

# **PREPARATION AND CHARACTERIZATION OF NANOFUIDS AND SOME INVESTIGATION IN BIOLOGICAL APPLICATIONS**

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## DECLARATION

I hereby declare that thesis entitled “**PREPARATION AND CHARACTERIZATION OF NANOFLUIDS AND SOME INVESTIGATION IN BIOLOGICAL APPLICATIONS**” is an authentic record of my study carried out as requirements for the award of degree of **M.Tech. (Chemical Engineering)** at **Thapar University, Patiala**, under the guidance of **Dr. D. Gangacharyulu**, Associate Professor, **Department of Chemical Engineering**, Thapar University, Patiala during **July 2009 to June 2010**. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree.

  
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## ABSTRACT

Nanofluids are suspensions of nanoparticles in base fluids, a new challenge for thermal sciences provided by nanotechnology. The tested fluids are prepared by dispersing the  $\text{Al}_2\text{O}_3$  into water at three different concentrations such as 1 %, 2 %, and 4 %. Thermophysical properties of nanofluids are measured by KD2 Pro, Ostwald viscometer and specific gravity bottle. Experimental results show that thermal conductivity of nanofluids is higher than the base fluid and with increase in temperature it is increased from 2 % to 44 % (for 1 % particle concentration), 3 % to 47 % (for 2 % particle concentration) and 5 % to 48 % (for 4 % particle concentration). The result indicates that the volumetric specific heat of  $\text{Al}_2\text{O}_3$  nanofluid decreases gradually with increasing volume concentration of nanoparticles and with increasing temperature too. Viscosity and density of nanofluids are decreasing with increase in temperature but with increase in particle concentration, viscosity is decreased but density increased. Nanoparticle PCR is a novel method to optimize DNA amplification. With the addition of gold nanoparticles in PCR mixture, it enhanced the efficiency of PCR by reducing the time cycle. Results shows that PCR efficiency is more enhanced with increase in size of nanoparticles and by reducing the enzyme dilution. Nanofluids have not shown satisfactory results with large size of DNA i.e. 2.6 kb. PCR mixture forms immediately precipitate when  $\text{Al}_2\text{O}_3$  is added in the mixture so  $\text{Al}_2\text{O}_3$  is not fit for PCR.

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## ABBREVIATIONS

CHF	Critical heat flux
DI	Deionized water
DLS	Dynamic light scattering
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
DMHP	Disk-shaped miniature heat pipe
EtBr	Ethidium bromide
HPLC	High performance liquid chromatography
nm	Nanometer
PCR	Polymerase chain reaction
PCS	Photon correlation spectroscopy
SANSS	Submerged arc nano-synthesis system
SANS	Small angle neutron scattering
SAXS	Small angle x-ray scattering
SEM	Scanning electron microscope
SPM	Scanning probe microscopy
TEM	Transmission electron microscope
TAE	Tris base, acetic acid and EDTA
U.V	Ultra violet
XPCS	X-ray photon correlation spectroscopy

## NOMENCLATURE AND SYMBOLS

<b>Symbol</b>	<b>Description</b>
$c_p$	Volumetric specific heat, MJ/ (m <sup>3</sup> .K)
$\rho$	Density, kg/m <sup>3</sup>
$k$	Thermal conductivity, W/(m.K)
$\mu$	Viscosity, cP
$t$	Time, seconds
$T$	Temperature, °C
%	Percentage
°C	Degree Celcius
ng	Nanograms
Cu	Copper
Si	Silicon
uA	Microampere
$\mu$ L	Micro liters
nM	nanomolar
W	Watt
K	Kelvin
MJ	Mega Joules
cP	centipoise
Al <sub>2</sub> O <sub>3</sub>	Aluminium oxide
AuNPs	Gold nanoparticles

# CHAPTER – 1

## INTRODUCTION

Development of many industrial and new technologies is limited by existing thermal management, and need for high performance cooling. Many industrial applications need ultrahigh-performance cooling systems to miniaturize the thermal systems. Nanofluids as a new, innovative class of heat transfer fluids represent a rapidly emerging field where nano science and thermal engineering coexist. In this context the requirement is addressed by two means, i.e., one is by geometry, and the second one is by fluids being used. The latest literature reveals that the using of nanofluids in thermal systems as well as in biomedical systems is one of the best options.

### 1.1 Nanofluids

Nanofluids are suspensions of nanoparticles in base fluids, a new challenge for thermal sciences provided by nanotechnology. Nanofluids have unique features different from conventional solid-liquid mixtures in which mm or  $\mu\text{m}$  sized particles of metals and non metals are dispersed. Due to their excellent characteristics, nanofluids find wide applications in enhancing heat transfer [1].

Nanofluids (Nanoparticle fluid suspensions) is the term coined by Choi (1995) to describe this new class of nanotechnology based heat transfer fluids that exhibit thermal properties superior to those of their host fluids or conventional particle fluid suspension [2]. Nanofluids are stable colloidal suspensions of nanoparticles, nanofibers, nanocomposites in common base fluids. Nanoparticles are very small, nanometer-sized particles with dimensions usually less than 100 nm. The smallest nanoparticles, only a few nanometers in diameter, contain only a few thousand atoms. These nanoparticles can possess properties that are substantially different from their parent materials. Similarly, nanofluids may have properties that are substantially different from their base fluids, like much higher thermal conductivity, among others [3]. The goal of nanofluids is to achieve the highest possible thermal properties at the smallest possible concentrations (preferably  $< 1\%$  by volume) by uniform dispersion and stable suspension of nanoparticles (preferably  $< 10\text{ nm}$ ) in host fluids. Three methods of generating these nanofluids are available: creating them from chemical precipitation, purchasing the nanoparticles in powder form and mixing them with the base fluid, and direct purchase of prepared nanofluids.

Characterization of nanofluids includes colloidal stability, size distribution, concentration, and elemental composition. Quality control of the nanofluids to be used for heat transfer testing is crucial; an exact knowledge of the fluid constituents is a key to uncovering mechanisms responsible for heat transport enhancement.

## 1.2 Nanoparticles and Base fluids

Nanoparticles are sized between 1 and 100 nanometers. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials. Nanoclusters have at least one dimension between 1 and 10 nanometers and a narrow size distribution [4, 5]. Nanopowders [6] are agglomerates of ultra fine particles, nanoparticles, or nanoclusters. Nanometer-sized single crystals, or single-domain ultra fine particles, are often referred to as nanocrystals. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures.

A bulk material should have constant physical properties regardless of its size, but at the nano-scale size-dependent properties are often observed. Thus, the properties of materials change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant. For bulk materials larger than one micrometer (or micron), the percentage of atoms at the surface is insignificant in relation to the number of atoms in the bulk of the material. The interesting and sometimes unexpected properties of nanoparticles are therefore largely due to the large surface area of the material, which dominates the contributions made by the small bulk of the material. Suspensions of nanoparticles are possible since the interaction of the particle surface with the solvent is strong enough to overcome density differences, which otherwise usually result in a material either sinking or floating in a liquid. Nanoparticles also often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects. For example, gold nanoparticles appear deep red to black in solution. Nanoparticles have a very high surface area to volume ratio, which provides a tremendous driving force for diffusion, especially at elevated temperatures. Sintering can take place at lower temperatures, over shorter time scales than for larger particles.

Aluminium oxide is an amphoteric oxide of aluminium with the chemical formula  $\text{Al}_2\text{O}_3$ . It is also commonly referred to as alumina, corundum, sapphire, ruby or aloxite in the mining, ceramic and materials science communities. This theoretically does not affect the density of the final product, though flow difficulties and the tendency of nanoparticles to agglomerate complicates matters. The large surface area to volume ratio also reduces the incipient melting temperature of nanoparticles [7]. Common nanoparticles which are used now a days are Oxide ceramics –  $\text{Al}_2\text{O}_3$ , CuO, Metal carbides – SiC, Nitrides – AlN, SiN, Metals – Al, Cu, Nonmetals – Graphite, carbon nanotubes, Layered – Al +  $\text{Al}_2\text{O}_3$ , Cu + C, PCM – S/S, Functionalized nanoparticles.

Common host fluids include water, ethylene or triethyleneglycols and other coolants, oil and other lubricants, bio-fluids, polymer solutions, and organic liquids. Nanoparticles are typically made of

chemically stable metals, metal oxides or carbon in various forms. The size of the nanoparticles imparts some unique characteristics to these fluids, including greatly enhanced energy, momentum and mass transfer, as well as reduced tendency for sedimentation and erosion of the containing surfaces. Nanofluids are being investigated for numerous applications, including cooling, manufacturing, chemical and pharmaceutical processes, medical treatments, cosmetics, etc.

### **1.3 Nanofluids Properties**

Nanofluids were found to exhibit higher thermo-physical properties such as thermal conductivity, thermal diffusivity and viscosity than those of base fluids. Heat transfer enhancement in a solid–fluid two-phase flow has been investigated for many years. Research on gas particle flow [8-11] showed that by adding particles, especially small particles in gas, the convection heat transfer coefficient can be greatly increased. The enhancement of heat transfer, in addition to the possible increase in the effective thermal conductivity, was mainly due to the reduced thickness of the thermal boundary layer. In the processes involving liquid–vapor phase change, particles may also reduce the thickness of the gas layer near the wall. The particles used in the previous studies were on the scale of a micrometer or larger. It is very likely that the motion of nanoparticles in the fluid will also enhance heat transfer. Therefore, more studies are needed on heat transfer enhancement in nanoparticle–fluid mixtures. Thermal conductivities of nanoparticle–fluid mixtures have been reported by Masuda et al. [12], Artus [13], and Eastman et al. [14] adding a small volume fraction of metal or metal oxide powders in fluids increased the thermal conductivities of the particle–fluid mixtures over those of the base fluids.

The viscosity of nanofluids, which is the key influencing factor in heat transfer [15–17], has not received as much attention. The experimental and simulation results indicate that nanoparticle size is of crucial importance to the viscosity of the nanofluid due to aggregation. As the nanoparticle size decreases, the viscosity becomes much more dependent on the volume fraction. Moreover, when the nanoparticle diameter is smaller than 20 nm, the viscosity is closely related to the pH of the nanofluid, and fluctuates with pH values from 5 and Although viscosity influences interfacial phenomena and flow characteristics, very few studies have been performed on the effective viscosity of nanofluids as a function of volumetric loading of nanoparticles [18–20]. The primary results from these studies indicate that the viscosity of a nanofluid increases with increasing nanoparticle volume fraction. In addition to the very few experimental studies, no theoretical model is available for the prediction of the effective viscosity of nanofluids as a function of temperature and particle volume fraction. It is worthwhile to study temperature-dependent viscosity of nanofluids for their potential applications especially in microfluidic systems.

As a thermodynamic property, the specific heat capacity of a nanofluid is important to dictate the nanoparticle and fluid temperature changes, which affect the temperature field of the nanofluid and hence the heat transfer and flow status. Other thermophysical properties, such as thermal diffusivity and Prandtl number need the knowledge of specific heat capacity, too.

#### **1.4 Nanofluids in Biological Applications**

In biology there are numerous examples of systems which far exceed any man-made machine in terms of efficiency, precision, and complexity. We would like to be able to take advantage of the engineering that Nature has done for thousands of years and directly manipulate biological molecules. Our goals are to create nanoscale interfaces to biology to control biological processes. This requires not only exploiting the unique size and material dependent properties of nanoparticles but also understanding and engineering their interface to biology, which is a crucial part of their implementation in any biological application.

In biomedical applications, PCR is one of the most popular tools in molecular diagnosis. It can amplify DNA samples to a detectable signal level within a short period of time and amplify a single or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. However, primer–dimer and GC-rich regions of the template and the PCR system's heating/cooling ratio may interfere with the efficiency of the PCR. To increase the efficiency and yield of the PCR, two key components of PCR should be considered; reagent and equipment. The reagents include the *Taq* DNA polymerase, primers and the template. The *Taq* DNA polymerase derived from the thermophilic bacterium *Thermus aquaticus* has shown the best activity at 72°C. Primer design, buffer content and the occurrence of primer–dimer have also been reported to affect PCR efficiency. Recently, PCR machines showing rapid heating-cooling responses have been developed.

The thermoelectric heating of the PCR used had to be restricted by the Peltier-effect because of the large thermal flux relative to the slow heating and cooling rates. To overcome this limitation, machines were developed with increased surface/volume ratio and decreased content volume, such as the capillary and the microchip PCR. The surface-to-volume ratio (S/V ratio) of the PCR chamber has been increased from 1.5 to 8.0 to 20.4 as the PCR machines were developed from conventional PCR equipment to capillary PCR machines to microchip-based PCR equipment. This change, however, increased the efficiency of the PCR only 5- to 10-fold, and the improvements to these machines pushed the thermal efficiency to its limit [21].

The nanoparticle is a novel material and has many physical properties which are different from bulk materials. In addition to their reaction with DNA, AuNPs also interact with other bio-

molecules, especially DNA polymerase. Because of the binding properties of AuNPs with proteins, interactions between AuNPs and DNA or polymerase can be applied to a variety of immunoassay [22, 23]. There are many strategies available for preparation of protein- AuNPs complexes [24-27]. It is reasonable to deduce that interactions between AuNPs and polymerase can be used to modulate and optimize PCR process. The reduced PCR amplification caused by excess AuNPs was due to the interaction between DNA polymerase and AuNPs, and increasing the polymerase concentration in the system could avoid the amplification-restraining effect.

### **1.5 Why nanoparticles use in PCR**

PCR is a technique which is carried out at three different temperatures. Initializing step which is at 94-96 °C, denaturation is at 94-98 °C, annealing is at 50-65 °C and final elongation is at 75-80 °C. All steps are carried out at high temperatures. If nanoparticles interfere with PCR product, then due to unique properties of nanoparticles such as high thermal conductivity, it may affect the PCR. Gold nanoparticles have found broad and important applications in biology; for example, a variety of ultra sensitive biosensor strategies have been reported which take advantage of gold nanoparticles. Gold nanoparticles are nontoxic, biocompatible nano materials that can be either obtained from commercial sources or conveniently produced in laboratories, and owing to the availability of versatile chemistry for functionalization at their surface as well as their unique optoelectronic properties gold nanoparticles provide a particularly useful platform for attachment of biomolecules. However, to the best of our knowledge, the use of gold nanoparticles to improve the performance of PCR has not been reported. It was also found that AuNPs affected the concentration-activity curve of DNA polymerase, and adjusting the AuNPs and polymerase concentrations could increase yield of the desired PCR product.

Based on the results, we propose that AuNPs improve PCR process through modulating the concentration of free-polymerase. We believe that AuNPs are an important component in PCR, and the study on interaction between AuNPs and polymerase will help to elucidate mechanisms of nanoparticle PCR, so as to explore new ways of optimizing PCR for applying nanoparticle PCR into biomedicine.

### **1.6 Conclusion**

Research work on the concept, its thermo-physical properties, heat transfer enhancement mechanism, and application of the nanofluids is still in its primary stage. This study provides a review of research in this field with focus on thermal properties of nanofluids as well as in biomedical applications to increase the efficiency of PCR.

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## **CHAPTER: 2**

### **LITERATURE REVIEW**

#### **2.1 Introduction**

Various studies on nanofluids were carried out by many researchers in the past. This chapter reviews the previous published literatures, which lays foundation and basis for further work in this investigation. This helps to give a better understanding about the topic and also acts as a guideline for this thesis. The major focus of the following study is on the nanofluids and its applications in various areas. This section deals with literature review on nanofluids, its preparation and characterization, thermo-physical properties of nanofluids and its biological applications.

#### **2.2 Nanofluids Preparation and Characterization**

##### **2.2.1 Preparation**

Choi et al. [1] first prepared nanofluids by mixing nano particles with fluids. Since then, there has been rapid development in the synthesis techniques for nanofluids. However, there is not yet a standard preparation method for nanofluids. Different studies prepared nanofluids using different approaches. There are two fundamental methods to obtain nanofluids:

- a). Two-step process. C.G. Granqvist, and R.A. Buhrman [2] found the two- step process. First, nanoparticles are produced as a dry powder, typically by inert gas condensation. The second step involves dispersion of dry nanoparticle powder into a base fluid, like water, oil or ethylene-glycol. Romano et al [3] reported an advantage of the two-step process is that the inert-gas condensation technique has been scaled up to commercial nanopowder production. A deficiency of this method is the tendency of nanopowder to agglomerate during storage and dispersion in the base fluids, particularly with heavier metallic nanoparticles. Surfactants and other surface-stabilization additives can be used to achieve more homogeneous and more stable suspensions. In addition to mechanical mixing, ultra-sonic mixers can be used to break up agglomerates and give more uniform dispersions.
- b). Chemical approach [4 and 5] using wet technology, a single-step approach, is emerging as a powerful method for growing nanostructures of different metals, semiconductors, non- metals, and hybrid systems. Laser ablation is another much sought, single-step technique that simultaneously makes and disperses nanoparticles directly in the base fluids. A variety of nanofluids have been prepared by laser ablation method [6-9] by ablating solid metals, semiconductors, etc which are submerged in the base fluid (water, lubrication oils, etc). By creating a nanofluid in this way, stable nanofluids resulted without using any property-changing dispersants. This method is also useful for further splitting of nanoparticles present in the

nanofluids to study effect of particle size on thermal conductivity. A.K. & Raykar et al. [10] gives another one step approach that is adopted for nanofluids synthesis based on microwave irradiation and is a quick method of nanofluid synthesis. Lo. C et al used submerged arc nano-synthesis system (SANSS) for preparing nanofluids [11, 12].

### **2.2.2 Characterization**

Imaging analysis of nanofluids is done using electron microscope (SEM/TEM, TEM being preferred over SEM for nanofluids) and most of the reported studies makes use of TEM for characterizing nanofluids. Cryogenic transmission electron microscopy might provide a powerful characterization method, but few laboratories are equipped to apply this technique. Scanning probe microscopy (SPM) has not found much use for characterizing nanofluids. A simplistic approach makes use of particle size analyzer based on dynamic light scattering (DLS). Robert J. Hunter [13] has used DLS systems for particle size distribution to augment the results with TEM as main characterization tool. A valuable characterization tool for the structure of suspensions at nanoscale is the small angle x-ray scattering (SAXS) and small angle neutron scattering (SANS). In these techniques a nanofluid is illuminated by a collimated x-ray or neutron beam and the intensity of scattering is measured as a function of angle. Lurio et al. [14] measured equilibrium dynamics by using a relatively recent x-ray scattering technique called x-ray photon correlation spectroscopy (XPCS), which is based on the traditional laser based photon correlation spectroscopy (PCS). In the XPCS technique a sample is illuminated with a fully coherent x-ray beam; instead of the more typical situation in which the incident x-rays consist of a large number of incoherent regions. The effect of coherent illumination is to obtain a speckle pattern superimposed on the usual SAXS pattern.

### **2.3 Nanofluid Thermal conductivity, Viscosity, and Specific heat**

Choi made the first measurement of thermal conductivity of nanofluid. Many experimental results of nanofluids thermal conductivity has been reported and summarized [15, 16]. The main characteristics are:

1. Thermal conductivities are far above those of the base fluids and above the traditional solid-liquid suspensions,
2. A strong temperature dependence of the thermal conductivity, and
3. A strong thermal conductivity to nanoparticle-concentration dependence.

Carbon nanotubes (about 1 % volume of 25 nm particles) produced the highest increase of 150% in thermal conductivity, while copper nanoparticles (about 0.5 % volume of 10 nm size) increased the

base fluid thermal conductivity by 40 %. In comparison, the thermal conductivity increase for oxide nanoparticles is an order of magnitude smaller.

Ravi Prasher et al. [17] performed the experiments and give results on the viscosity of alumina-based nanofluids which are reported for various shear rates, temperature, nanoparticle diameter, and nanoparticle volume fraction. From the data it seems that the increase in the nanofluid viscosity is higher than the enhancement in the thermal conductivity as reported in the literature. It is shown, however, that the viscosity has to be increased by more than a factor of 4-relative to the increase in thermal conductivity to make the nanofluid thermal performance worse than that of the base fluid. But still there is great advantage in using nanofluids.

Le-Ping Zhou et al [18] shows that the specific heat capacities of nanofluids are different from that of base fluid and vary with the size and volume concentration of nanoparticles. The specific heat capacity of CuO nanofluid decreases gradually with increasing volume concentration of nanoparticles. In most of the cases, however, there are no experimental data available for specific heat capacity, such that two kinds of models have been generally adopted to deduce it from the value of base fluid and nanoparticles. One is macroscopic, that the specific heat capacity of a nanofluid is equal to the volume average of the specific heat capacities of base fluid and nanoparticles. The other is microscopic, which assumes the base fluid and the nanoparticles are in their thermal equilibrium, respectively. Using the volumetric heat capacity instead, the volumetric heat capacity of a nanofluid can be expressed as the sum of the volumetric heat capacities for base fluid and nanoparticles, using their respective volume concentrations.

Sheng-Qi Zhou and Rui Ni [19] investigate the specific heat  $c_p$  of water-based  $Al_2O_3$  nanofluid with a differential scanning calorimeter. The result indicates that the specific heat  $c_p$  of nanofluid decreases gradually as the nanoparticle volume fraction increases from 0.0% to 21.7%. The relationship between them exhibits good agreement with the prediction from the thermal equilibrium model. The other simple mixing model fails to predict the specific heat of nanofluid.

## **2.4 Applications of Nanofluids**

Nanofluids are an exciting new category of more efficient heat transfer fluids that have the potential to greatly improve upon thermal management systems in a wide variety of applications, owing to their increased thermal conductivity and long term stability [20]. Some of the applications of nanofluids are as follows:

### 2.4.1 Cooling Applications

**Crystal Silicon Mirror Cooling** - One of the first applications of research in the field of nanofluids is for developing an advanced cooling technology to cool crystal silicon mirrors used in high intensity x-ray sources. Lee and Choi [21] carried out analysis to estimate the performance of microchannel heat exchangers with water, liquid nitrogen, and nanofluids as the working fluid.

**Electronics Cooling** – Chien et al. [22] were probably the first to show experimentally that the thermal performance of heat pipes can be enhanced by nearly a factor 2 when nanofluids are used. Water based nanofluids are used containing 17 nm gold nanoparticle as the working fluid in a disk-shaped miniature heat pipe (DMHP). Then, measured the thermal resistance of the DMHP with both nanofluids and deionized (DI) water. The results show that thermal resistance of a DMHP is reduced significantly (40%) when nanofluids are used instead of DI water.

**Vehicle cooling** – Nanoparticles can be dispersed not only in coolants and engine oils, but in transmission fluids, gear oils, and other fluids and lubricants. These may provide better overall thermal management and better lubrication. Tzeng et al. [23] were probably the first to apply nanofluid research in cooling a real-world automatic power transmission system. They dispersed CuO and Al<sub>2</sub>O<sub>3</sub> nanoparticles into automatic transmission oil to investigate the optimum possible compositions of a nanofluid for higher heat transfer performance.

**Transformer Cooling**- The power generation industry is interested in transformer cooling application of nanofluids for reducing transformer size and weight. If the heat transfer capability of existing transformers can be increased, many of the upgrades may not be necessary. Xuan and Li and Yu et al. [24, 25] have demonstrated that the heat transfer properties of transformer oil can be improved by using nanoparticle additives.

**Space and Nuclear Systems Cooling**- You et al. [26] have discovered the unprecedented phenomenon that nanofluids can double or triple the CHF in pool boiling. Kim et al. [27] found that the high surface wettability caused by nanoparticle deposition can explain this remarkable thermal property of nanofluid. The work is important in developing realistic predictive models of the CHF in nanofluids. The ability to greatly increase the CHF, the upper heat flux limit in nucleate boiling systems, is of paramount practical importance to ultra-high heat flux devices that use nucleate boiling, such as high power lasers and nuclear reactor components. Therefore nanofluids opened up exciting possibilities for raising chip power in electronic devices or simplifying cooling requirements for space applications.

#### **2.4.2. Tribological Applications**

Nanofluid technology can help develop better oil and lubricants. Recent nanofluid activity involves the use of nanoparticle in lubricants to enhance tribological properties of lubricants, such as load carrying capacity and antiwear and friction reducing properties between moving mechanical components. Que et al. [28] performed experimentally in lubrication application and reported that surface modified nanoparticles stably dispersed in mineral oils are very effective in reducing wear and enhancing load carrying capacity.

#### **2.4.2 Biomedical Applications**

Nanofluids was originally developed primarily for thermal management application such as engine, microelectronics, and photonic. However, nanofluids can be formulated for a variety of other uses for faster cooling. Nanofluids are being developed for medical applications, including cancer therapy. Jordan et al. [29] suggested that iron based nanoparticles can be used as delivery vehicles for drugs or radiation without damaging near by healthy tissue by guiding the particles up the bloodstream to a tumor with magnets. In contrast to cooling, nanofluids could be used to produce higher temperatures around tumors, to kill cancerous cells without affecting nearby healthy cells.

#### **2.5 Nanofluids in biological applications**

Conjugation of proteins to nanoparticles has numerous applications in sensing, imaging, delivery, catalysis, therapy and control of protein structure and activity. M-E Aubin-Tam and K Hamad-Schifferli [30] characterizing the nanoparticle protein interface. Site-specific labeling is desirable in order to control the protein orientation on the nanoparticle, which is crucial in many applications such as fluorescence resonance energy transfer. A specific protein residue is linked directly to the nanoparticle core or to the ligand. Characterization studies of nanoparticles protein complexes show that the structure and function are influenced by the chemistry of the nanoparticle ligand, the nanoparticle size, the nanoparticle material, the stoichiometry of the conjugates, the labeling site on the protein and the nature of the linkage.

Marie-Eve Aubin-Tam and Kimberly Hamad-Schifferli [31] reported the effect of nanoparticle ligand charge on the structure of a covalently, site-specifically linked protein. Au nanoparticles with positive, negative, and neutral ligands were appended to a specific cysteine, C102, of *Saccharomyces cerevisiae* cytochrome c. Conjugates were purified by HPLC or gel electrophoresis. Circular dichroism spectroscopy shows that changing the nanoparticle ligand dramatically influences the attached cytochrome c structure. The protein retains its structure with neutral ligands

but denatures in the presence of charged species. This is rationalized by the electrostatic interaction of amino acids in the local vicinity of C102 with the end groups of the ligand.

Das [32] briefed about nanofluids and its applications in two aspects (a) its utility in engineering as a heat transfer fluid and (b) its usage in biomedical applications such as that of cancer treatment. By this two different properties of nanofluids are analyzed. For Laser ablation treatment of tumors the significant property required is the absorption to the laser irradiation by nanoparticles to localize the heating it is seen that gold rods have this feature and a finite element analysis to predict the temperature profiles occurring during laser irradiation gives an excellent prediction of the observations made experimentally.

Min Li, Yu-Cheng Lin, Chao-Chin Wu and Hsiao-Sheng Liu [33] found that the PCR could be dramatically enhanced by Au nanoparticles. With the addition of 0.7 nM of 13 nm Au nanoparticles into the PCR reagent, the PCR efficiency was increased. Especially when maintaining the same or higher amplification yields, the reaction time could be shortened, and the heating/ cooling rates could be increased. The excellent heat transfer property of the nanoparticles should be the major factor in improving the PCR efficiency. Different PCR systems, DNA polymerases, DNA sizes and complex samples were compared in this study. Our results demonstrated that Au nanoparticles increase the sensitivity of PCR detection 5 to 10 fold in a slower PCR system (i.e. conventional PCR) and at least 10<sup>4</sup> fold in a quicker PCR system(i.e. real-time PCR). After the PCR time was shortened by half, the 100 copies/ml DNA were detectable in real-time PCR with gold colloid added, however, atleast 10<sup>6</sup> copies/ml of DNA were needed to reach a detectable signal level using the PCR reagent without gold colloid. This innovation could improve the PCR efficiency using non expensive polymerases, and general PCR reagent. It is a new viewpoint in PCR, that nanoparticles can be used to enhance PCR efficiency and shorten reaction times.

MI LiJuan, ZHU HongPing, ZHANG XiaoDong, HU Jun, & FAN ChunHai [34] suggested that nanoparticle PCR is a novel method to optimize DNA amplification. It performs well in improving specificity, enhancing sensitivity and speed. Several mechanisms were proposed in previous studies: one was based on the interaction between gold nanoparticles (AuNPs) and DNA while the other was attributed to the heat transfer property of AuNPs. In this paper, we propose that the interaction between AuNPs and DNA polymerase can significantly influence PCR. First, the addition of DNA polymerase can eliminate the inhibitory effects of excess AuNPs. Second, the addition of AuNPs will increase yield of the desired PCR product and make the optimum concentration of DNA polymerase move to higher value. Third, while excess polymerase might inhibit amplification efficiency, AuNPs can reverse this process and the yield of PCR

amplification. Based on these results we propose a possible mechanism that AuNPs might modulate the activity of polymerase and improve PCR amplification.

## Conclusion

The literature review carried out in this chapter highlights about nanofluids, its preparation and characterization and thermophysical properties. The applications of nanofluids in heat transfer and in biomedical area have also high lightened. In today's science, nanofluids play a major role in various areas due to their unique characteristics. According to the literature, still there very few researches are carried out on nanofluids properties and its applications. In this thesis, our main work is to find out the thermophysical properties of nanofluids and then implement it in biological applications.

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## **CHAPTER: 3**

### **EXPERIMENTAL SETUP**

Nanoparticles are very small, nanometer-sized particles which are stable colloidal suspensions in common base fluids such as water, oil, ethylene-glycol mixtures (antifreeze), polymer solutions, etc. To measure its thermophysical properties various equipments are used such as thermal property analyzer are used to measure thermal conductivity and volumetric heat capacity, and U-Tube viscometer as used to measure viscosity. Further, to check the efficiency of PCR, nanofluids are added in PCR reagent and then results are verified by agarose gel electrophoresis. These techniques are discussed briefly in this chapter.

#### **3.1 Ultra Sonicator**

Sonication is the act of applying sound (usually ultrasound) energy to agitate particles in a sample, for various purposes. In the laboratory, it is usually applied using an ultrasonic bath known as a sonicator. Sonication can be used to speed dissolution, by breaking intermolecular interactions. It is especially useful when it is not possible to stir the sample. Sonication is commonly used in nanotechnology for evenly dispersing nanoparticles in liquids.



Figure 3.1: Ultra sonicator

### 3.2 Thermal Property analyzer

The KD2 Pro is a hand held device used to measure thermal properties. It consists of handheld controller and sensors that can be inserted into the medium. The single needle sensors measure thermal conductivity and resistivity; while the dual needle sensor also measures volumetric specific heat capacity and diffusivity. It has been designed for ease of use and maximum functionality.

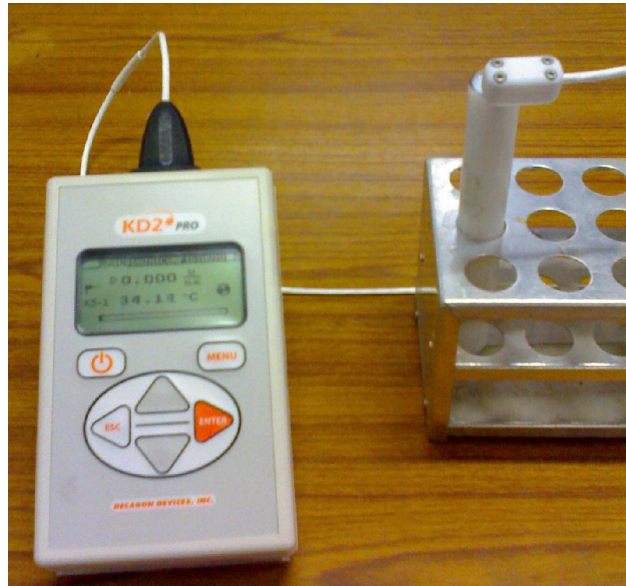


Figure 3.2: KD2 Pro

#### Specifications

Operating environment:

Controller: 0 to 50 °C

Sensors: -50 to +150 °C

Power: 4 AA cells

Battery life: At least 500 readings in constant use or 3 years with no use (battery drain in sleep mode < 50 uA)

Case size: 15.5 cm x 9.5 cm x 3.5 cm

Display: 3 cm x 6 cm, 128 x 64 pixel graphics LCD

Keypad: 6 key, sealed membrane

Data storage: 4095 measurements in flash memory (both raw and processed data are stored for download)

Interface: 9-pin serial

Read modes: Manual and Auto Read

## Sensors:

### KS-1 single needle

The small (60 mm long, 1.3 mm diameter) single needle KS-1 sensor measures thermal conductivity and thermal resistivity. It is designed primarily for liquid samples and insulating materials (thermal conductivity  $< 0.1 \text{ W/(m.K)}$ ). The KS-1 sensor applies a very small amount of heat to the needle which helps to prevent free convection in liquid samples. However, the small size of the needle and typically short heating time make the KS-1 a poor choice for granular samples such as soil and powders where contact resistance can be an important source of error. In insulating materials, the errors from contact resistance become negligible making the KS-1 sensor a good choice.

Size: 1.3mm diameter x 60mm long

Range: 0.02 to 2.00 W/(m.K) (thermal conductivity)

50 to 5000 °C cm/W (thermal resistivity)

Accuracy (Conductivity):  $\pm 5\%$  from 0.2 – 2 W/(m.K)

$\pm 0.01 \text{ W/(m.K)}$  from 0.02 - 0.2 W/(m.K)

Cable length: 0.8 m



Figure 3.3: KS-1 sensor needle

### TR-1 single needle

The large (100 mm long, 2.4 mm diameter) single needle TR-1 sensor measures thermal conductivity and thermal resistivity. It is designed primarily for soil, concrete, rock, and other granular or solid materials. The relatively large diameter and typically longer heating time of the

TR-1 sensor minimize errors from contact resistance in granular samples or solid samples with pilot holes. The TR-1 needle heats the sample significantly more than the KS-1 sensor, which allows it to measure higher thermal conductivity samples, but means that you not measure liquid samples with the TR-1 sensor. The large diameter of the TR-1 is more robust than the KS-1, meaning that it is less likely to be damaged by normal usage conditions in soil or other solid materials. Additionally, the dimensions of the TR-1 sensor conform to the specifications for the Lab Probe called out by the IEEE 442-03 Guide for soil Thermal resistivity measurements.

Size: 2.4 mm diameter x 100 mm long

Range: 0.10 to 4.00 W/m.K (Thermal conductivity)

25 to 1000 °C cm /W (Thermal resistivity)

Accuracy (conductivity):  $\pm 10\%$  from 0.2 – 4 W/ (m.K)

$\pm 0.02$  W/ (m.K) from 0.1 – 0.2 W/ (m.K)

Cable length: 0.8 m

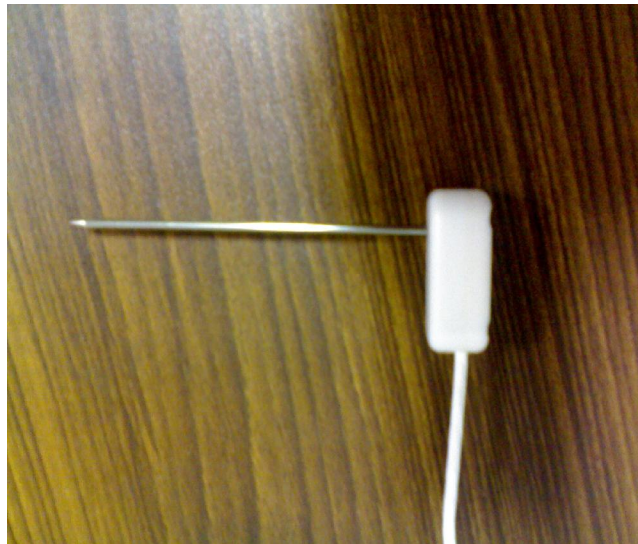


Figure 3.4: TR-1 sensor needle

### **SH-1 dual needle**

The dual needle SH-1 sensor measures volumetric heat capacity, thermal diffusivity, thermal conductivity, and thermal resistivity. The SH-1 is compatible with most solid and granular materials, but should not be used in liquids due to the large heat pulse and possible resulting free convection in liquid samples.

Size: 1.3 mm diameter x 30 mm long, 6 mm spacing

Range: 0.02 to 2.00 W/ (m.K) (thermal conductivity)

50 to 5000 °C cm/W (thermal resistivity)

0.1 to 1 mm<sup>2</sup>/s (diffusivity)

0.5 to 4 mJ/ (m<sup>3</sup>K) (volumetric specific heat)

Accuracy: (conductivity)  $\pm 10\%$  from 0.2-2W/ (m.K)

$\pm 0.01$  W/ (m.K) from 0.02 -0.2 W/ (m.K)

(Diffusivity)  $\pm 10\%$  at conductivities above 0.1 W/ (m.K)

(Volumetric Specific Heat)  $\pm 10\%$  at conductivities above 0.1 W/ (m.K)

Cable length: 0.8 m

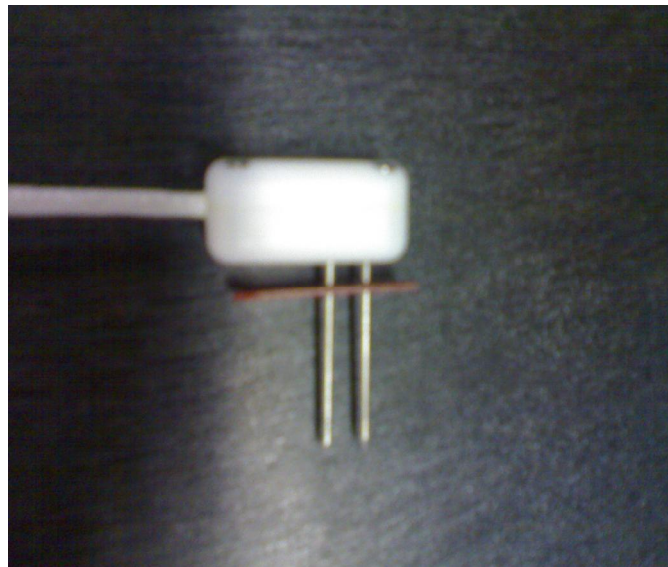


Figure 3.5: SH-1 sensor needle

### 3.3 U-Tube (Ostwald viscometer) viscometer

This is used to measure viscosity of fluids. These devices also are known as glass capillary viscometers or Ostwald viscometers, named after Wilhelm Ostwald. Another version is the Ubbelohde viscometer, which consists of a U-shaped glass tube held vertically in a controlled temperature bath. In one arm of the U is a vertical section of precise narrow bore (the capillary). Above this is a bulb; with is another bulb lower down on the other arm. In use, liquid is drawn into the upper bulb by suction, and then allowed to flow down through the capillary into the lower bulb. Two marks (one above and one below the upper bulb) indicate a known volume. The time taken for the level of the liquid to pass between these marks is proportional to the kinematic viscosity. Most commercial units are provided with a conversion factor, or can be calibrated by a fluid of known properties. The time required for the test liquid to flow through a capillary of a known diameter of a

certain factor between two marked points is measured. By multiplying the time taken by the factor of the viscometer, the kinematic viscosity is obtained. It can be calculated as:

$$\frac{\mu (\text{Fluid})}{\mu (\text{Water})} = \frac{t (\text{Fluid}) * \rho (\text{Fluid})}{t (\text{Water}) * \rho (\text{Water})}$$



Figure 3.6: Ostwald viscometer

### 3.4 Specific gravity Bottle

Specific gravity bottle is used to measure the density of any fluid. Relative density, or specific gravity is the ratio of the density (mass of a unit volume) of a substance to the density of a given reference material. Specific gravity usually means relative density with respect to water. Relative density (RD) or specific gravity (SG) is a dimensionless quantity, as it is the ratio of either densities or weights:

$$RD = \frac{\rho_{\text{substance}}}{\rho_{\text{reference}}}$$

A specific gravity bottle holds a known volume of liquid at a specified temperature. The bottle is weighed, filled with a liquid whose specific gravity is to be found and then again weighed. The difference in weights is divided by the weight of an equal volume of water to give the specific gravity of the liquid. Note that since water has a density of  $1 \text{ g/cm}^3$ , the specific gravity is the same as the density of the material measured in  $\text{g/cm}^3$ .

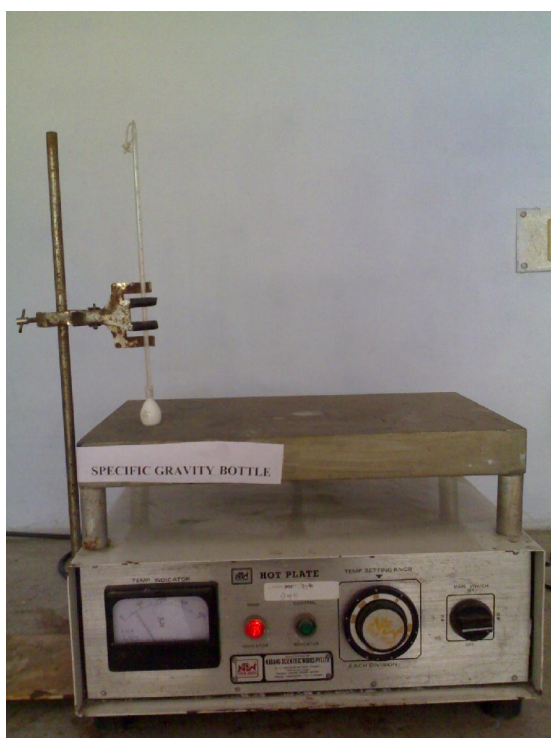


Figure 3.7: Specific gravity bottle

### 3.5 Biorad PCR

Polymerase chain reaction (PCR) is a technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase (after which the method is named) are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR is used to amplify a specific region of a DNA strand (the DNA target). Most PCR methods typically amplify DNA fragments of up to ~10 kilo base pairs (kb), although some techniques allow for

amplification of fragments up to 40 kb in size. A basic PCR set up requires several components and reagents. These components include:

- DNA template that contains the DNA region (target) to be amplified.
- Two primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target.
- Taq polymerase or another DNA polymerase with a temperature optimum at around 70 °C.
- Deoxynucleoside triphosphates (dNTPs; also very commonly and erroneously called deoxynucleotide triphosphates), the building blocks from which the DNA polymerases synthesizes a new DNA strand.
- Buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
- Divalent cations, magnesium or manganese ions; generally  $Mg^{2+}$  is used, but  $Mn^{2+}$  can be utilized for PCR-mediated DNA mutagenesis, as higher  $Mn^{2+}$  concentration increases the error rate during DNA synthesis.
- Monovalent cation potassium ions.

### **Procedure for Operation of PCR**

The PCR usually consists of a series of 20 to 40 repeated temperature changes called cycles; each cycle typically consists of 2-3 discrete temperature steps [1]. Most commonly PCR is carried out with cycles that have three temperature steps. The cycling is often preceded by a single temperature step (called *hold*) at a high temperature ( $>90$  °C), and followed by one hold at the end for final product extension or brief storage. The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters. These include the enzyme used for DNA synthesis, the concentration of divalent ions and dNTPs in the reaction, and the melting temperature ( $T_m$ ) of the primers.

- Initialization step: This step consists of heating the reaction to a temperature of 94–96 °C (or 98 °C if extremely thermostable polymerases are used), which is held for 1–9 minutes.
- Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 94–98 °C for 20–30 seconds. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single strands of DNA.
- Annealing step: The reaction temperature is lowered to 50–65 °C for 20–40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically the annealing temperature is about 3-5 degrees Celsius below the  $T_m$  of the primers used. Stable DNA-DNA

hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. The polymerase binds to the primer-template hybrid and begins DNA synthesis.

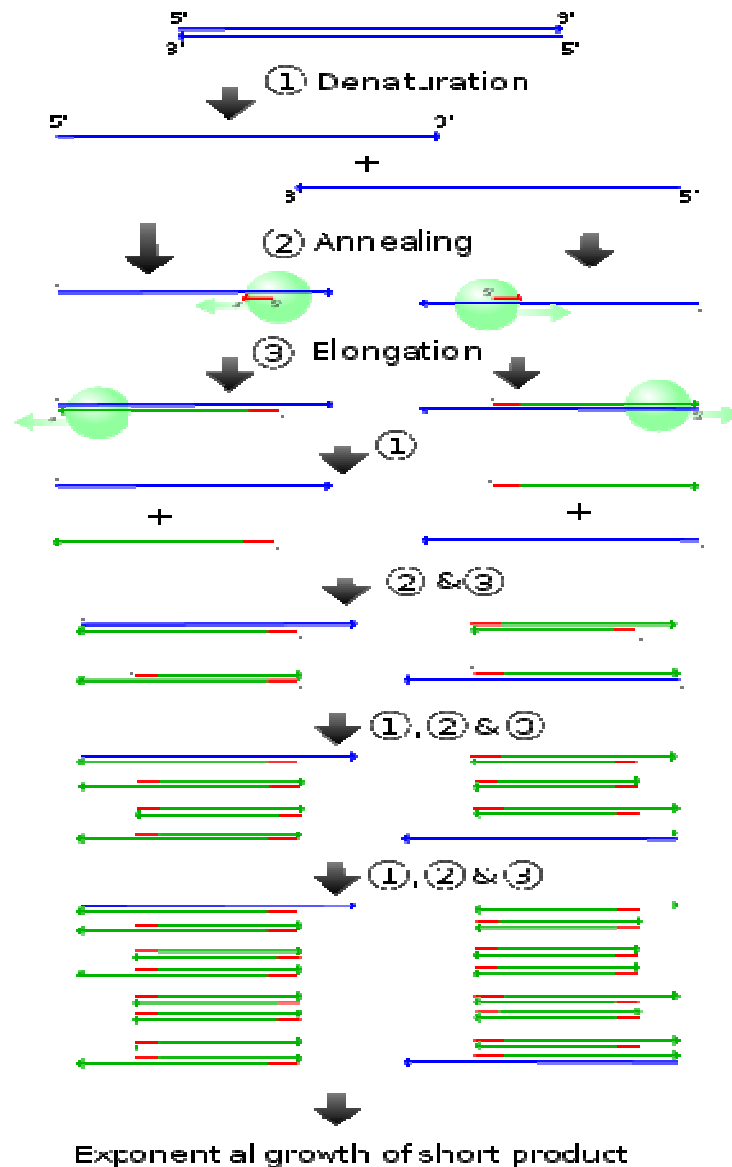


Figure 3.8: Schematic drawing of the PCR cycle. (1) Denaturing at 94–96 °C. (2) Annealing at ~65 °C (3) Elongation at 72 °C. Four cycles are shown here. The blue lines represent the DNA template to which primers (red arrows) anneal that are extended by the DNA polymerase (light green circles), to give shorter DNA products (green lines), which themselves are used as templates as PCR progresses.

- Extension/elongation step: The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75–80 °C and commonly a temperature of 72 °C is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to

the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified. As a rule-of-thumb, at its optimum temperature, the DNA polymerase will polymerize a thousand bases per minute. Under optimum conditions i.e. if there are no limitations due to limiting substrates or reagents, at each extension step, the amount of DNA target is doubled, leading to exponential (geometric) amplification of the specific DNA fragment.

- Final elongation: This single step is occasionally performed at a temperature of 70–74 °C for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.
- Final hold: This step at 4–15 °C for an indefinite time may be employed for short-term storage of the reaction.



Figure 3.9: Biorad PCR

### 3.6 Agarose gel electrophoresis

Agarose gel electrophoresis is a method used to separate DNA/proteins, or RNA molecules by size. This is achieved by moving negatively charged nucleic acid molecules through an agarose matrix with an electric field (electrophoresis). Shorter molecules move faster and migrate farther than longer ones.

#### Applications

- Estimation of the size of DNA molecules following restriction enzyme digestion, e.g. in restriction mapping of cloned DNA.
- Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting.
- Separation of restricted genomic DNA prior to Southern transfer or of RNA prior to Northern transfer.

The most important factor is the length of the DNA molecule, smaller molecules travel farther. But conformation of the DNA molecule is also a factor. To avoid this problem linear molecules are usually separated, usually DNA fragments from a restriction digest, linear DNA PCR products, or RNAs. Increasing the agarose concentration of a gel reduces the migration speed and enables separation of smaller DNA molecules. The higher the voltage, the faster the DNA moves. But voltage is limited by the fact that it heats and ultimately causes the gel to melt. High voltages also decrease the resolution (above about 5 to 8 V/cm).

The most common dye used to make DNA or RNA bands visible for agarose gel electrophoresis is ethidium bromide, usually abbreviated as EtBr. It fluoresces under UV light when intercalated into DNA (or RNA). By running DNA through an EtBr-treated gel and visualizing it with UV light, any band containing more than ~20 ng DNA becomes distinctly visible. EtBr is a known mutagen; however, safer alternatives are available.

There are a number of buffers used for agarose electrophoresis. The most common being: tris acetate EDTA (TAE), Tris/Borate/EDTA (TBE) and Sodium borate (SB). TAE has the lowest buffering capacity but provides the best resolution for larger DNA.

After electrophoresis the gel is illuminated with an ultraviolet lamp (usually by placing it on a light box, while using protective gear to limit exposure to ultraviolet radiation) to view the DNA bands. The ethidium bromide fluoresces reddish-orange in the presence of DNA. The DNA band can also be cut out of the gel, and can then be dissolved to retrieve the purified DNA. The gel can then be

photographed usually with a digital or polaroid camera. . Although the stained nucleic acid fluoresces reddish-orange, images are usually shown in black and white.

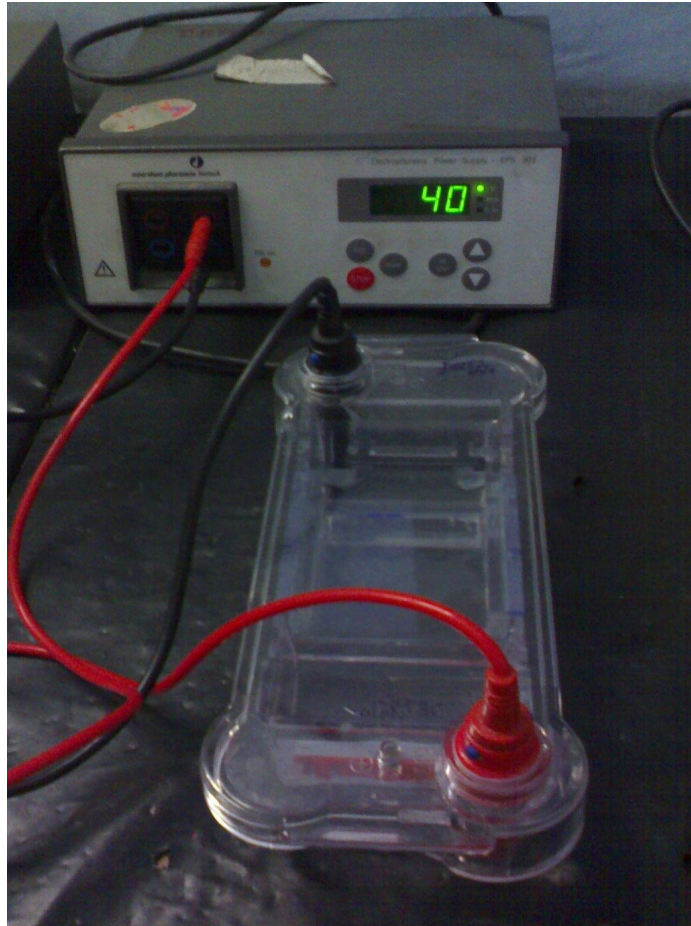


Figure 3.10: Agarose gel electrophoresis

### 3.7 Gel Documentation System

The Gel Doc XR system is a high-resolution gel documentation system for all of your everyday imaging needs. The 1.4 megapixel imaging system is fast and easy to use. Follow the onscreen steps and get your results with only three clicks of a mouse. The motorized zoom lens allows a hands-free approach to finding the perfect focus, zoom, and iris settings. The intuitive and easy-to-use Quantity One interface allows you to expose and print directly from the acquisition window. The Gel Doc XR provides a detection range of 3 orders of magnitude with a 12-bit CCD and utilizes a FireWire interface for fast data transfer.

- High-resolution imaging with 1.4 million pixels
- Motorized zoom lens for hands-free gel documentation
- Real-time imaging for quick positioning and focusing of samples

- Onscreen integration - No need to save an image; simply “freeze” the image at the desired intensity and print
- FireWire interface for rapid data transfer
- White light transilluminator allows documentation of visible dyes
- Upgradable to the Chemi Doc XRS system

The Gel Doc XR provides extra resolution for the acquisition, optimization, quantization, and documentation of all of your gel data.



Figure 3.11: Gel documentation system

**Reference:**

1. Rychlik W, Spencer WJ, and Rhoads RE (1990). Optimization of the annealing temperature for DNA amplification *in vitro*. *Nucl Acids Res*, Volume **18**. pp. 6409–6412.

## CHAPTER-4

### MATERIALS AND METHODOLOGY

#### 4.1 Introduction

The main aim of this thesis is to prepare nanofluids with  $\text{Al}_2\text{O}_3$  nanoparticles and water as fluid for improving the heat transfer characteristics of the fluid. These analyses are performed by measuring all thermo physical properties like thermal conductivity, specific heat, density and viscosity at different temperatures for the different volume concentrations of nanofluids.

The other main aim of this thesis is to implement nanofluids in its biomedical applications. Gold nanofluids are used to increase the efficiency of PCR by reducing the PCR time cycle, by changing the size of nanoparticles or by diluting the enzyme concentrations.

#### 4.2 Materials Required

##### 4.2.1 Materials used for preparing Nanofluids

- Nanoparticles-  $\text{Al}_2\text{O}_3$  (Average size- 45 nm)
- Distilled water

##### 4.2.2 Ingredients of PCR

Total reaction=50 $\mu\text{L}$

a) For control

- PCR water – 36.5  $\mu\text{L}$
- 10X Buffer- 5  $\mu\text{L}$
- dNTPs- 5  $\mu\text{L}$
- Forward Primer-2.5  $\mu\text{L}$
- Reverse Primer- 2.5  $\mu\text{L}$
- Template DNA-1  $\mu\text{L}$
- Taq DNA Polymerase (stored at -20 °C)

b) For 1% Gold (30nm or 60nm) or  $\text{Al}_2\text{O}_3$  Nanoparticles

- PCR water – 26.5  $\mu\text{L}$

- 10X Buffer- 5  $\mu$ L
- dNTPs- 5  $\mu$ L
- Forward Primer-2.5  $\mu$ L
- Reverse Primer- 2.5  $\mu$ L
- Template DNA-1  $\mu$ L
- Gold or Al<sub>2</sub>O<sub>3</sub> nanoparticles- 10  $\mu$ L
- Taq DNA Polymerase (stored at -20 °C)

c) For 0.5% Gold (30nm or 60nm) or Al<sub>2</sub>O<sub>3</sub> Nanoparticles

- PCR water – 31.5  $\mu$ L
- 10X Buffer- 5  $\mu$ L
- dNTPs- 5  $\mu$ L
- Forward Primer-2.5  $\mu$ L
- Reverse Primer- 2.5  $\mu$ L
- Template DNA-1  $\mu$ L
- Gold or Al<sub>2</sub>O<sub>3</sub> nanoparticles- 5  $\mu$ L
- Taq DNA Polymerase (stored at -20 °C)

d) For 0.5% Gold (30nm + 60nm)

- PCR water – 26.5  $\mu$ L
- 10X Buffer- 5  $\mu$ L
- dNTPs- 5  $\mu$ L
- Forward Primer-2.5  $\mu$ L
- Reverse Primer- 2.5  $\mu$ L
- Template DNA-1  $\mu$ L
- Gold (30 nm) nanoparticles- 5  $\mu$ L
- Gold (60 nm) nanoparticles- 5  $\mu$ L
- Taq DNA Polymerase (stored at -20 °C)

#### 4.2.3 Ingredients of 0.7 % Agarose Gel Electrophoresis

- Agarose powder-0.7 gm
- 5X TBE- 10 ml
- Double distilled water- 90 ml
- EtBr- 10  $\mu$ L
- Dye (Bromophenol blue)- 3  $\mu$ L
- 0.5X TBE

- A gel rack
- Nitrile rubber gloves
- A comb
- Power supply
- PCR product
- U.V Lamp or Gel Documentation System

Experiments were carried out in the Department of Biotechnology and Environmental sciences and its facilities have been used in the experimentation.

### **4.3 Methodology**

#### **4.3.1 Preparation of nanofluids**

Aluminium oxide ( $\text{Al}_2\text{O}_3$ ) nanoparticles are purchased from M/s. Alpha Aesar, A Johnson Matthey Chemicals India Pvt Ltd., Hyderabad. The size of nanoparticles is 45 nm as it was mentioned by the company. For its characterization, it can be tested under SEM in R&D of Thapar University, Patiala but due to some problem in SEM results are not satisfactory. Nanofluids are prepared by two step process. The resulting nanoparticles are then dispersed into the base fluid i.e. distilled water. Make the volume concentration of 1 %, 2 %, and 4 % by mixing 1 gm, 2 gm, and 4 gm of nanoparticles in 100 ml of distilled water. To make the nanoparticles more stable and remain more dispersed in water, ultra sonicator is used. Sonication had done for 3-4 hours before testing any thermo physical property of the nanofluids. By this nanoparticles become more evenly dispersed in distilled water.

#### **4.3.2 Thermo physical Properties Measurement**

For the measurement of Thermal conductivity and volumetric specific heat, KD2 Pro is used. Different sensors are used for the measurement of these properties. As nanofluids are relatively low viscosity fluid, so KS-1 sensor is used to measure the thermal conductivity. And for the measurement of volumetric specific heat SH-1 sensor is used. These properties can be measured at various ranges of temperatures such as from 30 °C to 90 °C. Nanofluids get heated by hot plate and wait till the steady state achieved at particular temperature. After attaining the steady state, dipped the sensor in the bottle and on the KD2 Pro. It takes 1 minute for thermal conductivity and 2 minutes for volumetric specific heat. After this note the readings at that particular temperature which shows on the screen. Same experiment is repeated for the different volume concentrations of nanofluids i.e. 1 %, 2 % and 4 % at different temperature.

For the measurement of density of nanofluids, specific gravity bottle method is used. Firstly, weigh the specific gravity bottle then fill with the distilled water. Weigh the bottle filled with water at different temperature ranges from 30 °C to 90 °C. Then, the filled bottle with nanofluids sample was weighed again at different temperatures. To calculate the specific gravity, take the ratio of the net weight of the nanofluid and distilled water. Distilled water is used as a reference because water is used as a base fluid for the preparation of nanofluids.

For the measurement of viscosity of nanofluids, Ostwald viscometer is used. As this apparatus is cheap and easily available, and still no one can find viscosity through this viscometer, this is used in the thesis work. There are two bulbs such as one is at lower section on one side and other bulb is at upper side of another side. Fill the lower bulb with water and then water is drawn into the upper bulb by suction, and then allowed to flow down through the capillary into the lower bulb. The time taken for the water to pass between the two marks of viscometer noted down. This is done at various temperature ranges i.e. 30 °C to 90 °C. Viscometer put in the water bath and then, maintains the steady state at 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C. Note down the time taken for fluid to pass between the two marks after attaining the steady state. Firstly, it was done for distilled water as distilled water is used as a base fluid for preparing nanofluids. Then, same method is done for the other samples i.e. 1 %, 2 %, 4 % volume concentrations of nanofluids and noted down the time at different temperatures. Then, calculate the dynamic viscosity by dividing it by density.

### **4.3.3 PCR with Nanoparticles**

Before starting the experiment, switch on the U.V light of laminar air flow for 15min. Take small eppendorf tubes for preparing the samples and label it. Make 50  $\mu$ L reaction by adding sterile water, buffer, dNTPs, forward and reverse primer, template DNA in the quantity as mentioned above in materials required. For reaction with nanoparticles, gold nanoparticles are added before adding the template DNA. AuNPs are purchased from IMTECH, Chandigarh. After adding all this, finally Taq DNA polymerase is added in the quantity of 3units/ $\mu$ L. But in one experiment in which we see the effect of enzyme dilution, this polymerase is added in the quantity of 1.5 units/ $\mu$ L. Then, closed the lid of tube and places the tube in Biorad PCR. Program is of 30 cycles:

1. For first experiment, PCR run at 94 °C for 1 min, 94 °C for 1 min, 55 °C for 2 min, 72 °C for 1min. In this size of DNA is 0.8 kb which is less than 1 kb. For this size of DNA, above time cycle is a standard time cycle.
2. For second experiment, time cycle reduced and it was 94 °C for 30 sec, 94 °C for 30 sec, 55 °C for 45 sec, 72 °C for 30 sec. Further, all experiment performed on same time cycle for 0.8 kb size of DNA.

3. When the size of DNA is increased i.e. 2.6 kb, PCR run at 94 °C for 30 sec, 94 °C for 45 sec, 55 °C for 45 sec, 72 °C for 1 min.

#### **4.3.4 Agarose gel electrophoresis**

Make a 0.7 % agarose solution in 100 ml TAE, for 0.8 kb DNA fragments. For making 100 ml TAE, take 10 ml 5X buffer solution and 90 ml double distilled water as our requirement is to make 0.5X buffer solution. Carefully bring the solution just to the boil to dissolve the agarose, in a microwave oven. Let the solution cool down to about 40-45 °C at room temperature, or water bath. Stir or swirl the solution while cooling. Wear gloves from here on, as ethidium bromide is a mutagen.

Add 10 µl ethidium bromide stock per 100 ml gel solution for a final concentration of 1 µg/ml. Insert the comb at one side of the gel, about 5-10 mm from the end of the gel. Stir the solution to disperse the ethidium bromide, and then pour it into the gel rack. When the gel has cooled down and become solid, carefully remove the comb. The holes that remain in the gel are the wells or slots. TAE buffer is filled in the gel rack so that gel must be completely covered with the buffer, with the slots at the end electrode that will have the negative current.

After the gel has been prepared, use a micropipette to inject DNA ladder and PCR product. Inject 15µl DNA ladder into the first slot of gel. Dilute the 3 µl DNA sample with 8 µl distilled water and add 3 µl EtBr dye so that it can be visible under U.V light. A current is applied. The DNA moves toward the positive anode due to the negative charges on its phosphate backbone.

Small DNA strands move fast, large DNA strands move slowly through the gel. The DNA is not normally visible during this process, so the marker dye is added to the DNA to avoid the DNA being run entirely off the gel. The marker dye has a low molecular weight, and migrates faster than the DNA, so as long as the marker has not run past the end of the gel, the DNA will still be in the gel. Finally, U.V lamp or Gel documentation system is used to see the results.

## CHAPTER-5

### RESULTS AND DISCUSSIONS

Various experiments were performed during thesis work with the help of various apparatus and techniques as mentioned in previous chapter. In this chapter we discuss all the results which we get from the experiments like viscosity measurement, density measurement, thermal conductivity measurement and PCR.

#### 5.1 Thermal conductivity measurement

Thermal conductivity can be measured by thermal property analyzer i.e. KD2 Pro by using KS-1 sensor needle as this needle is preferred for low viscosity fluids. It is measured at different ranges of temperature such as from 30 °C to 90 °C for the different concentration of nanoparticles such as 1 %, 2 % and 4 % and data calculated by experiment is mentioned in table 5.1, 5.2 and 5.3.

**Table 5.1 Values of Thermal conductivity at different temperatures for 1 % Al<sub>2</sub>O<sub>3</sub>**

S. No	Temperature, °C	Thermal conductivity (W/(m.K) 1 % Al <sub>2</sub> O <sub>3</sub> )
1	30.33	0.632
2	35.16	0.651
3	37.05	0.663
4	41.32	0.686
5	44.02	0.708
6	48.57	0.712
7	54.46	0.724
8	59.20	0.754
9	68.90	0.88
10	72.33	0.902
11	80.60	1.002
12	88.56	1.109

**Table 5.2 Values of Thermal conductivity at different temperatures for 2 % Al<sub>2</sub>O<sub>3</sub>**

S. No	Temperature, °C	Thermal conductivity (W/(m.K) 2 % Al <sub>2</sub> O <sub>3</sub> )
1	34.28	0.638
2	35.50	0.640
3	37.74	0.672
4	41.10	0.691
5	52.44	0.719
6	55.61	0.738
7	56.54	0.744
8	58.43	0.762
9	64.43	0.928
10	70.15	1.070
11	75.84	1.169
12	81.23	1.242
13	89.79	1.183

**Table 5.3 Values of Thermal conductivity at different temperatures for 4 % Al<sub>2</sub>O<sub>3</sub>**

S. No	Temperature, °C	Thermal conductivity (W/(m.K) 4 % Al <sub>2</sub> O <sub>3</sub> )
1	33.07	0.651
2	36.15	0.69
3	42.80	0.742
4	46.20	0.768
5	52.80	0.811
6	58.86	0.85
7	60.89	1.055
8	69.30	1.112
9	77.30	1.189
10	89.20	1.198

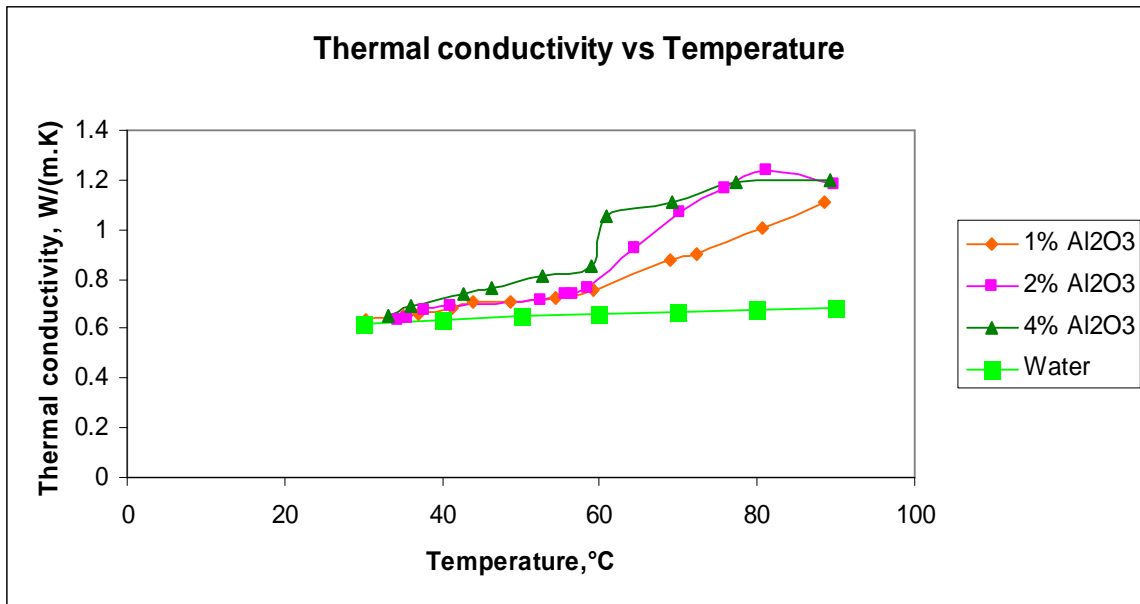


Figure 5.1: Thermal conductivity as a function of temperature for water-Al<sub>2</sub>O<sub>3</sub> nanofluids

The generalized polynomial equation of volumetric specific heat with temperature is as follows:

$$k = -1.241 + 0.1365T - 0.003108T^2 + 0.1311 * 10^{-4} T^3 + 0.3303 * 10^{-6} T^4 - 0.2542 * 10^{-8} T^5 - 0.1723 * 10^{-11} T^6 \quad (1)$$

$$k = 1.887 - 0.1215T + 0.4774 * 10^{-2} T^2 - 0.9952 * 10^{-4} T^3 + 0.1213 * 10^{-5} T^4 - 0.7490 * 10^{-8} T^5 + 0.1649 * 10^{-10} T^6 \quad (2)$$

$$k = -0.1353 + 0.04605 T - 0.6056 * 10^{-3} T^2 - 0.1139 * 10^{-4} T^3 + 0.3755 * 10^{-6} T^4 - 0.3178 * 10^{-8} T^5 + 0.8367 * 10^{-11} T^6 \quad (3)$$

Where k= Thermal conductivity, W/(m.K) and T= Temperature, °C.

Equations 1, 2, and 3 are the generalized polynomial equation for 1 %, 2 % and 4 % Al<sub>2</sub>O<sub>3</sub> derived from the data that was calculated by the experiment. From figure 5.1, it can be conclude that thermal conductivity of nanofluids shows great enhancement with increase in temperature. As well as with increase in concentrations of nanoparticles, thermal conductivity of nanofluids gets increased. It has been observed that 2 % to 5 % increase in thermal conductivity at 30 °C for the 1 %, 2 %, and 4 % water-Al<sub>2</sub>O<sub>3</sub> nanofluids. When the temperature is increased approximately up to 90°C, thermal conductivity get enhanced by 44 % to 48 % for the 1 %, 2 %, and 4 % water-Al<sub>2</sub>O<sub>3</sub> nanofluids. In nanofluid main mechanism of thermal conductivity enhancement can be thought as the stochastic motion of the nanoparticles. Presumable this Brownian like motion will be dependent on fluid temperature and so this amount of enhancement with temperatures is quite explicable for Al<sub>2</sub>O<sub>3</sub>. According to the graph, effect of temperature on thermal conductivity enhancement is even more than water and it rises from 2 % to 44 % (for 1 % particle concentration), 3 % to 47 % (for 2 % particle concentration) and 5 % to 48 % (for 4 % particle concentration). The measurement indicates that particle concentrations and temperature is an important parameter for thermal conductivity.

## 5.2 Volumetric specific heat measurement

Volumetric specific heat can be measured by thermal property analyzer i.e. KD2 Pro by using SH-1 sensor needle as this needle is preferred for this property measurement. It is measured at different ranges of temperature such as from 30 °C to 90 °C for the different concentration of nanoparticles such as 1 %, 2 % and 4 % and data calculated by experiment is mentioned in table 5.4, 5.5 and 5.6.

**Table 5.4 Values of Volumetric Specific heat at different temperatures for 1 % Al<sub>2</sub>O<sub>3</sub>**

S. No	Temperature, °C	Volumetric Specific heat (MJ/(m <sup>3</sup> .K) 1 % Al <sub>2</sub> O <sub>3</sub>
1	29.32	2.432
2	38.47	2.224
3	46.94	2.123
4	49.76	1.814
5	51.85	1.728
6	55.45	1.702
7	61.63	1.692
8	68.92	1.680
9	72.64	1.674
10	79.69	1.652
11	88.63	1.550

**Table 5.5 Values of Volumetric Specific heat at different temperatures for 2 % Al<sub>2</sub>O<sub>3</sub>**

S. No	Temperature, °C	Volumetric Specific heat (MJ/(m <sup>3</sup> .K) 2 % Al <sub>2</sub> O <sub>3</sub>
1	32.85	2.342
2	39.42	2.220
3	48.96	2.024
4	56.23	1.699
5	59.14	1.680
6	67.42	1.672
7	72.31	1.666
8	78.41	1.641
9	87.42	1.609

**Table 5.6 Values of Volumetric Specific heat at different temperatures for 4 % Al<sub>2</sub>O<sub>3</sub>**

S. No	Temperature, °C	Volumetric Specific heat (MJ/(m <sup>3</sup> .K) 4 % Al <sub>2</sub> O <sub>3</sub>
1	33.53	2.234
2	39.35	2.120
3	45.42	2.020
4	52.09	1.712
5	61.12	1.678
6	68.55	1.612
7	74.54	1.601
8	80.19	1.596
9	88.56	1.582

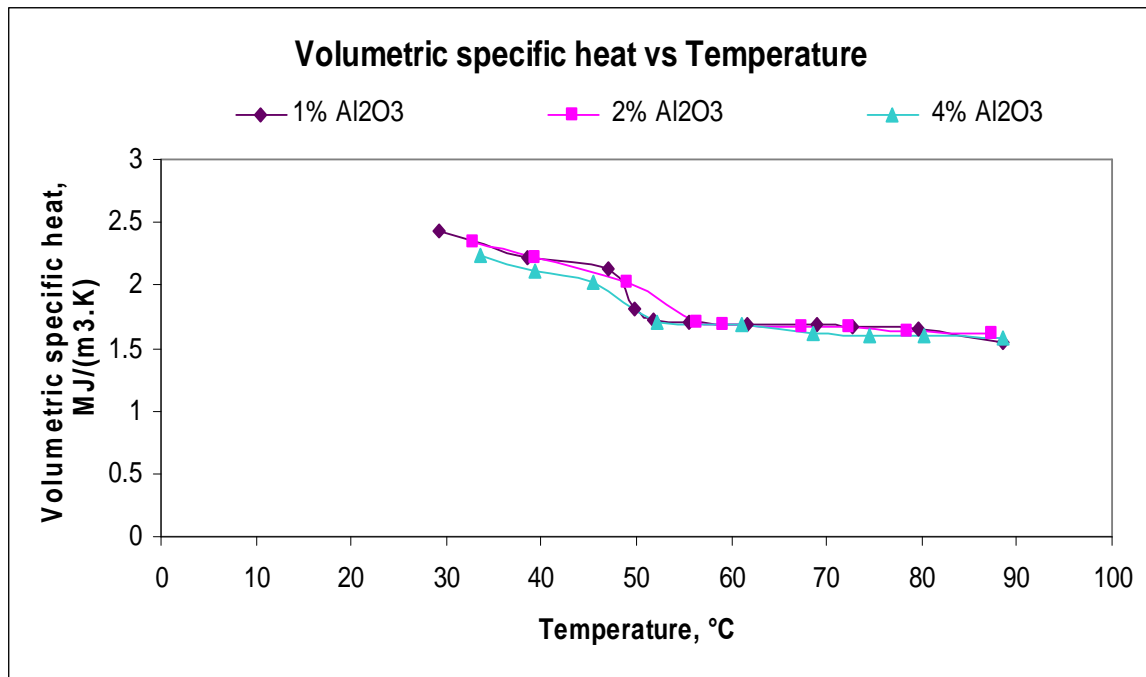


Figure 5.2: Volumetric specific heat as a function of temperature for water Al<sub>2</sub>O<sub>3</sub> nanofluids

The generalized polynomial equation of volumetric specific heat with temperature is as follows:

$$C_p = -1.092 + 0.3467 T - 0.01194 T^2 + 0.1831 * 10^{-3} T^3 - 0.1595 * 10^{-5} T^4 + 0.1012 * 10^{-7} T^5 - 0.3858 * 10^{-10} T^6 \quad (4)$$

$$C_p = -0.2093 * 10^{-3} + 0.2026 T - 0.6079 * 10^{-2} T^2 + 0.934 * 10^{-4} T^3 - 0.1333 * 10^{-5} T^4 + 0.1533 * 10^{-7} T^5 - 0.7271 * 10^{-10} T^6 \quad (5)$$

$$C_p = -0.7992 + 0.2946 T - 0.9854 * 10^{-2} T^2 + 0.1437 * 10^{-3} T^3 - 0.1084 * 10^{-5} T^4 + 0.5129 * 10^{-8} T^5 - 0.1561 * 10^{-3} \quad (6)$$

Where  $C_p$  = Volumetric specific heat, MJ/ (m<sup>3</sup>.K) and T= Temperature, °C.

Equations 1, 2, and 3 are the generalized polynomial equation for 1 %, 2 % and 4 % Al<sub>2</sub>O<sub>3</sub> derived from the data that was calculated by the experiment. The above figure 5.2 shows that effective heat flow curve of nanofluid is moved down as the temperature increases as well as nanoparticle volume fraction increases. The variation of volumetric specific heat for the different concentrations of nanofluids is very small. But there is great variation of volumetric specific heat with change in temperature. There is gradually decrease in volumetric specific heat as with increase in the temperature but above curve is slightly decreased when nanoparticle volume fraction increases from 1% to 4%. For a nanofluid of large nanoparticle volume fraction, this result implies that low heat energy is needed to obtain the same temperature increment.

### 5.3 Viscosity measurement

Viscosity can be measured by Ostwald viscometer at various ranges of temperatures such as 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, and 90 °C. It can be measured for distilled water, 1 %, 2 %, 4 % volume concentrations of Al<sub>2</sub>O<sub>3</sub>. The experimental values which are drawn by the experiment are mentioned in table 5.7, 5.8, 5.9, 5.10 and 5.11.

**Table 5.7: Time taken for Distilled water to cover the distance between two points marked on Ostwald viscometer.**

For Distilled water							
Time							
S. No	30 °C	40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
1	1min 55sec	1min 52sec	1min 48sec	1min 45sec	1min 42sec	1min 40sec	1min 38sec
2	1min 55sec	1min 51sec	1min 48sec	1min 45sec	1min 42sec	1min 40sec	1min 38sec
3	1min 56sec	1min 52sec	1min 48sec	1min 45sec	1min 41sec	1min 40sec	1min 38sec

**Table 5.8: Time taken for 1 % Al<sub>2</sub>O<sub>3</sub> to cover the distance between two points marked on Ostwald viscometer.**

1 % Al <sub>2</sub> O <sub>3</sub>							
Time							
S. No	30 °C	40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
1	1min 35sec	1min 27sec	1min 20sec	1min 14sec	1min 7sec	1min 2sec	59sec
2	1min 36sec	1min 27sec	1min 19sec	1min 14sec	1min 7sec	1min 2sec	59sec
3	1min 37sec	1min 26sec	1min 19sec	1min 15sec	1min 6sec	1min 1sec	59sec

**Table 5.9: Time taken for 2 % Al<sub>2</sub>O<sub>3</sub> to cover the distance between two points marked on Ostwald viscometer.**

2 % Al <sub>2</sub> O <sub>3</sub>							
Time							
S. No	30 °C	40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
1	1min 30sec	1min 16sec	1min 9sec	1min 2sec	57 sec	53 sec	50sec
2	1min 29sec	1min 15sec	1min 7sec	1min 1sec	56 sec	53 sec	50sec
3	1min 28sec	1min 15sec	1min 8sec	1min 2sec	57 sec	53 sec	49sec

**Table 5.10: Time taken for 4 % Al<sub>2</sub>O<sub>3</sub> to cover the distance between two points marked on Ostwald viscometer**

4 % Al <sub>2</sub> O <sub>3</sub>							
Time							
S. No	30 °C	40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
1	1min 23sec	1min 18sec	1min 13sec	1min 8sec	56 sec	52 sec	49 sec
2	1min 24sec	1min 17sec	1min 12sec	1min 8sec	55 sec	51 sec	50 sec
3	1min 25sec	1min 17sec	1min 12sec	1min 9sec	56 sec	53 sec	49 sec

**Table 5.11: Viscosity at different temperatures for 1 %, 2 %, and 4 % Al<sub>2</sub>O<sub>3</sub>**

S. No.	Temperature, °C	Viscosity,(cP) 1% Al <sub>2</sub> O <sub>3</sub>	Viscosity,(cP) 2 % Al <sub>2</sub> O <sub>3</sub>	Viscosity,(cP) 4% Al <sub>2</sub> O <sub>3</sub>
1	30	0.671	0.6293	0.5985
2	40	0.51024	0.4496	0.4644
3	50	0.404	0.351	0.3758
4	60	0.3318	0.278	0.308
5	70	0.2643	0.2259	0.222
6	80	0.2197	0.1892	0.186
7	90	0.18974	0.1595	0.159

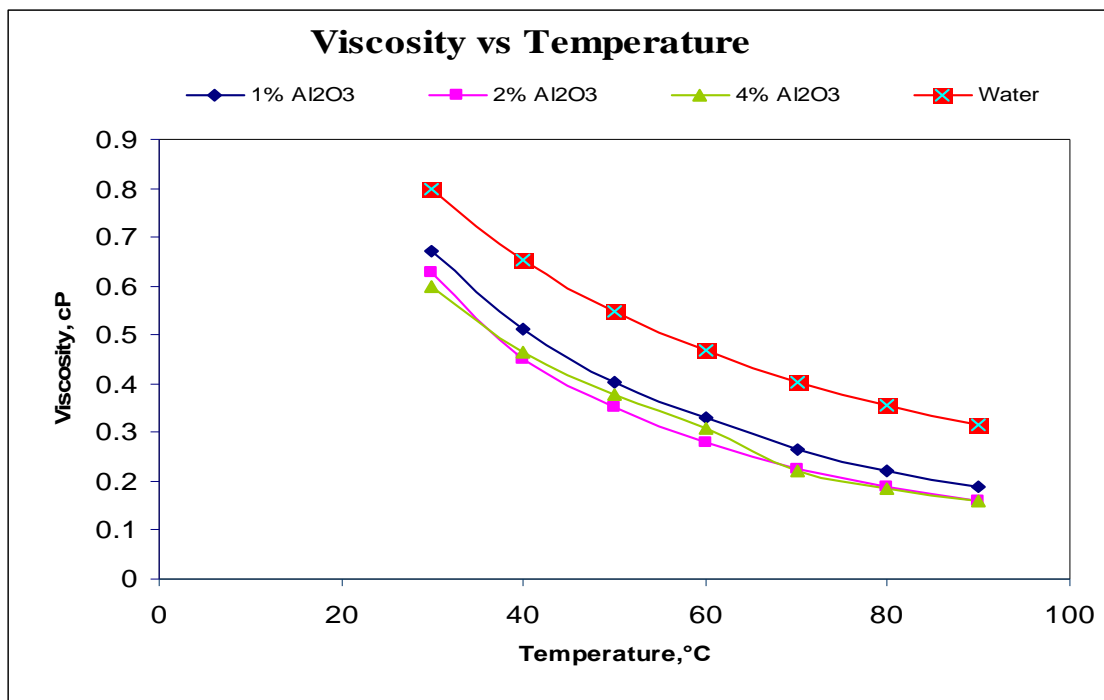


Figure 5.3: Variation of viscosity with increase in temperature for water- Al<sub>2</sub>O<sub>3</sub> nanofluids.

The generalized polynomial equation of viscosity with temperature is as follows:

$$\mu = 1.67 - 0.04373 T + 0.1835 * 10^{-4} T^2 + 0.2076 * 10^{-4} T^3 - 0.420 * 10^{-6} T^4 + 0.34 * 10^{-8} T^5 - 1.007 * 10^{-10} T^6 \quad (7)$$

$$\mu = 1.884 - 0.057 T + 0.2119 * 10^{-4} T^2 + 0.3167 * 10^{-4} T^3 - 0.07773 * 10^{-6} T^4 + 0.5849 * 10^{-8} T^5 - 0.1874 * 10^{-10} T^6 \quad (8)$$

$$\mu = 1.645 - 0.053 T + 0.2821 * 10^{-3} T^2 + 0.2558 * 10^{-4} T^3 - 0.6686 * 10^{-6} T^4 + 0.6396 * 10^{-8} T^5 - 0.2173 * 10^{-10} T^6 \quad (9)$$

Where  $\mu$  = Viscosity, cP and T= Temperature, °C

Equations 1, 2, and 3 are the generalized polynomial equation for 1%, 2% and 4% Al<sub>2</sub>O<sub>3</sub> derived from the data that was calculated by the experiment. From the above data and figure 5.3, it seems that viscosity is gradually decreased with increase in temperature as well as with increase in nanoparticle volume fraction. As compared with the viscosity of nanofluids with water, it shows that nanofluids become less viscous than water. And as further nanoparticle concentration increases, viscosity is decreased. From figure 5.3, behaviour of 2% and 4% nanofluids are seems to be same and but 1% water- Al<sub>2</sub>O<sub>3</sub> shows high viscous than other. Behaviour of viscosity of nanofluids with temperature is same as for water but this is quite uncertain that as the nanoparticles are added in water and further increases the concentrations, viscosity get decreased. In nanofluids, nanoparticles are not mixed with water while it present in suspended form. When nanofluids filled in Ostwald viscometer then due to the weight of nanoparticles which acts in downward direction may alter the falling speed of nanofluids. As the nanoparticle concentrations increases, more weight is acting in downward direction so due to this time taken to covered the distance get reduced. And due to this viscosity of nanofluids is less as compared with water and further it getting reduced by increasing the concentrations of nanoparticles.

#### 5.4 Density measurement

Density can be measured by specific gravity bottle at different ranges of temperature such as 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, and 90 °C for distilled water and different concentrations of nanofluids i.e. 1%, 2%, and 4%. Since water has a density of 1 g/cm<sup>3</sup>, the specific gravity is the same as the density of the material measured in g/cm<sup>3</sup>. All the experimental data are mentioned in table 5.12, 5.13 and 5.14.

Weight of specific gravity bottle= 8.973gm

**Table 5.12: Net weight of Distilled water and 1% Al<sub>2</sub>O<sub>3</sub> at various temperatures**

S. No	Temperatures, °C	Distilled water	1% Al <sub>2</sub> O <sub>3</sub>
1	30	14.833-8.973=5.86gm	14.893-8.973=5.920gm
2	40	14.829-8.973=5.856gm	14.869-8.973=5.869gm
3	50	14.821-8.973=5.848gm	14.854-8.973=5.881gm
4	60	14.811-8.973=5.838gm	14.834-8.973=5.861gm
5	70	14.794-8.973=5.821gm	14.819-8.973=5.846gm
6	80	14.788-8.973=5.815gm	14.813-8.973=5.838gm
7	90	14.780-8.973=5.807gm	14.804-8.973=5.829gm

**Table 5.13: Net weight of 2% Al<sub>2</sub>O<sub>3</sub> and 4% Al<sub>2</sub>O<sub>3</sub> at various temperatures**

S. No	Temperatures, °C	2% Al <sub>2</sub> O <sub>3</sub>	4% Al <sub>2</sub> O <sub>3</sub>
1	30	14.962-8.973=5.989gm	15.013-8.973=6.040gm
2	40	14.950-8.973=5.977gm	14.989-8.973=6.016gm
3	50	14.933-8.973=5.960gm	14.976-8.973=6.003gm
4	60	14.902-8.973=5.929gm	14.900-8.973=5.927gm
5	70	14.853-8.973=5.880gm	14.869-8.973=5.896gm
6	80	14.824-8.973=5.851gm	14.834-8.973=5.861gm
7	90	14.796-8.973=5.823gm	14.819-8.973=5.846gm

**Table 5.14 Value of density at different temperature for 1%, 2%, and 4% Al<sub>2</sub>O<sub>3</sub>**

S. No	Temperature, °C	Density,(gm/cm <sup>3</sup> ) 1% Al <sub>2</sub> O <sub>3</sub> (ρ/ρ <sub>w</sub> )	Density,(gm/cm <sup>3</sup> ) 2% Al <sub>2</sub> O <sub>3</sub> (ρ/ρ <sub>w</sub> )	Density,(gm/cm <sup>3</sup> ) 4% Al <sub>2</sub> O <sub>3</sub> (ρ/ρ <sub>w</sub> )
1	30	1010.2	1022.0	1030.0
2	40	1006.8	1020.66	1027.0
3	50	1005.6	1019.15	1026.0
4	60	1003.9	1015.0	1015.0
5	70	1004.29	1010.0	1012.0
6	80	1003.9	1006.0	1007.9
7	90	1003.7	1002.7	1006.0

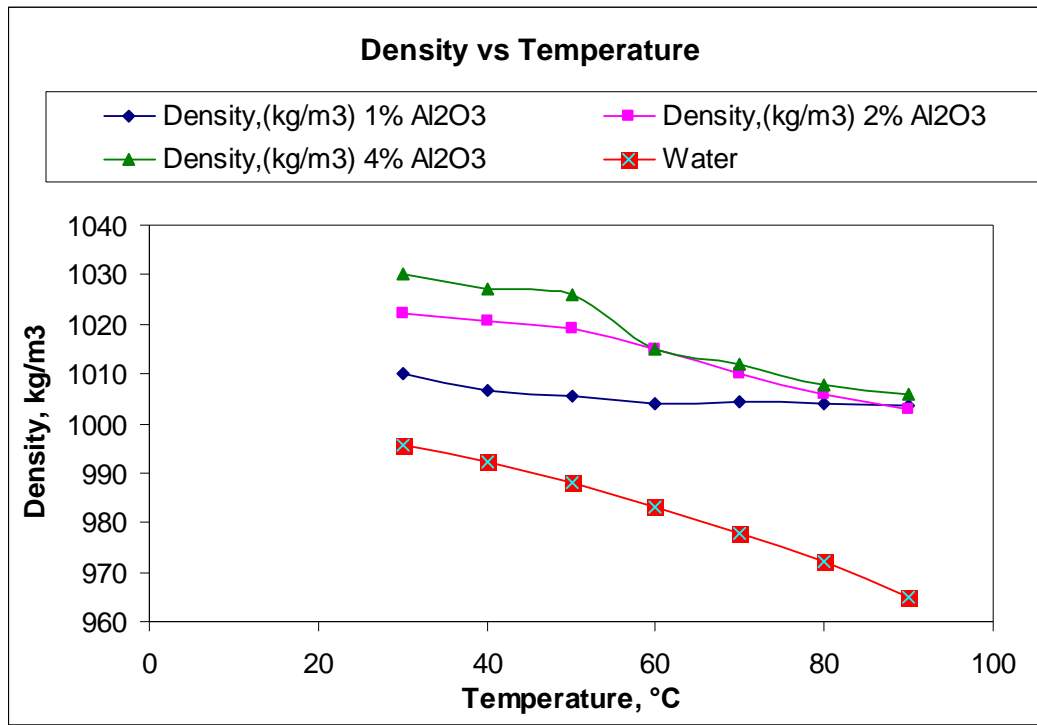


Figure 5.4: Variation of density with increase in temperature for water- Al<sub>2</sub>O<sub>3</sub> nanofluids

The generalized polynomial equation of density with temperature is as follows:

$$\rho = 1.051 - 0.004699 T + 0.2573 * 10^{-3} T^2 - 0.7934 * 10^{-5} T^3 + 0.1322 * 10^{-6} T^4 - 0.1106 * 10^{-8} T^5 + 0.3638 * 10^{-11} T^6 \quad (10)$$

$$\rho = 1.002 + 0.005821 T - 0.4628 * 10^{-3} T^2 + 0.1642 * 10^{-4} T^3 - 0.2922 * 10^{-6} T^4 + 0.2526 * 10^{-8} T^5 - 0.8445 * 10^{-11} T^6 \quad (11)$$

$$\rho = 1.067 - 0.004830 T + 0.232 * 10^{-3} T^2 - 0.5087 * 10^{-5} T^3 + 0.4929 * 10^{-7} T^4 - 0.1746 * 10^{-9} T^5 \quad (12)$$

Where  $\rho$  = Density,  $\text{kg/m}^3$ , and  $T$  = Temperature,  $^{\circ}\text{C}$ .

Equations 1, 2, and 3 are the generalized polynomial equation for 1%, 2% and 4%  $\text{Al}_2\text{O}_3$  derived from the data that was calculated by the experiment. According to figure 5.4, temperature has also impact on the density of nanofluids. Density of nanofluids is higher than the base fluids i.e. distilled water and with increase in temperature, density of nanofluids goes on decreasing. Further, if nanoparticle volume fraction increases from 1% to 4%, density is also increased. But at high range of temperature i.e. above  $80^{\circ}\text{C}$ , density of 1% to 4% nanofluids become almost same but still more than water as shown in figure 5.4.

## 5.5 Effect of time cycle on PCR

M 1 2 3 4

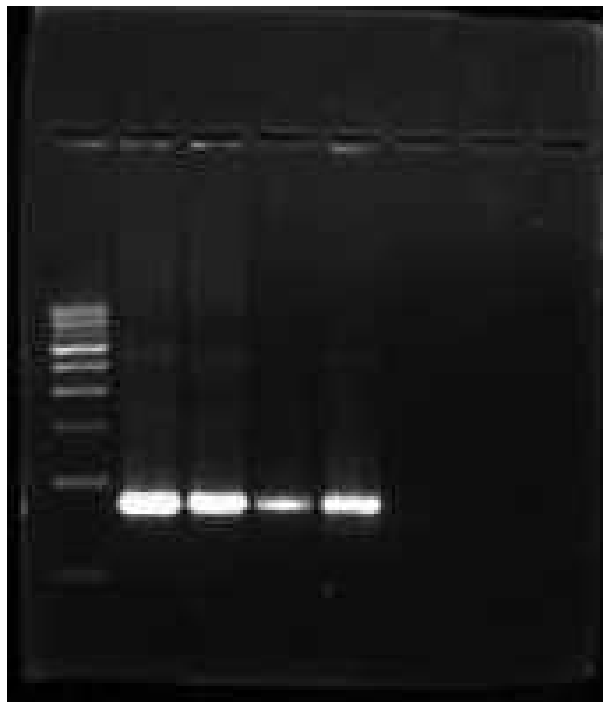


Figure 5.5: The effect of time cycle on PCR. PCR was performed by employing 0.8kb base pairs sequence, and PCR products were analyzed by agarose gel electrophoresis. Lane M is for markers; Lane1 is for control in which time cycle is 1min, 1min, 2min, 1min; Lane2 shows the result of PCR performed with gold particles (1%) with time cycle 1min, 1min, 2min, 1min; Lane 3 is for control with time cycle 30sec, 30sec, 45sec, 30sec. Lane4 shows the result of PCR performed with gold particles (1%) with time cycle 30sec, 30sec, 45sec, 30sec.

In the above gel picture, PCR was performed for 2 different time cycles. Firstly, PCR experiment is run on their normal cycle i.e. 1minute for initial denaturation, 1min for denaturation, 2min for annealing and 1min for elongation. This is a normal time cycle for the small size of DNA such as less than 1kb. Two samples were prepared for this experiment; one is control without gold nanoparticles and other is with 0.1volume percent gold nanoparticles. In the above picture, intensities of lane 1 and lane 2 are same because this time is enough time for the amplification of DNA. That's why; gold particles don't show their effect on PCR. In next experiment, time cycle was reduced and it was 30sec for initial denaturation, 30sec for denaturation, 45sec for annealing, and 30sec for elongation as this is a minimum time for amplification of DNA. Lane 3 is without gold nanoparticles and lane 4 is with 0.1volume percent gold nanoparticles and the effect of nanoparticles is clearly visible. Intensity of lane 4 band is quite high than lane 3 which shows that gold particles enhance the efficiency of PCR by increased the amplification. This is due to the unique property of gold nanoparticles i.e. thermal conductivity which increases the heat transfer at various temperatures. So, from this result it can be conclude that for small size of DNA i.e. less

than 1kb; time cycle is 30sec for initial denaturation, 30sec for denaturation, 45sec for annealing and 30sec for elongation and further all experiments are carried out on same time cycle.

### 5.6 Effect of gold nanoparticles concentration on PCR

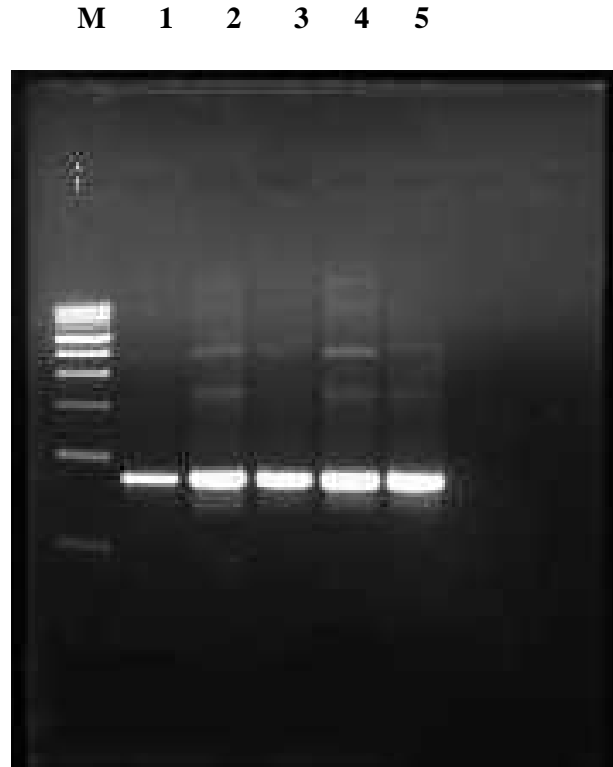


Figure 5.6: Effect of gold nanoparticles concentration on PCR. Lane M is for markers; Lane 1 shows the result of PCR without gold nanoparticles; Lane 2 shows the result of PCR with 0.5% gold nanoparticles (30nm). Lane 3 shows the result of PCR with 1% gold nanoparticles (30nm). Lane 4 shows the result of PCR with 0.5% gold nanoparticles (60nm). Lane 5 shows the result of PCR with 1% gold nanoparticles (60nm).

This experiment is carried out to see the effect of concentration of gold nanoparticles as in previous experiment 1% gold nanoparticles are used but in this experiment 0.5% gold nanoparticles are used and compare with the 1% gold nanoparticles. In this experiment, different sizes of gold nanoparticles are also used. This picture shows that effect of 1% and 0.5% is same both for 30nm and 60nm sized nanoparticles. So, from this it can be conclude that 0.5 volume percent gold nanoparticles can be used instead of 1% for any size of gold nanoparticles.

## 5.7 Effect of size of nanoparticles on PCR

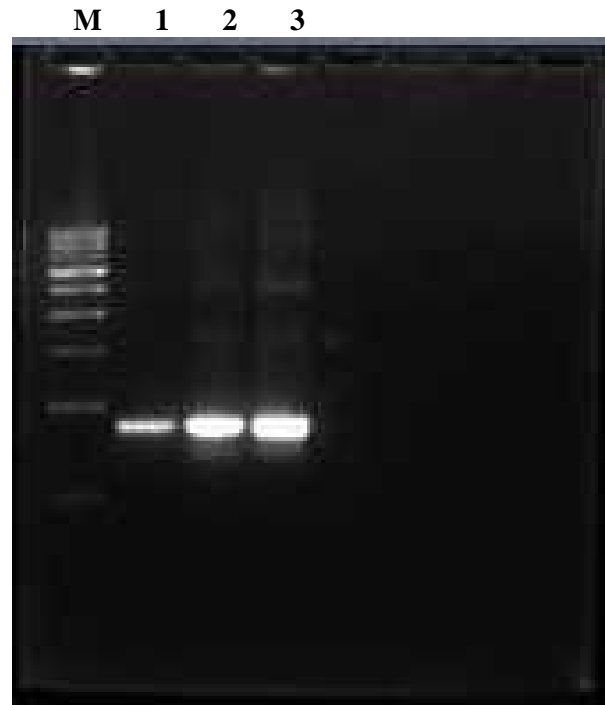


Figure 5.7: Effect of the size of gold nanoparticles on PCR. Lane M is for marker; Lane 1 shows the result of PCR without gold nanoparticles; Lane 2 having 0.5% of 30nm gold nanoparticles; Lane 3 having 0.5% of 60nm gold nanoparticles

According to the previous experiments, it was concluded that 0.5% gold nanoparticles can be used and time cycle is 30sec, 30sec, 45sec, 30sec and in this experiment effect of size of nanoparticles can be observed. Lane 1 is for control without gold nanoparticles, lane 2 is for 0.5% gold nanoparticles having sized 30nm, and lane 3 is for 0.5 % gold nanoparticles having sized 60nm. It is clearly visible in above gel picture that by increasing the size of gold nanoparticles, efficiency of PCR also increased. The band for 30nm sized particle is comparatively small than 60nm sized particle. So, it can be concluded that as we increasing the size of nanoparticles, efficiency of PCR also increased.

## 5.8 Comparison the effect of gold and Al<sub>2</sub>O<sub>3</sub> nanoparticles on PCR



Figure 5.8: Comparison the effect of gold and Al<sub>2</sub>O<sub>3</sub> nanoparticles on PCR. Lane M is for marker; Lane 1 is for control without gold nanoparticles; Lane 2 having 0.5% of 30nm gold nanoparticles; Lane 3 having 0.5% of 40nm Al<sub>2</sub>O<sub>3</sub>; Lane 4 having 0.5% of gold and Al<sub>2</sub>O<sub>3</sub> in combination.

In this experiment, different nanoparticles are used i.e. Gold, Al<sub>2</sub>O<sub>3</sub> and Gold + Al<sub>2</sub>O<sub>3</sub> nanoparticles. Lane 1 is for control without gold nanoparticles; Lane 2 having 0.5% of 30nm gold nanoparticles; Lane 3 having 0.5% of 40nm Al<sub>2</sub>O<sub>3</sub>; Lane 4 having 0.5% of gold and Al<sub>2</sub>O<sub>3</sub> in combination. From above picture it can conclude that gold particles are best for biological purposes instead of using Al<sub>2</sub>O<sub>3</sub> nanoparticles. Gold particles show their activity in PCR while Al<sub>2</sub>O<sub>3</sub> nanoparticles inhibit the activity of PCR. It is due to the reaction between ions of buffer solutions and Al<sub>2</sub>O<sub>3</sub> nanoparticles. When Al<sub>2</sub>O<sub>3</sub> nanoparticles added then instantly it forms precipitate due to which PCR reaction is not carried out and this can easily be seen in the above picture as lane 3 and lane 4 shows no band in gel. So, it is recommended that in future Al<sub>2</sub>O<sub>3</sub> nanoparticles cannot used in PCR applications.

## 5.9 Comparison the effect of size of DNA with gold nanoparticles on PCR

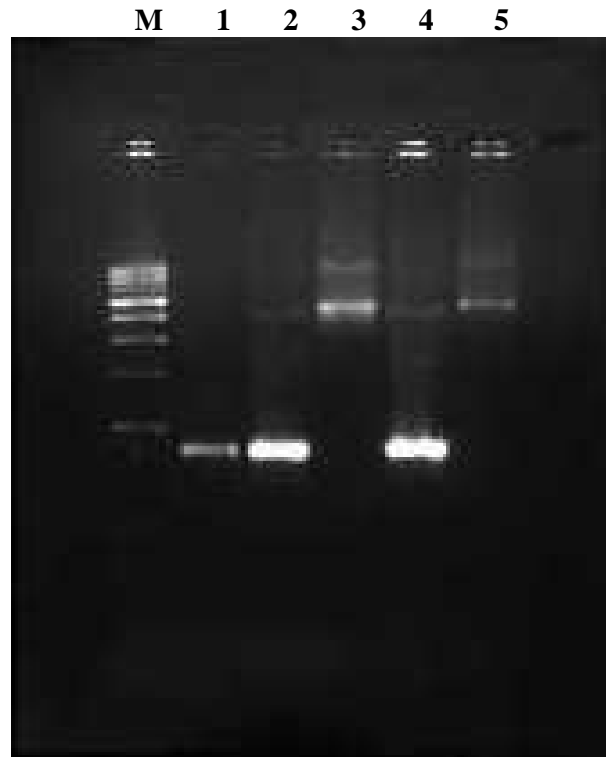


Figure 5.9: Comparison the effect of size of DNA. Lane M is for marker; Lane 1 is for control without gold nanoparticles; Lane 2 shows the result 0.5% of 30nm gold nanoparticles having 0.8kb base pair DNA; Lane 3 shows the result 0.5% of 30nm gold nanoparticles having 2.6kb base pair DNA; Lane 4 shows the result 0.5% of 60nm gold nanoparticles having 0.8kb base pair DNA; Lane 5 shows the result 0.5% of 60nm gold nanoparticles having 2.6kb base pair DNA.

The above picture shows the effect of size of DNA while adding gold nanoparticles. In this experiment 0.8kb and 2.6kb size of DNA is used. Lane 1 is for control without gold nanoparticles; lane 2 is for 0.8kb template DNA having 30nm gold nanoparticles; Lane 3 is for 2.6kb template DNA having 30nm gold nanoparticles; Lane 4 is for 0.8kb template DNA having 60nm gold nanoparticles; Lane 5 is for 2.6kb template DNA having 60nm gold nanoparticles. The above picture clears that gold particles show visible effect on 0.8kb size of DNA but don't have considerable effect on 2.6kb size of DNA.

## 5.10 Effect of enzyme dilution on PCR

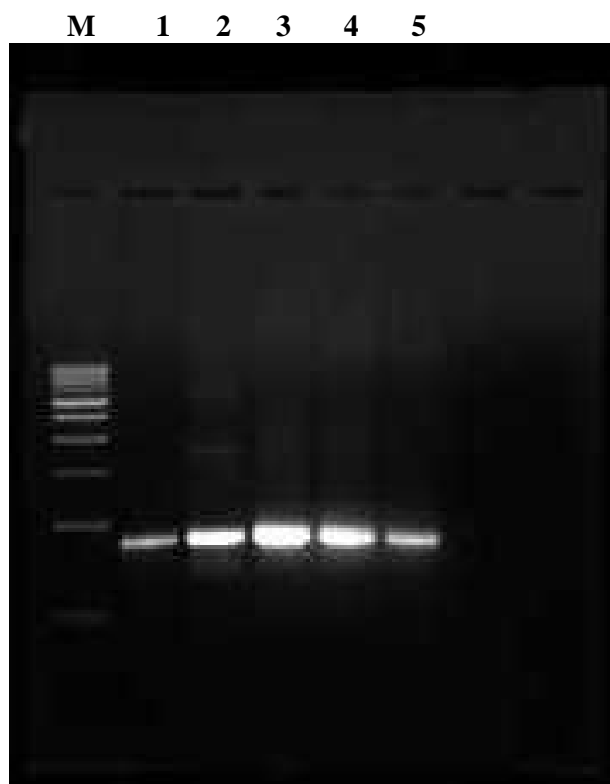


Figure 5.10: Effect of enzyme dilution on PCR. Lane M is for marker; Lane 1 is for control without gold nanoparticles; lane 2 is for 0.5% gold nanoparticles (30nm) with 3units/ $\mu$ L enzyme concentration; lane3 is for 0.5% gold nanoparticles (30nm) with 1.5units/ $\mu$ L enzyme concentration; lane 4 is for 0.5% gold nanoparticles (60nm) with 1.5units/ $\mu$ L enzyme concentration; lane 5 is for 0.5% gold nanoparticles (60nm + 30 nm) with 1.5units/ $\mu$ L enzyme concentration.

The above picture shows the effect of enzyme dilution on PCR. In lane 2, 3 unit/  $\mu$ L enzyme was used with gold nanoparticles and in lane 3, 1.5 units/  $\mu$ L enzyme was used and the effect is clear visible that PCR having more efficiency when enzyme get more diluted i.e. 1.5 units/  $\mu$ L. In lane 4, 60nm sized particle and lane 5, (60nm+30nm) both nanoparticles was used with 1.5 unit/  $\mu$ L enzyme concentration and the efficiency is not good. It is less than 30nm having 1.5unit/  $\mu$ L enzyme concentration. So, it can be concluded that by decreasing the concentration of enzyme, gold particles become more effective in PCR if their size is small but if size of gold particles increased or if we used in the combination by mixing two different size of gold nanoparticles, then their efficiency get reduced as compared with small size of gold particles. So, it's better to used low concentration enzyme for small size of nanoparticles but for larger size of nanoparticles, prescribed concentration can be used.

## CHAPTER-6

### CONCLUSION

All results are concluded on the basis of the experiment performed for measuring the thermophysical properties like thermal conductivity, volumetric specific heat, viscosity and density besides analysis the efficiency of PCR. This chapter summarizes the conclusion drawn in this report.

1. Nanofluids are dilute colloidal suspensions with nano-sized particles. It can be prepared by dispersing the nanoparticles in base fluid and then put the samples in ultra sonicator to make the nanoparticles more suspended in the fluid and it also increases the stability of nanofluid.
2. The effective thermal conductivity and volumetric specific heat of nanofluids can be measured by thermal property analyzer such as KD2 Pro at different ranges of temperature. The experimental results show that a dramatic increase in the enhancement of thermal conductivity of  $\text{Al}_2\text{O}_3$  – water mixture takes place with increase in temperature. Effect of temperature on thermal conductivity enhancement is even more than water and it rises from 2 % to 44 % (for 1 % particle concentration), 3 % to 47 % (for 2 % particle concentration) and 5 % to 48 % (for 4 % particle concentration). The results indicate that particle volume fraction is also an important parameter for nanofluids. With increase in particle volume concentration, thermal conductivity of nanofluids also increased.
3. It has also been found that volumetric specific heat of nanofluid decrease with increasing nano particle volume fraction and temperature. But the effect of nanoparticle volume fraction is very small as compared with temperature on volumetric specific heat.
4. Viscosity and density of nanofluids can be measured by Ostwald viscometer and specific gravity bottle respectively. From the experimental results, it is observed that nanofluids have lower viscosity and higher thermal conductivity. Viscosity and density both are decreases with increases in temperature. These properties are also depending upon the nanoparticle volume concentration present in the base fluid. Viscosity decreases and density increases by increasing the particle volume concentration of nanofluids.
5. Due to its unique thermophysical properties such as higher thermal conductivity and lower viscosity, nanofluids can implement in various heat transfer and biological applications. In this thesis, nanofluids are implemented in biological applications. PCR is an apparatus in which DNA amplification can be done at various sets of temperature ranges from 50 °C to 95 °C. As

nanofluids having high thermal conductivity means high heat transfer so nanofluids are added with PCR sample to check the effect of nanofluids on PCR.

6. It is demonstrated that effect in the presence of gold nanoparticles, PCR amplification can be optimized with respect to both yields and specificity. While adding the appropriate concentrations of gold nanoparticles it reduces the time cycle of PCR and it increases the efficiency too by increasing the amplification of DNA. Size of nanofluids has also shown significant effect on PCR. As size of nanoparticles increased, PCR efficiency also gets increased.
7. Size of template DNA also shows significant effect on PCR. If the size of DNA is small i.e. less than 1 kb then nanoparticles show their activity in PCR but if the size of DNA is greater than 2 kb then nanoparticles do not show their activity in PCR. From the results it can also be conclude that by reducing the enzyme dilution, it increases the efficiency of PCR if nanoparticle size is small i.e. less than 30 nm but if a size is near 60 nm than it shows inverse effect on PCR and efficiency get reduced.
8. From the results it can also be conclude that gold nanoparticles are best for biological applications instead of using  $\text{Al}_2\text{O}_3$  nanoparticles.  $\text{Al}_2\text{O}_3$  nanoparticles forms immediately precipitate when it was added in the PCR mixture.

## SCOPE FOR FUTURE STUDIES

During the present thesis work, it is thought that the following areas may be considered for further investigations:

1. Few properties like Thermal conductivity, volumetric specific heat, viscosity and density are measured at different ranges of temperature. Many more investigation is required in this field. In future, properties like thermal resistivity, diffusivity can also be measured at different ranges of temperatures for different nanoparticles. More study is needed to determine the extent of such temperature-dependent behavior.
2. Thermophysical properties can be measured by changing the nanoparticles and its size or by changing the base fluid at different nanoparticle volume fraction.
3. The ongoing studies on the interaction between AuNPs with other components in PCR mixtures will further reveal the mechanisms of nanoparticle PCR. Further investigation into the effect of gold nanoparticles on these PCR reactions is required.
4. Nanofluids can be used in heat transfer applications to improve the heat exchange rate in heat exchangers.