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AN OVERVIEW ON MICROFLUDIC TECHNOLOGY IN PHARMACEUTICAL ANALYSIS

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ABSTRACT

In the current trends of the analytical chemistry, miniaturization is one of the fast developing research areas. In this miniaturization of analytical techniques, the microfluidics technology plays a very important role. Microfluidics technology means, it is a device that performs one or several laboratory functions on a single chip of only millimeters to a few square centimeters in size. It deals with the handling. Manipulation and controlling of very small fluid volumes like picoliters. Modern developments in the design and utilization of microfluidics devices for fluid transport have found many applications, ranging from the life sciences industries for pharmaceuticals and biomedicine (drug design, delivery and detection, diagnostic devices) to industrial applications of combinatorial synthesis (such as rapid chemical analysis and high throughput screening).There is great interest today in the possibility that prefabricated, miniaturized laboratories will fill this need. A large number of analytical micro fabricated devices have been developed since the concept µ-TAS was introduced. In pharmaceutical and bio analytical research, micro devices have widely been developed for proteomics, genomics, clinical diagnostics and drug discovery.

Keywords: Micro fluidic system, Combinatorial synthesis, Fluid transport.

INTRODUCTION

In the current trends of the analytical chemistry, miniaturization is one of the fast developing research areas. In this miniaturization of analytical techniques, the microfluidics technology plays a very important role. Then question comes that, what is mean by the microfluidics technology. It is a device that performs one or several laboratory functions on a single chip of only millimeters to a few square centimeters in size. Basically it deals with the handling, manipulation and controlling of very small fluid volumes down to less than picoliters.

Such Microfluidics devices are circuits of very small closed channels and wells, etched onto a glass or plastic microchip. Pressure or electro kinetic forces push small volumes of fluids through selected pathways in a controlled manner. These mini-laboratories may include elements such as pumps, valves, mixing and reaction chambers and separation channels. Since that time the field of miniaturization is known as lab-on-a-chip or micro-total analysis system (µ-TAS).The driving force for this is an increasing demand for low-cost instruments which can rapidly analyse the compounds in very small volumes with a high level of automation. The integrated micro-analytical system can perform complete analysis cycle such as sample pre-treatment, chemical reaction, analytical separation, detection, and data handling on the same micro device.

Modern developments in the design and utilization of microfluidics devices for fluid transport have found many applications, ranging from the life sciences industries for pharmaceuticals and biomedicine (drug design, delivery and detection, diagnostic devices) to industrial applications of combinatorial synthesis (such as

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rapid chemical analysis and high throughput screening). There is great interest today in the possibility that prefabricated, miniaturized laboratories will fill this need. A large number of analytical micro fabricated devices have been developed since the concept µ-TAS was introduced. In pharmaceutical and bio analytical research, micro devices have widely been developed for proteomics, genomics, clinical diagnostics and drug discovery.

THEORY INVOLVED

Microfluidics is the miniaturization of biological separation and assay techniques so that multiple experiments can be performed in parallel on a device small enough to fit in the palm of your hand. Very small quantities of media, reagents and even nanoparticles are flow through narrow channels on the device where they are delivered, manipulated, and analyzed by such techniques as fluorescence detection. Multiple components, such as pumps, valves, and heaters can be integrated into the system to enable the easy manipulation of fluids. As a result, devices with microfluidic channels can be used to perform experiments with higher throughput than conventional approaches, with using minimum reagents and achieving fast reaction times. All possible unit operation happening in microfluidic chip are shown in figure.1

INSTRUMENTATION

In order to initiate, maintain and control flow through a microchannel network a number of functional elements are necessary such as pumps, valves, mixer, and heater. Such integrated microfluidics device then perform standard laboratory function like sample handling, mixing, reaction, separation and detection of analyte of interest.

i) Micro fluidic chips

Chip material should have some properties like chemical inertness, optical transparency and biocompatibility. Typical analytical micro devices are made up of glass-, silicon-, or polymer-based chips ranging in overall size from mm-to-cm scale with individual structures such as separation channels in µm-scale. Silicon has limited chemical stability and lack of transparency that's why it is eliminate as choice of material for microfluidics devices in drug discovery applications. The another substrate for microchip are polymers like poly(methylmethacrate),poly(dimethylsiloxane),
construction of polymer based chip is very simple and it can be prepared in any chemical laboratory. Glass based microchip is very famous because it has certain properties. Glass is less expensive than silicon, superior optical properties compared to other material. Glass material also has excellent shelf life. But cracking and breakage are main disadvantages of glass material using as chip material.

ii) Micro pumps

In order to move fluids through the microfluidic channel network we can apply external energy sources (pressurized air containers), simple physical principles (capillary action, gravity, surface tension, electro osmosis) or truly integrated miniaturized pumps. When choosing particular pumps for microfluidics devices we must considering following points about pump:

- as little flow rate pulsation as possible
- flow rate adjustable over a certain range
- flow rate is stable on fluctuating pressures
- resistant to chemicals, works for long periods of time at higher temperatures conditions
- production cost as low as possible

A precise control on flow rate is important to get accurate and reliable result of analysis.

In microfluidic system the sample or fluid transport is governed by various fundamental flow mechanisms, let us discuss some important method of movement of fluid in microfluidics chips.

1) Pressure driven flow

This flow of liquid is carried out by applying pressure gradient in a microchannel or capillaries. This pressure may be external or internal sources such as syringe pumps. Pressure driven platform provide advantages of processing multiple sample which is very much important on line monitoring such as water quality control. This pressure can also use in generation and manipulation of droplets of two-phase microfluidic system. Example of such pressure driven device is HPLC-chip.

2) Electro kinetic flow

Electrokinetic flow of liquid is an effect that occurs in heterogeneous fluids or in porous bodies filled with fluid. Heterogeneous means a fluid containing particles. Particles can be solid, liquid or gas bubbles with sizes on the scale of a micrometer or nanometer. There is a common source of all these effects the so-called interfacial 'double layer' of charges. Influence of an external force on the diffuse layer generates tangential motion of a fluid with respect to an adjacent charged surface. This force might be electric, pressuregradient, concentrationgradient,gravity. This kind of flow includes:

Electrophoresis is a movement of particles in porous body under influence on elecric field.

Electroosmosis is a movement of liquid in porous body under influence of electric field. It is an important principle in chemical separation techniques such as capillary electrophoresis. Based on electrosmotic flow we can able to measure and record volume up to picolitre range.

iii) Micro mixers

In micro fluidic systems fluid flow should be laminar because size of micro channels in chip are very

small, so diffusion is the main mass transfer process for mixing components and controlling the reaction rates. The required channel length of chip for complete mixing depends on several parameters such as flow velocity, diffusivity of the sample loaded and the width of the channel. Decreasing the channel width or depth will decrease the mixing time. Micromixers are divided in two types on the basis of principle followed to perform mixing at the microscale, passive and active micromixers. Passive micromixer does not require external energy, and mixing process depends on diffusion only. On the other hand, active mixer requires energy input from external sources (such as electrokinetic force).

iv) Micro valves

Micro valves are the very important component in fully integrated microfluidic analytical systems. These components control the transport of small volumes of liquids in the system and play a role in the success of the integrated analytical steps. Valves allow controlling the passage of fluids (flow or no flow), determining the direction of flow, and can help to adjust the rate of flow. An ideal valve for microfluidic system should possess some characteristics such as:

- zero leakage (when in its closed state, no flow gets through the valve)
- zero power consumption (ideally, no energy is necessary to switch between the open and closed state, or to maintain one of the states)
- zero dead volume (the valve does not introduce unnecessary extra volumes)
- zero response time (the transition from one state to another is as fast as possible)
- ability to operate with fluids of any density, viscosity and chemical properties.

TYPES OF MICROFLUIDIC SYSTEMS

Continuous flow micro fluidic system:

Micro fluidics system can perform typical laboratory operations with very low consumption of reagents and extremely short reaction times. In continuous flow micro fluidics, the flow of fluid is laminar type due to small channel size. Such systems have a lot of limitations:

- Consumption of sample and reagent is limited by the size of the micro channel
- Mixing in the channel is limited by diffusion
- Dispersion, which is associated with pressure-driven laminar flow, leads to some problems (e.g., dilution and cross-contamination of the samples)

Droplet manipulation type micro fluidic systems:

These limitations are overcome by the forming droplets of sample and reagent in immiscible carrier. Such fine droplets are able to provide rapid chemical and biological reactions. For such type of manipulation we can apply forces generated by electric field, there are two main methods for manipulation of droplets which are Electro wetting and Dielectrophoresis.

1) Electrowetting

Electrowetting involves modifying the surface tension of liquids on a solid surface using a voltage. By using this principle we are able to break droplets of two-phase system into very smaller droplets. The universal applicability of moving droplets by this mechanism was shown with several media such as ionic liquids, aqueous surfactant solutions, and also biological fluids like whole blood, serum, plasma, urine, saliva, sweat, and tear fluid.

A droplet of conducting liquid initially forms contact angle with a solid hydrophobic insulator, then application of voltage (V) between the droplet and a counter-electrode underneath the insulator reduces the solid-liquid interfacial energy, leading to a reduction in internal angle (ø) between droplets with insulating surface. And hence improved wetting of the solid by the droplet.

2) Dielectrophoresis

It is another method of manipulating droplets based on electrically neutral but polarizable fluid. This dielectrophoresis system contains coplanar electrodes (i.e. smooth and insulating substrate), covered by a thin dielectric layer and the fluid involved has higher dielectric permittivity than its surrounding fluid. When the fluid remains on the hydrophobic substrate, it can be transported and divided into hemispherical nanodroplets by short application of voltage and appropriate change in electrode connections.

High-throughput microfluidic sample-introduction systems:

Microfluidic technology provide us recent development in continuos sample introduction techniques. They are very useful in high throghput analysis. Automated continuoes introduction of series of samples to microfluidc chip with fast speed and low sample consumption require a interfacing system between different samples. This sample introducing into microfluidic chip i.e. from outside macroenvironent to miniaturised system. This interfacing system is very tough challenge for successfull devolopment of lab on chip devices. In presesnt situation posssible modes of sample introduction shows in figure.7

In mode A, sample and reagent solutions are loaded in the on-chip reservoirs connected with microchannels. It gives the advantages of a high degree of integration and easy operation in transferring the solution in the reservoir to the microchannels. The number of on-chip sample reservoirs is depending upon the chip size and the ability of peripheral equipment.

In second mode B the sample introduced directly through capillary attached to microchip. It provides

sequential analysis of multiple samples. Mode C involves the use of an on-chip sampling probe for continuous sample introduction, which substitutes the on-chip sample reservoir in mode A and the sample introduction channel in mode B. With such a design, the sample consumption in the sample introduction process can be reduced. In mode D of sample introduction is performed by many steps. It is based on auto-sampling but cost and size of device will increases. Such sample introduction system is very helpful in increasing the speed of analysis and applicable for miniaturized flow injection analysis, sequential-injection analysis, capillary electrophoresis and liquid-liquid extraction.

ADVANTAGES OF MICROFLUIDIC TECHNOLOGY

In developing countries, the availability of higher analytical tools is not available which are very costly. That's why the miniaturization trends come in to picture. A well designed microchip platform provides us generic and consistent way of integration and automation. This platform allows parallel means side by side analytical steps. So as to perform such complicated analytical processes it need to have many micro component such as micropumps, microvalves, micromixer and many more. This set of multicomponent elements should able to perform some basic fluid handling or unit operation as like as conventional laboratories.

Microfluidic analytical devices have certain specific characteristics therefore they give advantages over the conventional analytical techniques, since the need increases towards the miniaturization. Table.1 shows such characteristics of microfluidics devices and their advantages.

APPLICATION OF MICROFLUIDIC TECHNOLOGY

1) On-chip high performance liquid chromatography (HPLC)

HPLC-chip consist the sample enrichment and separation columns of a nanoflow LC system with the inter-connections and spray tip used in electrospray mass spectrometry directly on the polymer chip. The second component of the HPLC-Chip technology is the the HPLC-Chip/MS interface. A chip is inserted into the interface, which mounts on mass spectrometer.

Advantages of on-chip HPLC over conventional HPLC

- New HPLC-Chip technology is a reusable microfluidic polymer chip and it issmallerthan a credit card, the HPLC-chip consist the sample enrichment and separation columns of a nanoflow LC system.
- The technology eliminates 50% of the traditional fittings and connections required in a nanoflow LC/MS system, and also reducing the possibility of leaks and dead volumes and significantly improving ease of use, sensitivity, productivity and reliability of analysis.
- This HPLC-Chip has maximum sensitivity with minimal sample sizes.
- The HPLC-Chip integrates sample preparation, separation, and electrospray tip on a single chip.
- It also includes a sprayer that connected efficiently with a mass spectrometer, allowing separated compounds such as peptides to then be identified and quantified via mass spectrometry.
- This highly integrated, automated system is able to improve the analysis of complex samples of unknown composition, increasing productivity and throughput. Compared with conventional column-based nano flow HPLC,
- HPLC-Chip offers greater reliability and robustness and higher sensitivity.

This recent HPLC-Chip technology has many applications including proteomics research, pharmaceutical development, compound analysis, drug metabolism and pharmacokinetic, food safety, environmental monitoring.

2) Automated sample enrichment with HPLC-chip

For biological samples enerichment step is important to get good detection.Such integration of the enrichment column, analytical columns, connecting capillaries and nanospray emitter directly on to the micro-fluidic HPLC-Chip gives us high sensitivity and enhanced chromatographic performance.Hence we can improved peptide and protein identification by this technique.

This enrichment column is used to concentrate the sample and also allowsloading the sample at higher flow rates reducing the overall injection cycle time. In addition, biological samples can be desalted on the enrichment column to minimize the introduction of salt in the MS source.

For conventional nanocolumn LC/MS system, valve control is typically performed using a valve event time to time. But it can create unnecessarily large data files and requires re-optimization of the valve event timetable each time the sample injection volume is modified. This process is time consuming and improper settings can significantly reduces the efficiency of the enrichment process. But in new sample loading called as intelligent samlpe loading in chip,sample can be loaded using higher flow rates onto the enrichment column,flushed onto the analytical column, and then analyzed by nanoflowLC/MS.

This novel feature eliminates the need to adjust the method when changing the injection volume and saves time by using the shortest possible injection cycle. Thus automation of the entire process ensures that the operator can change injection volumes without the need to re-optimize method parameters for sample loading.

Now we will discuss some case studies on HPLC microfluidics technology.

Case study 1

Detection of ketamine and norketamine simultaneously in human hairs by HPLC-Chip–MS/MSConventionally urine testing is the most popular method to check the drug Abuse, but researchers developed method for such abused drugs like ketamine and its metabolite norketamine in human hairs samples.

They developed a quantitative HPLC-Chip–MS/MS method for simultaneous measurement of ketamine and its metabolite norketamine in human hair. In this experiment, Ketamine and norketamine extracted from hair by acid hydrolysis, and then enriched means concentrate sample by organic solvent extraction. In this experiment chromatographic separation was achieved in 15 min, with the drug identification and quantification by a tandem mass spectrometer. The limit of detection (LOD) and the limit of quantification (LOQ) for ketamine and norketamine was found 0.5 and 1 pg /mg of hair, respectively. This method has several advantages over the conventional GC–MS or LC–MS: -

- Without the chemical derivatization, the running time and cost is reduced.
- The sensitivity of ketamine increased by about 1000-fold as compared to GC–MS, or close to 200-fold as compared to standardized LC–MS
- The amount of hair required could be reduced from 10 mg (old method) to only 2 mg (about 2 strands of 3-cm hair at the current method).

And most important in this is reduction of sample size did not affect the sensitivity.

Case study 2

Drug metabolism and pharmacokinetic study on HPLC-chip

For low level quantification of molecules in complex biological matrices the ultrahigh capacity HPLC-Chip (UHC-Chip) is created by Agilent. This is ideal for drug metabolism and pharmacokinetic (DMPK) studies where the volume of blood is minimum as a simple because small animal used in this study are unable to give us blood sample for quantitative study. But using combination of the UHC-Chip and triple quadrupole MS provides enhanced sensitivity and robustness of low level quantification of compounds of interest from complex biological matrices, specifically from less than 10µL of blood samples.

UHC-Chip with triple quadrapole MS for drug metabolism and pharmacokinetic studies has
Advantages such as:

- It is 100timesmore sensitive comparing to the conventional electrospray at higher flow rate.
- Equally effective in analyzing compounds with a wide range of hydrophilicity.
- It is Compatible with the emerging dried blood spot sample Preparation method.

RECENT DELEVPMENTS IN MICRO FLUIDIC SYSTEMS

1) On-chip capillary electrophoresis (ce)

The new development in microchip CE that requires a low sample volume to provide fast and high resolution separations as compared to conventional capillary electrophoresis devices. This electrophoresis on microfluidic chip is mainly used in DNA analysis. But now a days it is used in many other biological, environmental and industrial applications.

The microfluidic platform gives us new way to transform complex traditional analysis methods into simple and efficient microscale analytical methods. As compare to conventional capillary electrophoresis, on-chips CE is fast and selective.The first commercially available automated microfluidic electrophoresis platform (Agilent 2100 bioa-nalyzer, Agilent Technologies, USA). It is used for DNA, RNA and protein analysis.

This technique is used as analytical tool in food samples, for example to detect the presence of low concentration food contaminants and residues, such as (bio) toxins, chemical residues and pathogens . Microfluidic CE has shown to be powerful for the determination of biogenic amines in fermented beverages.

Pre-treatment-free fast ultraviolet detection with a disposable micro fluidic device

This detection techniques is developed for the indusrial chemicals such as melamine. It is a triazine based industrial chemical found in pet food and many milk products. Due to the harm to health caused by its illegal addition in milk products, its determination is necessary. The traditional method for screening melamine in milk products or animal tissues was high performance liquid chromatography (HPLC) with ultraviolet (UV) detection Or GC/MS. But all these methods mentioned above need complex sample pretreatments such as extraction, preconcentration and derivatization. The sample pretreatment procedure is time-consuming, and always needs toxic solvents, e.g. dichloromethane, nitrile, methanol or trichloroacetic acid. These extraction process gives low recovery of melamine.

New Microfluidic electrophoresis device is a powerful tool for analytical application due to its low consumption of reagents, short separation time, high separation efficiency, and low cost. It consist high-voltage power supply, a UV detector and data processor. This is environ-mental friendly method shows high sensitivity, acceptable recovery and satisfactory accuracy for milk samples. It could be used in both a professional laboratory and the market for fast, green and convenient monitoring of the quality of different foods.

2) A Novel Micro fluidic Device For Liquid/Liquid Extraction In Gas-Chromatography

Liquid liquid extraction is one of the important step for sample preparation technique for aqueous samples in gas chromatography. Practically it has number of disadvantages such as :

- It uses large volume of aqueous sample and expensive toxic organic solvents
- It is also laborious, difficult to automate and time consuming

But the novel method developed for this using microfluidic technology. Such micro fluidic device perform continuous liquid extraction.

It is based on molecular diffusion between two laminar flow in narrow channel. It shows excellent reproducibility and consistency. The results obtained from this microfluidic device shows good linearity and repeatability.

This extraction is basically deepend upon component called as micro extractor as like micropump micro valve in micro fluidic chip. This microfluidic chip have three inlet and one outlet for sample and reagents.
Examples:

i.Amphetamine in urine sample

The amphetamine is substance of abuse.The identification of amphetamine was successfully carrid out by using micro extractor with gas chromatography.

ii. volatile aromatic compounds in water sample

Environmental comtaminants such as benzene, toulene, ethyl benzene. These hazardous compounds arealso detected by this technique.

For detection of the separated components a standard FID (Flame Ionization Detector) and a lightweight Photoionization Detector were coupled to the column. Figure shows a chromatogram of the separated components of the gas mixture.

3) Microfluidic System For High-Throughput Forced Degradation Studies Of Pharmaceuticals

Forced degradation studies are used to identify reactions which may occur to degrade a processed product. Usually conducted before final formulation, forced degradation uses external stresses to rapidly screen material stabilities.
Typical stresses includes:

- pH (acid/base)
- Temperature
- Oxidation
- Concentration
- Light

By using microfluidic device for stress study,we can able toexpose the material rapidly heated to the desired temperature and cooled to ambient afterwards. Because this is coupled with accurate flow rate control.Hence we can achive the very constant and sharp temperature exposure to material to be exposed. Such flow reactor microfluidic systems are able to test a number of different materials (by using the reagent addition system), together with online analytical support (using the HPLC) means that rapid screening of temperature exposure based degradation studies are easily performed by this microfluidic flow reactor systems. In addition of secondary materials (such as acids and bases) to the flow stream allows additional stresses to be included in the study. The solid phase columns gives us the additional possibility of investigation of packing material effects on the substrate.This microfluidic based flow reactor system have benefits like:

- A sample of reaction mixture can be taken, diluted and analyzed by HPLC in an entirely automated fashion.
- Extremely quick reaction times, reagent additions, temperature changes and quenches are possible.
- Pressure sensors continually monitor every flow channel hence chances of blockage are reduced.
- Heat reactions up to 250°C or cool down to 0°C.

4) Micro fluidic Chip with Mass SpectrometryMicro fluidic chip with MALDI analysis for proteomics

Mass spectrometry (MS) is a primary analytical tool for proteomics research, especially when coupled with separation techniques Matrix assisted laser desorption/ionization (MALDI) is fast and efficient and has a high tolerance to nonvolatile buffers and impurities. A novel microfluidic chip for parallel processing of protein digests for MALDIis developed. The microfluidic chip is available for mass spectrometry. Spinning the chip leads to a centrifugal force that was used to move liquids through multiple microstructures. For example, samples can be concentrated and desalted in 96 micro columns packed with 15 µm C_{18} beads with salts and impurities directed to a waste outlet by the centrifugal force. Samples are eluted from the column using a matrix solution controlled by the disk rotation speed. After the micro fluidic analysis, the chip is mounted in the mass spectrometer for MALDI. This micro fluidic chip technology used for the detection of phosphopeptides at the femtomole level.

Micro fluidic chip with ESI-MS

This Microfluidic chip made up of parylene,silicon,poly dis methyl siloxane. This chip ntegrated with gradient pump injector, mixer, rreverse phase separation column, electrodes and ESI nozzle. By using this chip peptide mixture obtained from bovine serum albumin(BSA) digestion seperatedand detected in mass spectrometry.

Table 1. Specific characteristics of microfluidics based analytical device and their advantages

S. No	Specific characteristics	Advantages
1	Require low volume of sample and reagent	Reduces the cost of analysis and waste generation
2	Large surface-to-volume ratio	Enhances mass and heat transfer, shortening the analysis time and increase the performance of analysis
3	Portability	Allowing on-site analysis, disposability and low cost construction
4	Integration of multiple process	Allowing assay automation and improving analytical performance, useful to unskilled operator also
5	High throughput analysis	Potential to multiplexing of different assays and parallelization

Figure 1. Unit operation in micro fluidic chip

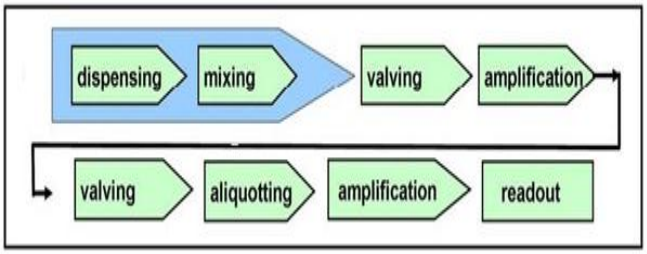


Figure 2. Schematic layout of sample driven by syringe pump

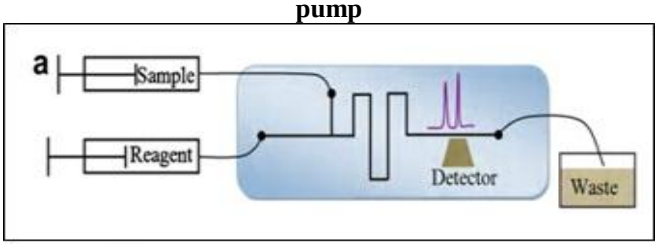


Figure 3. Basic electro osmotic flow and electrophoresis

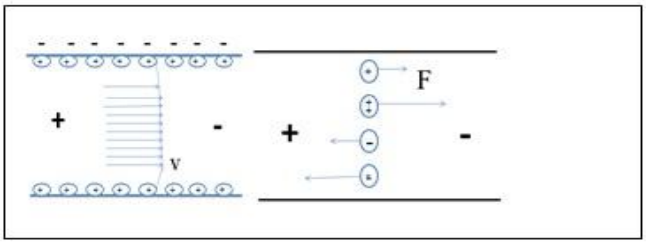


Figure 4.Operating principle of microfluidic diffusion

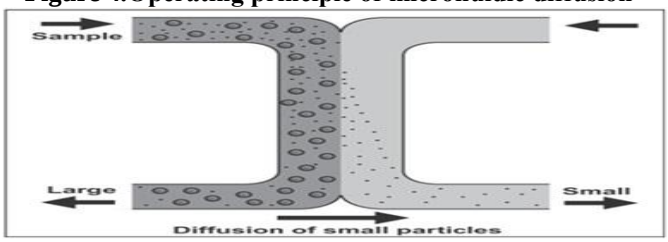


Figure 5. The Electrowetting effect

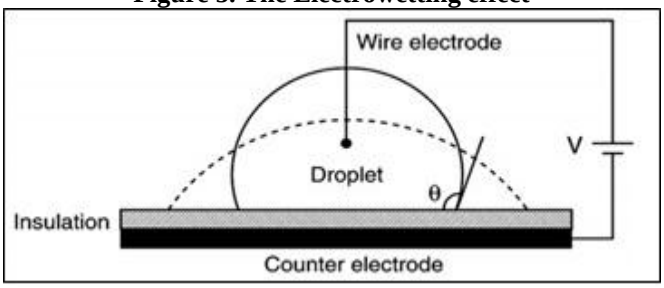


Figure 6.Dielectrophoresis with planar electrode

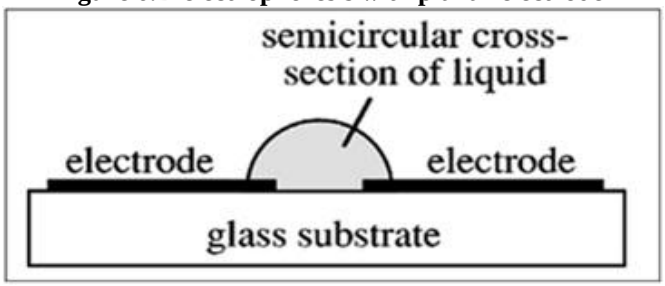
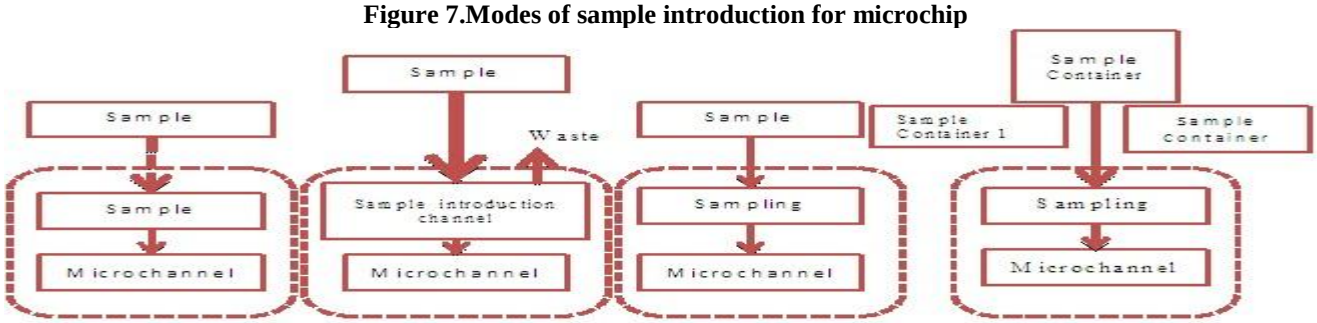


Figure 7.Modes of sample introduction for microchip



CONCLUSION

Microfluidictechnologyiswidely used in many scientific fields such as clinical diagnosis,biotechnology, and analytical chemistry. On-chip microfluidic systems have potential to improve the performance of analytical devices. We get decreasing detection limits, reduced price and faster and easier measurement in routine analysis.

Microfluidics technology also provides high throughput and large scale analysis. But detection principle may not scale down in every situation. Many analytical techniques are still developing at present condition. we hope, in future this technology will prove very dynamic for the analytical chemistry.

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