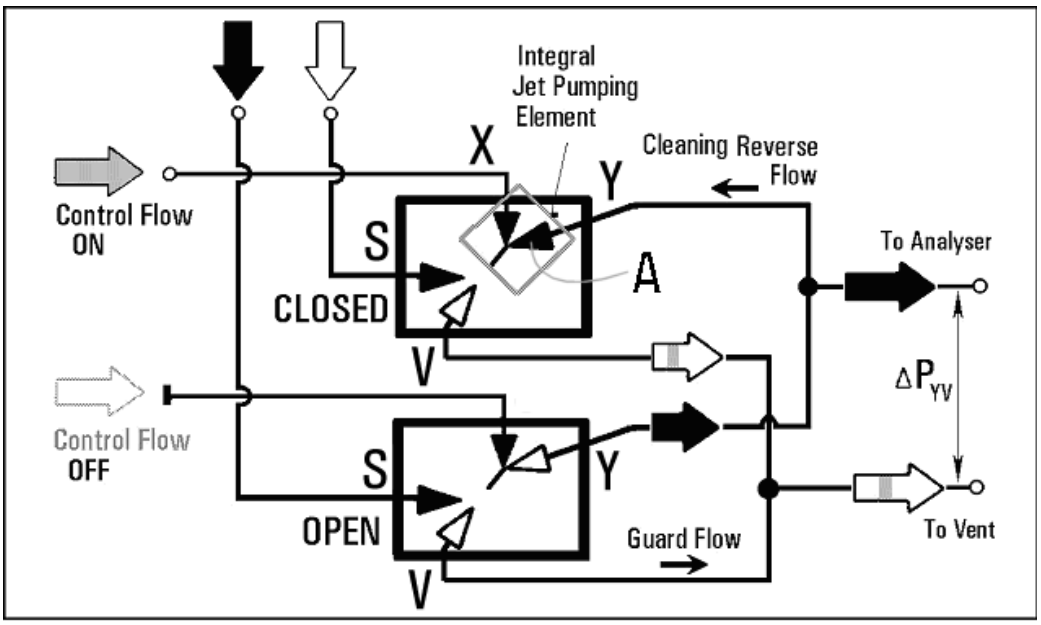
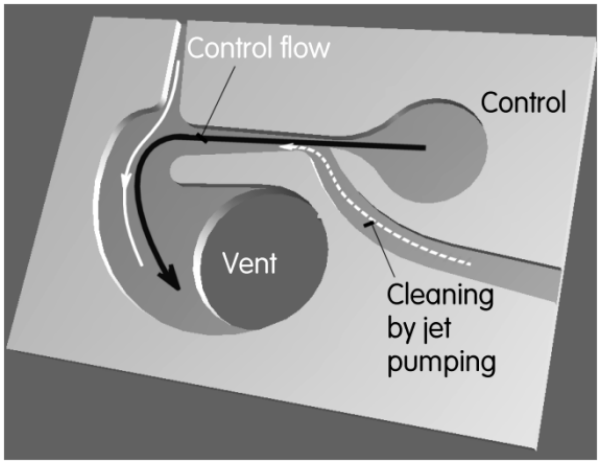


Fig. 30 Schematic representation of a part (two neighbour valves) of the low Re sampling unit. Note the jet pumps integrated with each valve. One of the valves (top) is in the CLOSED state, the other valve (below) is in the OPEN state. The OPEN state flows are driven by the pressure difference ΔP_{YV} between the analyser and the vent, while the CLOSED state is dominated by the powerful X flow. Note the change in character of the element A in the CLOSED state when the flow in it reverses and it loses its diffuser character (which, at the small Reynolds numbers in questions, is more or less only symbolic anyway).

It should be said that historically the whole field of fluidics emerged (in 1960's) after the invention of the pure-fluidics amplifiers as the key devices. The valves presented in Fig. 21 are in principle nothing else that a version of such amplifiers. The requirement of no contamination – met by arranging for the small auxiliary flows – meant it was necessary to generate the jet pumping effect. This, however, called for a jet flow with turbulent character. This is not achievable with the weak sample flow. It was therefore decided to design the valves with the " fractional gain", i.e. with the ratio of the controlled flow to the control flow



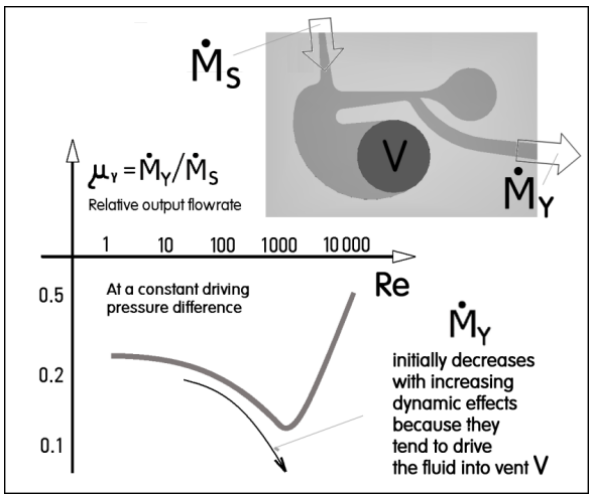


Fig. 31 (Left) The control (black) and sample (white) fluid flows in the final version of the very low Re valve in the CLOSED state. The jet-pumping effect of the powerful control flow cleans the "dead" volume between the sampling unit and the analyser.

Fig. 32 (Right) The relocation of the supply channel (change from B to C in Fig. 27) resulted in an unusual character of the transition into the subdynamic regime, presented here in terms of the dependence of the relative output flowrate on the Reynolds number Re.

(the ratio termed flow gain of the device) much less than 1.0. In contrast to the small available flow rate of the reactant products samples, there was no limitation on the power of the control flow (- the nitrogen flow from and external source: a high pressure cylinder).

The basic idea of the new microfluidic valves is presented in Fig. 26. There is no jet deflection – and, in fact no generation of the jet, which at the very low Reynolds numbers would not reach to any useful distance from its nozzle anyway. There is just the flowpath of the sample from the supply terminal S to the output terminal Y, obstacles-free in the OPEN state (top of Fig. 26). In the CLOSED state (bottom part of Fig. 26) this flowpath is practically blocked by the powerful control flow, the Reynolds number of which is high enough for its being turbulent and capable thus of entraining both the fluid from S (so that the pressure inside the reactor upstream from S does not increase) as well as from Y – where is generated the small cleaning return flow. The actual geometry of the valve resulted from several development stages (Fig. 27) necessitated by adopting the positions of the terminals dictated by other components of the unit being taken over from the earlier high-Re version. Some of the changes were actually demanded by the requirement of generating the guard flow – Figs. 28 and 29. The behaviour of the valve in its two regimes OPEN and CLOSED is shown in Figs, 28 and 31. It may be also instructive to follow the role of the terminals X, Y, S and V in the schematic representation in Fig. 30, which aims especially at showing the jet pump component as an integral part of the valve. The representation in Fig. 30 follows the conventions of standard high-Re fluidics with nozzles represented by black triangles and diffusers as white triangles. Of course, at the almost creeping flows associated with the small Re conditions nozzles and diffuser lose the basic importance they enjoy in high-Re fluidics.

Most laborious in the development of these microfluidic versions of valves was securing the correct magnitude of the guard flow into the vent in the OPEN state. It was necessary to ensure this flow does exist, but is not too large because it would mean loosing too much sample gas. Fig. 27 shows, by comparison of the case B with the shape C one of the approaches leading to an increase of the guard flow: the relocation of the inlet of the sample into the valve into a position nearer to the vent, whereby the guard glow rate was increased.

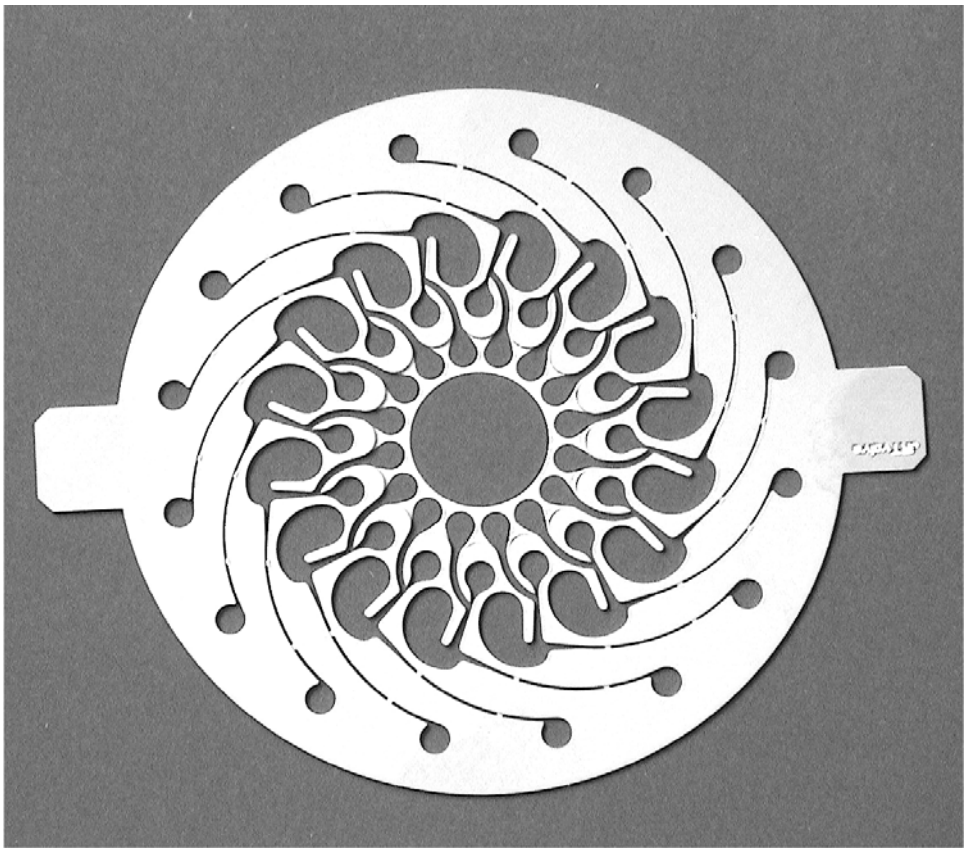


Fig. 32 (Right) A photograph of the microfluidic 16 channel sampling unit. The diameter of the 0.25 mm thin stainless steel plate is 82 mm.

Computed dependence in Fig. 32 of the ratio of the output flow rate $\dot{M}_Y$ to the supply flow rate $\dot{M}_S$ (the guard flow being the difference between the two) shows the complicating factor of dependence on Reynolds number.

The final version of the valve plate of the microfluidic sampling unit is presented in the photograph in Fig. 33. Apart from the valves the plate also contains laminar-flow resistors incorporated to ensure more even distribution of the flows (and reducing the high pressure needed in the reactor to the lower values prevailing in the valves).

8. Conclusions

Paper surveys progress in combinatorial chemistry, biological testing, and related areas that necessarily have to handle a large number of samples has been recently very rapid. It became one of the important applications of present-day microfluidics. The effort already brings its results: in some areas were accumulated quite extensive combinatorial libraries often leading to useful optimisations. The results are unfortunately not discussed in literature as often and as much as these achievements would deserve. Part of the explanation is the fact that some the results are considered a highly valued intellectual property of the organisations that financed the extensive and therefore costly investigations. Of course, considering the huge amounts of possible useful and marketable combinations that were not yet tested (and in foreseeable future obviously will be not tested – because of their huge numbers) there is a vast space open for continuing research and perhaps even new approaches. Substantial progress has been made in evaluation of the accumulated data, where the mathematics of the search for the optimum is complicated by such aspects as there being usually not a single optimality criterion but a number of them, some actually mutually contradictory.

The microscale aspect of the facilities for combinatorial chemistry has already been proven to be the main road that is to be followed. Earlier designs, quite simple from the present point of view, with the tested substances typically positioned in the titre-tray wells and moving robotic probes, have generally been by now replaced by the approach using no-moving-part fluidic circuits – and more often also new microfluidic valve designs, because the necessarily small size (required for performing many tests simultaneously) leads inevitably to low Reynolds number operation where the classical fluidic designs cease to be useful.

As is shown in this paper, the problem of avoiding the cross-contamination between the samples made the fluidic and microfluidic circuits more complex than was originally expected and special fluid flow control valves have been developed to tackle this issue.

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