CHAPTER – 2
LITERATURE SURVEY

2.1 INTRODUCTION

Particle separation is a necessary preparatory step in most biological Micro-assays and common in micro-chemical processing. In the biomedical field, separation techniques are used for fundamental cell studies where isolation of pure cell types is essential. It is a key activity of diagnostic and analytic tools. Micro-separation techniques are also needed for the detection of cancer cells or the accumulation and enhanced count of some types of cells and bacteria. For agrochemical, cosmetic and pharmaceutical domains, these techniques permit separation, after a chemical reaction. In the food industry, potentially harmful bacterial activity is carefully monitored. Separation and enrichment of bacteria are necessary. They are basically needed for analysis. Monitoring of biological weapons is an important activity in the defence sector. In this field, separation is required to detect threatening agents such as Anthrax. All these examples account for the tremendous need for portable, low-cost separation micro devices in a wide range of fields. Thus separation of particles is significant for the proper monitoring of vital factors, parameters associated with therein.

There are two types of separation techniques of micro particles

a. Continuous separation

b. Non-continuous separation

• In continuous separation different separation methods are highlighted namely (1) Optical separation (2) magnetic separation (3) Electrical separation (4) Fluidic separation (5) Dielectrophoretic virtual pillar array separation (6) Other type separations.

• In Non-continuous separation, several separation methods are identified namely (1) Filtering type separation. (2) vibration type separation. (3) Centrifugal separation or centrifugation type separation.
2.2 LITERATURE SURVEY

The overall survey of micro-particle separation can be classified into two categories and also depicts the different methods of micro particle separation techniques, its separation procedures and properties involved are shown in below figure 2.1.

![Diagram showing types of blood separation techniques]

Fig. 2.1 Types of blood separation techniques
2.2.1 Classified survey of continuous separation techniques

The classified survey of continuous separation techniques is explained below.

- **Optical Separation**

  The newest type of optical separation technique is optical fractionation. Dholakia and associates [15] and Grier and Associates [16] gave evidence of the idea of optical fractionation. This strategy utilizes the current advances in control of optical tweezers. Optical tweezers is a control apparatus created by Ashkin[17], where a firmly focused single laser beam is used to trap a single particle. They have been generally utilized from that point onwards to trap single particles in micro-fluidic systems. Advances in optical control permitted the creation of throughput of 40 particles for every second. Despite the fact that this throughput does not achieve the throughput of conventional Fluorescence-Activated Cell Sorters (FACS), it is asserted that optical fractionation has the potential for higher throughput and might discover specialty application areas [18]. FACS is a typical separation method where particles are optically interrogated one by one and coordinated into various outlets contingent upon the grilled result. However, FACS requires extremely costly apparatus and fluorescence labelling.

  Optical separation presents, for the most part, major advantages in terms of sensitivity, selectivity as well as versatility and permits the sorting of particles in a continuous flow. However, the need for laser sources hinders the easy portability of such systems although the integration of vertical cavity surface emitting lasers arrays offers real opportunity [19]. A real need for miniaturization of the apparatus is necessary in this field. The scale up of such optical and fluidics microsystems is also a major issue. Then, according to their refractive indices the optical separation of micro-droplets is presented by Jin Ho Jung [20]. The behaviour of the droplets was characterized in terms of the optical force and the hydrodynamic effects present upon illumination of the droplets in a direction normal to the flow direction in a rectangular micro-fluidic channel. The optical forces acting on the droplets and the resultant droplet trajectories were analyzed and compared with the numerically predicted values.
Optical fractionation system is able to separate only one particle from a mixture.

- **Magnetic separation**

  Magnetic separation is a method of separating mixture of two materials with one part possessing magnetic properties. Some metals like cobalt, nickel and iron have magnetic properties while metals like aluminium, Silver and gold do not have magnetic properties. Magnetic elements are prone to be attracted to a magnet. A Magnetic Separation is a very slow process of separation [3, 8, 9].

  Magnetism has many uses in biological and chemical assays such as in drug labelling and targeting, transport, mixing and also separation [21]. In the magnetic separation technique, arranged particles have either inherent attractive properties or are labelled with magnetic beads. On account of an immunological separation, coated antibodies are bound particularly with antigens of the target cells in an operation called labelling.

  The technique of a conventional magnetic separation system is a straightforward and long-established process in which a magnet is put in vicinity of a column containing the cells to be separated. Magnetically labelled cells are retained in the column, whereas non-labelled cells are flushed with the buffer allowing the immunological separation of species. The column is separated from the magnet in the next step and flushed to permit the accumulation of the assorted particles. This sort of partition is named magnetic activated cell sorting (MACS). A MACS is, likewise, a technique patented by Miltenyi Biotec. However it is broadly accessible monetarily under different names from manufacturers like Dexter or Stem Cell [22, 23]. The efficiency rate of the protocol is often better than 95% [24]. The technique of MACS has been shown at the micro scale keeping in mind the end goal to decrease the volume of the apparatus and to include other functions downstream. Benefits and drawbacks arise from the miniaturisation. Miniaturised magnets allow stronger and more precise magnetic fields because of the vicinity of the magnets to the micro-channels. But, the fabrication of integrated micro magnets requires various and costly manufacturing techniques [25]. The utilization of permanent magnets allows for
portable (no electric connection) and autonomous devices, but if the separation is not completely continuous, the evacuation of the magnet to flush the channel can cause troubles. Electromagnets have the advantage to be easily turned on and off, nonetheless, their fabrication is more muddled and hampers the energetic autonomy of the system. A case of integrated micro MACS is demonstrated by Deng et al. [26], in which arrays of posts were produced at the base of a micro-channel to trap magnetic particles.

This gadget demonstrated more effectiveness compared to macro equivalent. Hence, MACS is a batch procedure and might slow down further downstream analysis and limit the collection yield. A few attempts have been made to fabricate a continuous flow of magnetic separation devices. A procedure called 'on-chip free-flow magnetophoresis was exhibited by Pamme et al [27, 28]. In this illustration, a blend of various magnetic particles and non-magnetic particles is aligned along the wall of a micro channel. A micro magnet put upon the channel gives a non-homogeneous magnetic field gradient transverse to the laminar flow. Depending upon their size and magnetic properties, particles are deflected from their path. The inclusion of spacers permits the gathering of particles in isolated outlets. A continuous flow, magnetic separation device for the advancement of fatal cells from maternal blood has been depicted in a patent by Blankenstein [29]. This device utilizes the same working principle as the preceding illustration despite the fact that the cells require to be labelled. Another sort of continuous magnetic separation was exhibited by Inglis et al [30]. In this example, a few ferromagnetic strips fabricated in a micro-channel provide an array-like magnetic field pattern at a given angle towards the direction of the flow. Cells specifically labelled with magnetic nano particles deflect from the flow path to follow the strips. This system convincingly exhibits the separation of white blood cells (WBCs) from human blood. In another example, Han and Frazier [31] utilized an external magnetic field to actuate a ferromagnetic wire integrated with a micro-channel. Depending on their internal properties, particles will be repulsed or attracted by the wire and are collected at different outlets.

Magnetic separation is very interesting in terms of portability and autonomy. Magnetic fields have never been reported to damage biological particles and therefore
allow the gentle sorting of cells. However, the manufacturability of the magnetic-activated separation mechanism can hamper the development of such devices.

The characteristics of the blood cell motion are analysed by solving a coupled electromagnetic fluid and particle by Myung Ki Baek [32]. Here, the forces acting on blood cells are obtained from magnetic field and fluid field distributions, both of which are numerically calculated by the finite element method. These forces consist of the magnetophoretic force, drag force, buoyancy and gravity. These are the driving terms in Newton’s equation for the particle motion.

Direct magnetic separations of red blood cells from whole blood HMVC been carried out using a continuous magnetic separation method based on high gradient magnetic separation (HGMS) and a gas-permeable membrane with nitrogen gas by M. Takayasu [33].

- **Electrical separation**

The majority of the principal separation procedures for micro particles have utilized electrical power. Surely, electric field-based manipulation is appropriate at the micro-scale because of the ease with which high electric fields can be delivered with micron size gaps and voltage of several volts only. Furthermore, in the last few years the diversification of micro electromechanical system areas such as chemistry and biology has increased the tendency to combine electrical micro systems within micro-channels. There are two primary types of electric field-based manipulation, based upon the properties of the particles to be arranged. Electrophoresis, the movement of charged particles in a uniform electric field, is an exceptionally notable system to separate and transport various types of charged particles. A detailed review of electrophoresis can be found in the literature article [34].

Dielectrophoresis, frequently mistook for electrophoresis, is explained by Pohl [35] as the translational motion of neutral matter caused by polarisation effects in anon-uniform electric field. This procedure has pulled scientific attention because of its ability to manipulate neutral but polarisable particles.
Dielectrophoresis has first been applied to separate cells according to their size or dielectric properties. A number of early articles report the separation or the advancement of particles utilizing castellated or inter digitised planar electrodes giving an inhomogeneous electric field [36, 37]. These procedures sometimes referred to as stop-flow techniques, often relate to binary separation where a mixture is split into two sub-populations: one usually retained in the channel [38]. At times, particles moving toward DEP affinity and size are difficult or impossible to separate. To sort this limitation, Yang et.al [39] coupled antibody recognition and a dielectrophoretic stop-flow technique. In this illustration, antibodies particular to the targeted bacterial cells are coated above inter-digitated DEP arrays, separated by a thin layer of SiO$_2$. The blend of bacterial cells is infused into the channel. At the point when the DEP is activated, every one of the cells is concentrated over the electrodes against the flow. Meanwhile, the targeted cells bond themselves with the antibodies. At the point when the DEP is deactivated, the unbound cells flow away, leaving the directed cells isolated in the channel. These methods report high efficiencies, near that are the unadulterated recollection of each type of particle and are adopted for high product value and low volume application.

Some drawbacks can nevertheless be reported. To begin with, cells are often trapped with positive dielectrophoresis (pDEP) and without watchful control of the voltage and frequency. It may encounter high electric fields that can destroy them. A guide to the values of frequencies and applied electric fields affecting the cells’ viability is given in [40]. In [39] the cells caught by pDEP remain viable, yet an anomalous protein creation is accounted. Generally, just a single subpopulation can also be extricated from a heterogeneous mixture. At last, despite the fact that liquid is constantly drawn through the chip, voltages continuously must be turned on and off to gather the isolated subpopulations which can hamper the continuality of the procedure.

Hydrodynamic powers have been coupled to dielectrophoresis to create continuous particle separation. This method utilizes electrodes to suspend particles to various heights relying upon their dielectric properties. The inclusion of a parabolic flow enables the particles to be dragged away at various velocities. Separation of
erythrocytes and latex beads was performed by Roussel et al [41]. The associates of Gascoyne reported separation of various sorts of leucocytes with purity after separation up to 98% [42]. This procedure utilizes negative dielectrophoresis to suspend particles over the electrodes and thus protect vulnerable biological particles from high electric fields. But, it has limitations in separation performance. Cells of a type can contaminate the cell populace of another sort [42]. Different sorts of electrode arrangements have been successfully tested. Choi and Park [43] proposed a trapezoidal planar electrode array providing specific electric field geometry in a micro-channel. Particles focused on one side of the channel are deflected more or less by the electric field depending on their properties. Towards the end of the micro-channel, the inclusion of spacers helps gather different particles.

Liy and Kaler [44] revealed an ingenious ‘isomotive’ electrode arrangement for continuous-flow separation. Isomotive refers to a particular electric field where a particle encounters a constant dielectrophoretic force at any point in the field, a setup depicted by Pohl and Pethig [45]. This geometry gives a superior separation of species, based only on their dielectric properties.

The utilization of dielectrophoretic barriers has likewise been generally and effectively demonstrated. A dielectrophoretic barrier refers to electrodes mounted at the top and base in a micro channel. This design makes an electrical field barrier which diverts particles from the direction of fluid flow. To separate two distinct species, the frequency and magnitude of the voltage is set to such an extent that one species displays dielectrophoretic powers and can be deflected, though other species experience the electrical field barrier without encountering any force [46]. Fuhr and associates [47] were the principal group to report such an arrangement in 1998 with a serial and parallel noncontact manipulation at high velocity. From 1998, a few patents and articles have been distributed by the same group in collaboration with the German organization Evotec technologies and incorporate treatment, separating, sorting or confinement of diverse kinds of biological or synthetic particles [48, 49]. In a publication by Schnelle, a article separation is reported utilizing dielectrophoretic forces induced with curved electrodes and hydrodynamic forces [50].
In [51], a detailed experimental and theoretical study of DEP barriers shows a strong dependency between the channel height and the threshold velocity above which particles may penetrate the barrier. Decreasing the channel height leads to a better separation efficiency. In [52], a system named Nano Vir Detect is described. This adaptable module utilizes standardised biochips packaging and has independently tuneable functions such as focusing, separation, holding of micron or submicron-sized particles and which can be adaptable to a large number of different particles. Electrodes are made out of nano-porous materials to build the electrode capacitance. This is not only grows the frequency range of dielectrophoretic deflection but also enables the system to be utilized with higher conductivity medium which is of high interest particularly with biological specimens flowing in relatively high conductivity liquids. Other teams report also the successful implementation of dielectrophoretic sorters, using improved DEP barriers [53-55]. Although the new configurations double the maximum flow of speed compared to a classical strip-like electrode. The plane electrodes show more temperature rise than the classical geometry, which is a major drawback for sensitive biological or chemical applications.

A focusing device has been fabricated by Morgan et al. utilizing a quadropolar electrode arrangement [56]. In [57], a tripolar electrode arrangement for separation the purpose is exhibited. In this application, two tri-polar strips like electrode arrangements are installed on each side of the base of the channel. Subsequently, particles encountering positive DEP are relied to flow to stream close the electrodes, while particles with negative DEP affinity refocused along the central axis of the channel. This simple geometry won't be so legitimate for continuous flow, as particles with positive DEP will most likely adhere to the electrodes if the stream is as well flow is slow, hampering the accumulation of the species. A high speed cell-dipping system is proposed in [58]. A classic pair of strip-like electrodes is mounted at the top and bottom of a 20 mm high micro- channel. A lot of particles are transferred from one reagent to the next in under 0.5s at a flow speed of 300 µm/s. A substantial dissemination of the dye appeared as the second flow can be seen after the primary barrier and may be a result of some electro kinetic forces or a perturbation in the flow
caused by the gathering of particles on the tip of the barrier. The inclusion of a space between the two streams, as expressed in Fuhr's[47] patent, can help to avoid this perturbation.

In recent years, new ideas have been proposed to improve performance of DEP separations systems. Different channel geometry for dielectrophoretic focusing of particles is presented by Leu E S et.al [59]. In this device, a blend of particles is separated in a single particle stream with a mix of DEP forces and hydrodynamic forces in a funnel like channel. Although the results is obviously useful in terms of particle analysis. The electrodes need to be embedded on the channel side walls, which is a laborious fabrication step. An interesting insight to improve separation is proposed by Hu XY and associates [60]. In this approach, labelling of cells with particles that differ in polarization reaction upgrades the sorting action. The stream speed of 300 µm/s in a 20 µm high channel can be acquired. However, this procedure requires a supplementary step of labelling. Park et al. [61] detailed a fan-shaped like electrode geometry making a unique asymmetric electric field gradient. In this idea, the applied field is continuously varied due to half-circular type of micro-channel. This geometry enhances the discrimination power of the device also. But, this approach prompts a higher temperature rise.

The utilization of alleged electrode less DEP was accounted. In this method, a homogeneous electric field is made inside a micro-channel by presenting electrodes at the inlet and outlet. Insulating obstacles are placed within the channel rendering the electric field non-uniform, which leads to the creation of a dielectrophoretic force. The Electrode less DEP avoids electrode fouling and electrode destruction sometimes reported in DEP manipulation. Initially this principle was applied with the arrays of insulating posts in a micro-channel [62, 63]. In this type of arrangement, particles sensitive to DEP are caught between posts, while others flow along. This outcomes in a non-continuous system as the voltage must be turned down to recollect the trapped particle. Utilizing a similar idea, Barbulovic-Nad et al. exhibit a unique device consolidating an oil droplet at one point of the side wall of a micro-channel. Particles approach the base of the droplet in a similar manner and are redirected more or less at
the top of the bubble in the highest electric field zone. This permits the parallel accumulation of different particles [64].

The separation can be tuned by changing the size of the droplet. Demierre and associates [65] proposed recently a device embedding large electrodes insulated from the main micro-channel by thin, dead-end channels. These viable techniques nevertheless require high voltages and relatively create high electric fields, sometimes surpassing the threshold of safe cell manipulation. Another type of electrical partition, known as voyaging wave dielectrophoresis (TW-DEP), has been illustrated. In TW-DEP, particles are transported across arrays of inter-digitated electrodes being energised with sinusoidal electric signals [39]. A travelling electric field, created by phase shifting of the signals, instigates a dipole moving towards a path opposite to the course of the electrodes array. As the speed and direction of the motion depends on the particles’ dielectric properties, separation of different species can be achieved. Recently, Pethig et al. [66] exhibited the separation of T-cells from monocytes with a superposition of TW-DEP signals.

The system of signal superposition leads to a reduction in the length of the electrode array needed to achieve separation. To summarize, the use of an electric field for particle separation is a relatively old established technique. Among the different kinds of micro devices using electric field-based separation in a continuous flow, examples include DEP fractionation, DEP barriers and TW-DEP. Many different electrode arrangements related to these reliable and efficient techniques can be found in the literature. Moreover, it has been demonstrated that dielectrophoretic barriers have the potential for greater exploitation. Nevertheless, the use of electrodes introduces an important fabrication step which increases the cost of the micro device which might hamper mass-manufacturability

M. Dhindsa [67] has demonstrated controlled electrostatic depletion and accumulation of liquid ions in micro-scale channels by applying voltage across a dielectric coating on the channel walls. A 100 μm fluorescent ionic dye (FITC) was flown through a series of oxidized (SiO₂) Si microscale channels (each 10 μm wide, 15 μm deep and 1cm long).
**Fluidic separation**

To handle the issue of outer force field requirement in separation devices, researchers have proposed recently some techniques which can be termed ‘fluidic-only’ separation. In these partition techniques, particles are arranged solely by size, often using only the geometries of micro-channels and hydrodynamic forces. In 2004, Yamada and Seki [68] proposed a pinched-flow separation device. A blend of various measured particles is infused into a support. The different-sized particles is injected into a buffer, and are aligned to a sidewall of a pinched segment which is expanded along these lines; At this point, hydrodynamic forces act diversely on particles, deflecting the smaller ones far from the bigger ones. Flow rates of the two inlets and the angle between the two segments are determining factors to sort the different sizes of particles. In 2005, Yamada et al. [69] proposed a two-step system called 'hydrodynamic filtration' to dispose of the need of a second buffer to control the separation; concentration and classification are performed at the same time.

A liquid containing particles is infused in the principle micro-channel having multiple branch points and side channels. Particles do not enter the sub-channels when the relative flow rates at the branch point are low. This permits in a initial step the withdrawal of the liquid and the concentration of the cells. The increasing stepwise of the relative flow rates at each branch point permits the accumulation of initially small particles and then the large particles. The size limit of the sorted particles is determined by a precise distribution of the flow rate at each branch. This procedure was demonstrated by the enrichment of the WBCs. Although, RBC remains present in the fraction of interest. The relative concentration of WBCs in relation to RBCs was increased by 29 times. Fluidic-only separation technique is especially pertinent on account of blood plasma separation, where the axial migration of blood cells in micro-channels, beneath 300 mm is utilized for sorting [70-72]. Another system called deterministic parallel displacement was set up and tried by Huang et al. [73]. In this technique, micro-posts are set in rows inside a micro-channel. Each row of posts is shifted from the other by a separation which somewhat sets the critical separating size. The asymmetric bifurcation of laminar flow around obstacles leads particle to choose their path deterministically on the basis of their size.
A small particle will have a zigzag displacement path, whereas a large particle will tend to flow straight. After a number of rows, the particles can be collected separately. This technique is financially savvy and can separate in parallel to a substantial number of various different-sized particles with a precision of up to 10 nm. The technique was effectively tried for the separation of WBCs and RBCs [74]. But, because of a high risk of clogging because of the numbers of posts employed and the narrow gaps between them, Devis [75] Huang and associates [76] exhibited later an advanced device including additional regions alongside the active sorting arrays. These extra regions accumulate the bigger particles that have been sorted. Although these regions also contain pillars to maintain the same pressure drop across the system, the gaps between the pillars are made larger to avoid clogging. This device expels all the particles bigger than 1 mm from blood, permitting the accumulation of unadulterated undiluted plasma and it is patented.

Recently Li et al. [77] labelled CD4þ T helper lymphocytes with 25 mm polystyrene beads in a blend of WBCs infused in a lateral displacement system. Up to 91% of the lymphocytes were separated from the other type of WBCs. This article shows the possibility of cell subtype sorting with the continuous-flow lateral displacement method. ‘Liquid – liquid extraction’ is another fluidic-only separation method broadly in the chemical and biological industries. The concept exploits the preferential affinity or differential diffusion coefficients of solid compounds in a laminar flow of two liquids streams H-filters created by Yager et al. [78] They have utilized this method to extract molecular analyses from whole blood. Nam et al. [79] proposed a micro-system using this technique. In this device, the injection of cells in a thin stream between two phases enables the cells to partition in the preferred solvent.

The sorting efficiency of the micro-device was reported to be 97% which is higher than its macro-scale counterpart. But, it is not an appealable method, as just two of particles can be separated at the same time, using sometimes very specific solvents. Liquid–extraction is particularly adapted to chemical extraction, such as extraction of hydrocarbons from oil in the petrochemical industry or extraction of organic compounds from extra-terrestrial dissolved minerals in space exploration [80, 81].
To summarize, these fluidic-only separation methods have the great advantage of not requiring any external field. Therefore the manufacturing of these devices relies only on micro-channel networks. Manufacturing of these channels can be accomplished by means of hot-embossing or micro injection moulding. This holds the potential for cost effective and mass-manufacturable devices, which is particularly relevant in the case of point-of-care devices. But fluidic-only separation devices, with the exception of the last example, can separate particles only by size.

James Hanotu [82] in his study where micro bubbles are produced by fluidic oscillation via a no-moving part diverter valve to cut down the energy consumption considerably. Micro-bubbles are applied for the separation of emulsified oil in a process known as micro flotation. The mean bubble size generated by fluidic oscillation from the 50 ml pore diffuser was 100 ml, otherwise coarse bubbles were produced under steady flow.

- **Dielectrophoretic virtual pillar array separation**

These methods also do the separation by size difference and have an important advantage which doesn’t require additional labelling because biological cells have different sizes according to their own species. However size dependent particle separation using microstructures such as micro-pillar array has clogging problem in microstructure, which can cause malfunction of devices. In order to reduce clogging in micro pillar arrays, recently it is proposed virtual pillar array induced by negative dielectrophoretic forces and verified the capability of size dependent particle separation in it with polystyrene breeds. But the clogging problem cannot be avoided totally [83].

- **Thermal or acoustic separation techniques**

A system utilizing an external thermal field is exhibited in [84]. In this device, a temperature difference is applied across the micro-channel. A separation between particles happens due to the difference of thermal diffusion coefficients. This method does not have large separation efficiency and particles are not easily collectable at different outlets.
Acoustic separation can likewise be found in the research [85-87]. The last reference shows a separation efficiency almost 100% which is comparable with conventional blood cell separation by centrifugation. Acoustic procedures can be adapted with other methods to form hybrid systems. Undoubtedly, Wiklund et al. [88] exhibited recently a device incorporating short-range DEP control with long-range ultrasonic wave (USW) control. In this device, a DEP force is added by an electric field between co-planar electrodes at the base of the micro-channel. A transducer made of a piezo-ceramic component and a polymethyl methacrylate (PMMA) refractive edge is setup on the glass cover of the chip. The two consolidated forces allow particle trapping, arranging, concentration and separation with selectivity up to 90%. This technique had been already exhibited to some degree in a patent [89]. In the last work, USW was additionally proposed as a means for transportation of particles. The DEP/USW control looks appealing for high-throughput because of the use of long-range USW forces and high precision because of the use of short-range DEP forces applications.

2.2.2 Classified survey of non-continuous separation techniques

There are different types of non-continuous separation techniques which are presented below.

- **Filtering type separation**

Filtering type separation uses the permeable nonwoven material or membrane. The filters made of porous membrane trap the particles. This kind of filter is practically used for high-speed of separation and is not optimal for massive separation [3].

Hornsey VS and associates [90] have exhibited a method for the separation of whole blood into red cells and plasma by using the Sangofer® device which is a gravity-fed, hollow-fibre system. The components would then be compared with those produced by the use of more elaborate technical equipment.

Recently, a significant research effort has been invested by Yang XI and associates [91] into the development of micro-fluidic paper-based analytical devices
μPADs) implementing these conventional laboratory tests for point-of-care diagnostics in resource-limited settings. This paper describes the use of red blood cell (RBC) agglutination for separating plasma from finger-prick volumes of whole blood directly in paper, and demonstrates the utility of this approach by integrating plasma separation and a colorimetric assay in a single μPADs. Most of the blood becomes dead volume after filter pores are clogged by blood cells in the filtration.

- **Vibration type separation**

Vibration type separation technique is a collection of particle occurs at a maximum transverse velocity. The particles have a tendency towards antinodes by inertial motion inside a capillary.

Katsutoshi Ooe and associates [92] have developed new types of blood separation devices that use piezo-ceramic vibrators. The first device uses a capillary. One end of the capillary is fixed to the device frame, and the other is fixed to a piezo-ceramic vibrator. The vibrator transmits bending waves to the capillary. This device can process only a small amount of solution; therefore, it is not suitable for hemanalysis. In order to solve this problem, we developed a second device; this device has a pair of thin glass plates with a small gap as a substitute for the capillary used in the first device. These devices are based on the fact that particles heavier than water move toward transverse velocity antinodes while those lighter than water move toward velocity nodes.

- **Centrifugation type separation**

When there are smaller solid particles in a liquid, the particles easily pass through a filter paper. For such particles, separation using filtration technique fails. Mixtures with these kinds of smaller particle are separated by centrifugation. i.e, it is a technique of separating materials that are insoluble which are suspended in liquid. It is based on the density, shape and size of the particles, viscosity of the medium, and the speed of rotation. The principle is that the particles which are more dense are tend to go to the bottom and the particles which are lighter stay at the top when spun swiftly [5, 6].
Centrifuge is a term given to the apparatus that uses principle of centrifugation. It is a setup that has centrifuge tube holder called rotor. Equal amount of solid liquid mixtures which are inside centrifugal rotor balance is held by the rotor. When it is swiftly rotated vertically the centrifuge tubes rotate and due to the centrifugal force, the insoluble denser particles are separated from the liquid. When the rotation is stopped, solid particles are sedimented at the bottom where the liquid afloat on top in the centrifuge tube. This is the most widely used method to clinically separate blood components as it has leading advantages over the other types of separation techniques available.

The first known clinically used apparatus for blood separation is Chon centrifuge spectra developed by Dr. Chon in the early 1950’s. It is a device in which the clarified blood flows upwards into a separation chamber which rotates at about 2000rpm. When the chamber with blood is rotated and the separation process occurs [10, 11, 93]. Further development Latham re-designed the equipment and obtained a new simplified model of Chon centrifuge spectra. This model showed good results in comparison with Chon model which was the first commercial model to be put in the market [94, 95].

Further IBM has developed its own blood separator by name NCI-IBM. It was a more sophisticated model with better efficiencies compared to earlier models [5, 96]. Further many efficient commercial models like AMINCO Centrifuge-II [97], Fenwall CS3000 [98], IBM 2997 [99-101], Cobe Separator [102,103] have come into market which are continuous separators.

Amy P Wong, Malancha Gupta, et.al [104] proposed a process that the isolation of human blood plasma from human whole blood is achieved using egg beater as a centrifuge. Sometimes hand powered centrifuge finds an alternate at emergency situations.

Heberle SI, Brenner T et.al [105] proposed a Centrifuge technique of extraction of plasma from the sediment by a decanting structure. The technique supplies 2µl plasma from 5µl of whole blood at a moderate spinning frequency of 40Hz.
A research article by Mary Amasia[106] and Marc Madou [14] proposes that blood plasma separated using bulk volume centrifugal micro fluidic device. These devices for blood separation use a centrifugal disc based device using undiluted blood sample with added reagents. M.S. Salim, M.F. Abd Malek[107]Mohd Fareq et.al [108] proposed a time optimization model for centrifugation process in the human blood segregation which improves blood separation efficiency.

Kunshan Sun, Hyuntaek oh, et.al [15] proposed a blood component separation method that uses centrifugation. The proposed centrifugal separation of blood is done by creating firm barrier between densities bounded layers using UV curable thixotropic gel which gives an advantage over other techniques which uses gel that creates uneven barrier between the constituents of blood. Harald Anluf [109] has stated in his work a recent development in the centrifuge technology i.e. Centrifuges combine with other apparatus for the process of optimization. This technique is the combination of different apparatuses like a combination gravity thickener/clarifier (setting based) and pusher centrifuge.

Based on the above literature survey, the following facts can be outlined which will clearly exhibit the need for further research of the existing centrifuge based sedimentation techniques.

2.3 QUALITATIVE COMPARISON OF COST AND TRADE-OFF ISSUES OF DIFFERENT SEPARATION TECHNIQUES

The continuous separation techniques have relative technical drawbacks. The optical separation technique can separate particles based on size only that poses a limitation in whole blood separation. The optical separation technique uses advanced sensors which is expensive. Magnetic separation separates particles which possess magnetic properties. The magnetic separation technique is complex and expensive to implement practically. Electrical separation technique may over heat the particles in the mixture and will lead the particles to lose their generic properties and the electrodes on continuous usage may collect flux that is to be avoided in the separation process. Electric separation uses electrodes made of expensive metals for the
separation process. Fluidic separation technique separates mixtures based on
differential density in mixture and is not accurate in separating as the differential flow
of fluids may not be constant. Fluidic separation uses expensive hydro dynamic
equipment to create fluidic pressures which are very expensive. The Dielectrophoretic
Virtual pillar array does the separation based on size of particles induced by negative
dielectrophoretic forces and also makes use of polystyrene beads. It is complex
process, expensive and particles get clogged. Other separations are thermal separation
and acoustic separation. The thermal separation is dependent on thermal diffusion
coefficients of the micro particles of the fluid and separation is not efficient and
acoustic separation is complex and expensive. All these continuous separation
techniques use a large quantity of samples for separation as continuous flow of the
mixture is required to accomplish efficient separation.

The non-continuous techniques overcome the limitation of using large samples. A
non-continuous centrifugation technique has better advantages over the other
available techniques. Filtration technique is cheaper to implement but it may lead to
clogging of the filters and membranes used for the separation process. It uses gels and
reagents, which leads to the loss of generic properties of the mixture. Vibration
technique is relatively an expensive technique as it uses vibrating plates and consumes
more power in accomplishing separation. Vibration technique consumes more time
compared to any other separation technique for complete separation of the mixtures.
Comparative to other available separation techniques centrifugation technique is most
suitable for separation of density gradient mixtures. Hence it is most suitable for
separating whole blood as it is a density gradient liquid mixture. Centrifugation
technique is also cheaper compared to all available techniques.

As per the previous researchers research contributions are highlighted in the
classified survey for various types of separation techniques that are discussed and
exhibiting that few are expensive, few are complex and few are simple and all these
techniques are in a tabular way compared with the requirement of the low cost
alternative for the proposed new instrumentation system as shown in the comparative
Table 2.1.
Table 2.1 Qualitative comparisons of cost and trade-off issues of different separation techniques

<table>
<thead>
<tr>
<th>No</th>
<th>Type</th>
<th>Cost and Trade-off issues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Continuous separation techniques</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Optical separation</td>
<td>It separates blood based on size of particles only and that poses a limitation on detecting RBC and WBC in whole blood. The optical separation technique uses advanced sensors which are expensive. It is not suitable for proposed new instrumentation system.</td>
</tr>
<tr>
<td>2</td>
<td>Magnetic separation</td>
<td>It separates blood particles which possess magnetic properties. It is complex and expensive to implement practically. Thus it is not adapted for proposed new instrumentation system.</td>
</tr>
<tr>
<td>3</td>
<td>Electrical Separation</td>
<td>Electrical separation technique may overheat the particles in the blood and will make the particles to lose their generic properties. The electrodes may collect flux that is to be avoided in the separation process. Electrical separation uses electrodes made of expensive metals for the separation process.</td>
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<tr>
<td>4</td>
<td>Fluidic Separation</td>
<td>It separates blood based on differential density and is not accurate as the differential flow of fluids may not be constant. Fluidic separation uses expensive hydrodynamic equipment to create fluidic pressure. It is complex and expensive.</td>
</tr>
<tr>
<td>5</td>
<td>Dielectrophoretic Virtual pillar array</td>
<td>This method also does the separation based on size of particles induced by negative dielectrophoretic forces and also makes use of polystyrene beads. It is a complex process, expensive and particles get clogged. It is also not adaptable in the proposed new instrumentation system.</td>
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<tr>
<td>6</td>
<td>Thermal or acoustic separation</td>
<td>Other separations are thermal separation and acoustic separation. The former one is dependent on thermal diffusion of the micro particles of the blood and separation is not efficient. The second one is complex and expensive.</td>
</tr>
<tr>
<td></td>
<td><strong>Non Continuous separation techniques</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Filtration Technique</td>
<td>Filtration technique is cheaper to implement but it may lead to clogging of the filters and membranes used for the separation process. It uses gels and reagents, which leads to the loss of generic properties of the blood. It is mostly in use now in different instruments.</td>
</tr>
<tr>
<td>2</td>
<td>Vibration technique</td>
<td>Vibration technique is relatively an expensive technique as it uses vibrating plates and consumes more power in accomplishing separation. Vibration technique consumes more time when compared to any other separation technique for complete separation of the blood.</td>
</tr>
<tr>
<td>3</td>
<td>Centrifugation technique</td>
<td>As the name suggests this technique uses the principle of centrifugation and the equipments that employ this technique use more blood sample. A centrifugal disc is used for blood separation using whole blood sample with added reagents or gels. The blood particles are separated into sub-particles due to sedimentation process induced by rotation.</td>
</tr>
</tbody>
</table>
The continuous separation techniques use a large quantity of blood sample for separation and also a continuous flow of the blood to accomplish efficient separation. On the other hand the non-continuous separation techniques do not consume large quantity of blood sample for separation and moreover these do not require continuous flow of blood sample for efficient separation.

The literature review suggests that sufficient research has not been carried out to innovate and overcome the deficiencies or limitations of existing separation methods.

2.4 TECHNICAL AND RESEARCH GAPS ADDRESSED IN PROPOSED LOW COST INSTRUMENTATION SYSTEM

The instrumentations which use any of separation techniques presented in Table 2.1, have one or the other drawbacks or technical and research gaps. The following are most significant drawbacks or technical and research gaps.

**Drawbacks specific to separation techniques**

1. The optical separation technique uses advanced sensors which are expensive.
2. Magnetic separation works based on magnetic properties of blood particles. The process is complex.
3. Electrical separation overheats the blood particles. The generic properties of the particles will change.
4. Fluidic separation uses costly hydrodynamic equipment.
5. Diectrophoretic virtual pillar array is complex and expensive process.
6. Thermal or acoustic separation technique is not efficient because thermal diffusion of particles influence the results.
7. The filtration technique mainly poses the problem of clogging of the filters and membranes used for the separation process.
8. The vibration technique consumes more power and expensive in nature.
9. The instruments using centrifugation technique adopt procedures which require more blood more time and addition of reagents.
General drawbacks

The following are the general drawbacks in the instruments available in the market now.

1. Use of more blood sample
2. Addition of reagents, gels or diluting agents
3. Processing time is more.
4. Instruments work on AC supply.
5. The Instruments are expensive.
6. They are not portable
7. They are not easy to operate.
8. Service charges are more

The proposed new instrumentation systems (ARM based instrumentation system and FPGA based instrumentation system) adopt the separation technique of centrifugation and the design features and working procedure ensure that the technical and research gaps are addressed in them.

2.5 SUMMARY

In the literature survey, researchers have prescribed various concepts and methods for separation of micro particles of blood cells. The individual separation methods are elaborately studied and the pros and cons of each of these methods are identified. Then compared the techniques, cost and trade-off issues of these different separation methods and identified technical gaps and limitations of the present existing methods. The literature review suggests that sufficient research has not been carried out to innovate and overcome the deficiencies of existing separation methods.

A new instrumentation system for separation and detection of RBC and WBC components in whole blood is introduced by analysing cost, accuracy, and portability, time and power consumption to overcome the technical gaps and limitations of existing methods by using centrifuge technique. The next chapter is to describes about centrifugation, chopper and its need for the proposed new instrumentation system to achieve the aim are discussed in theoretical analysis.