Kaunas University of Technology
FACULTY OF MECHANICAL ENGINEERING AND DESIGN

Investigation of Young's Modulus and Hydrophobicity of PZT Composite Materials for Biosensor Applications

Master's Final Degree Project

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Mechanical Engineering (621H30001)

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Investigation of Young's Modulus and Hydrophobicity of PZT Composite Materials for Biosensor Applications

Declaration of Academic Integrity

I confirm that the final project of mine, Yatinkumar Rajeshbhai Patel, on the topic "Investigation of Young's Modulus and Hydrophobicity of PZT Composite Materials for Biosensor Application" is written completely by myself; all the provided data and research results are correct and have been obtained honestly. None of the parts of this thesis have been plagiarised from any printed, Internet-based or otherwise recorded sources. All direct and indirect quotations from external resources are indicated in the list of references. No monetary funds (unless required by law) have been paid to anyone for any contribution to this project.

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SUMMARY

The aim of the thesis is to investigate Young's modulus and hydrophobicity of the PZT composite material for biosensor application. Piezoelectric materials are popular because of their ability to convert the biological, chemical or mechanical response to the electrical signal by using piezoelectric or piezoresistive effect. In this thesis, three different types of piezoelectric composite materials with polymer composition (PVB, PS, and PMMA) are investigated and displaying its mechanical properties such as Young's modulus and Density. Also, simulation has been done using Comsol Multiphysics 5.0 software to verify the experimental results. Composite materials display the properties of the piezo effect that could be useful for the formation of microstructure on it. These microstructure can be used as innovative functional elements in a biomedical micro hydro-mechanical system such as microchannels. On the other hand, hydrophobicity plays an important role for biosensors which are working in the viscous (liquid) environment. Hydrophobicity investigation was done for PZT composite material (PVB, PS, and PMMA) with different liquids (Distilled water, Glycerine, Spirit, and Olive oil). For hydrophobicity identification of these PZT composite materials new experimental setup has been designed to measure contact angle, because Contact angle measurement is a convenient method for surface identification either it's hydrophobic or hydrophilic. Then by using image analysis software (ImageJ, DropSnake plugins) to analyze images to measure contact angle which is formed by liquid drops on PVB, PS, and PMMA composite surface. Moreover, the influence of different coating thickness of PZT composite material and different base materials on contact angle was investigated using same experiment platform.

Patel Yatinkumar Rajeshbhai, PZT kompozito, skirto biojutikliams, Jungo modulio ir hidrofobiškumo tyrimas. Magistro baigiamasis projektas / vadovas doc. dr. Giedrius Janušas; Kauno technologijos universitetas, Mechanikos inžinerijos ir dizaino fakultetas.
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SANTRAUKA

Darbo tikslas – nustatyti PZT kompozitinės medžiagos, naudojamos biojutikliuose, Jungo modulį ir hidrofobiškumą. Pjezoelektrinės medžiagos yra plačiai naudojamos įvairiuose biojutikliuose, nes gali konvertuoti cheminį ar mechaninį atsaką į elektros signalą, naudojant pjezoelektrinį arba pjezovaržinį efektą. Šiame darbe tiriami trys skirtingi pjezoelektriniai kompozitai, pagaminti maišant PZT daleles su polivinilbutiraliu, polistirenu ir polimetilmetakrilatu. Modeliavimas buvo atliktas naudojant "Comsol Multiphysics 5.0" programinę įrangą, siekiant patikrinti eksperimentinius rezultatus. Šios kompozitinės medžiagos gali būti naudojamos funkcinių elemetų formavimui, kurie gali būti naudojami kaip inovatyvūs funkciniai elementai biomedicininėse mikro hidro-mechaninėje sistemose, tokiose kaip mikrokanalia. Kitavertus hidrofobiškumas yra ypač svarbus biojutikliuose, kurie veikia klampioje (skystoje) aplinkoje. PZT kompozitinių medžiagų (PVB, PS ir PMMA) hidrofobiškumo tyrimas buvo atliktas su skirtingais skysčiais (distiliuotas vanduo, glicerinas, spiritas ir alyvuogių aliejus). Šių PZT kompozitinių medžiagų hidrofobiškumui nustatyti buvo sukurta nauja eksperimentinė sistema, skirta drėkinimo kampo matavimui, nes tai yra populiariausias paviršiaus savybių tyrimo metodas. Vaizdų apdorojimui ir analizei pasirinkta programinė įranga ImageJ. Šios įrangos papildinys DropSnake leidžia įvertinti kampą tarp skysčio ir PZT kompozito. Taip pat, naudojant tą pačią eksperimentinę įrangą, įvertinta PZT kompozitinės medžiagos dangos storio ir bandinių pagrindo įtaka drėkinimo kampui.

KAUNAS UNIVERSITY OF TECHNOLOGY

FACULTY OF MECHANICAL ENGINEERING AND DESIGN

Study programme MECHANICAL ENGINERING 621H30001

TASK ASSIGNMENT FOR FINAL DEGREE PROJECT OF MASTER STUDIES

Given to the student: Yatinkumar Rajeshbhai Patel

1. Title of the Project:

Investigation of Young's modulus and hydrophobicity of PZT composite materials for biosensor applications.

PZT kompozito, skirto biojutikliams, Jungo modulio ir hidrofobiškumo tyrimas.

Approved by the Dean's Order No. V25-11-5 of 2018 04 12

2. Aim and Tasks of the Project:

The aim:

The aim of the research is to develop and investigate the mechanical properties as Young's modulus and hydrophobicity of piezo composite materials for biosensor application.

Tasks:

To identify Young's modulus and density of the PZT composite materials.

To design experimental setup for contact angle measurement of PZT composite materials.

To investigate hydrophobicity of PZT composite materials.

3. Initial Data:

Review of previous research experiments for the different piezoelectric materials and PZT composite materials.

4. Main Requirements and Conditions:

Requirments: Prepare the specimens of PZT composite material; use Comsol Multiphysics for simulations.

5. Structure of the Text Part:

Introduction, Aim, Tasks, Litrature review, Experimental setup for Dynamic investigation of PZT composite material Properties and Design of experimental setup for contact angle measurements, Experimental results, Application of PZT compoiste material, Conclusion, List of references.

6. Structure of the Graphical Part:

Graphical parts include the specimen used for dynamic investigation and application of the proposed PZT composite material for biopartical transpotation.

7. Consultants of the Project:

—

Student: ....Yatinkumar Rajeshbhai Patel.. *2018 04 11*.

(Name, Surname, Signature, data)

Supervisor................................. Assoc. Prof. Dr. Giedrius Janušas... *2018 04 11*.

(Name, Surname, Signature, data)

Programme Director of the Study field.......Assoc. Prof. Kęstutis Pilkauskas....................... *2018 04 11*.

(Name, Surname, Signature, data)

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Introduction

Nowadays Biosensors are used and can be used in wide variety of applications. Some of that is presently used in the commercial market are blood glucose monitoring biosensor, pesticides in water contaminants biosensor, toxic level measurement, food industries, medication, laboratories, military, environmental industries and so on. Many materials were used in presently available biosensor device as an active sensing element like PDMS (Polydimethylsiloxane), PU, (Polyurethane), PZT (Lead zirconate titanate), SI (Silicon). But in the present world demand is increasing for micro and Nanotechnology in the field of biosensors, microchannels and drug delivery. Many types of research are going on to develop microdevices has an ability to measure very small scale component with the high level of accuracy. Piezoceramic and crystal materials are so popular to develop PZT biosensors but crystal and ceramics are tough in nature so it is not highly favorable for PZT cantilever and biosensor application. Also, PZT ceramics can easily degrade in the air and gaseous environment. Presently, PZT composite materials are popular to develop such a device because of the microstructure nature and flexible working range to provide real-time output and working in the viscous environment.

In this thesis report, three different types of new PZT composite materials were produced with PZT nanopowder and three different polymer binding polymers, polyvinyl butyral (PVB), polystyrene (PS) and polymethyl methacrylate (PMMA). In order to investigate Young's modulus and hydrophobicity of PZT composite material for biosensor application. Impulse test has been done to investigate Young’s modulus for PZT composite material. Also, simulation has been done using Comsol Multiphysics 5.0 software to obtain the Young's modulus for PZT composite material from measured frequency obtained from the experiment. Then compare the experimental results with theoretical results was done to validate the result.

In order to understand the hydrophobicity of the PZT composite material, new experimental setup was designed to measure the contact angle because contact angle is the easiest way to understand surface properties towards different liquids. Contact angle measurement has been done using four different liquids (Distilled water, Glycerin, Spirit, and Olive oil) on three surfaces of the PZT composite material PVB, PS, and PMMA. Images captured using the high-speed camera and analyzed using ImageJ (DropSnake plugins).

AIM

The aim of the research is to investigate the mechanical properties as Young's modulus and design new experimental setup to identify the hydrophobicity of piezo composite materials for biosensor application.

In order to achieve this aim, the following objectives were determined:

1. To identify Young's modulus and density of the PZT composite materials.

2. To design experimental setup for contact angle measurement of PZT composite materials.

3. To investigate hydrophobicity of PZT composite materials.

Nomenclature

PDMS – Polydimethylsiloxane

PVDF - Polyvinylidene fluoride

PU – Polyurethane

PZT – Lead zirconate titanate

PVB – Polyvinyl butyral

PS – Polystyrene

PMMA – Polymethyl methacrylate

PSA – Prostate-particular antigen

DNA – Deoxyribonucleic acid

Cd – Cadmium

U – Uranium

Zn – Zinc

Hg – Mercury

Ag – Silver

Cu – Copper

Pb – Lead

Ni – Nickel

Cr – Chromium

Fe – Iron

2A81G5 - Monoclonal antibody

LDH – Lactate dehydrogenase

PGA – Pyrrole polymetric material

Hg2+ - Inorganic mercury

Ag2+ - Ions

GLDH – Glutamate dehydrogenase

KCL - Potassium chloride

Mug/l – Microgram/liter

Mg/l – milligram / l

AchE – Acetylcholine esterase

RNA – Ribonucleic acid

OP – Organophosphorus pesticides

Cd2+ - Cadmium cation

Zn2+ - Zinc cation

Ppb – Part per billion

LAPS – Light addressable potentiometric sensors

PEM – Polyelectrolyte multilayer

AC – Alternating current

DC – Direct current

FET – Field-effect transistor

SiO2 – Silicon dioxide

Si3N4 – Silicon nitride

Vg – Gain voltage

Vd – Drain voltage

SPR – Surface Plasmon resonance

E. coli – Escherichia coli

Q – factor – Quality factor

LiNbO3 – Lithium niobate

PBLG – Poly(γ-benzyl-l-glutamate)

QCM – Quartz crystal membrane

CFU/mL – Colony-forming units per milliliter

Ng/mL – Nano grams Per Milliliter

µmol/L – Micromole/liter

PFU/mL – Plaque forming units

VOCs - Volatile compounds

γ – Surface tension

γ_{sv} – Solid–vapor interfacial energy

γ_{sl} – Interfacial tension between solid and liquid,

γ_{lv} – Liquid–vapor interfacial energy, (surface tension of the liquid)

LB-ADSA – Low-bond axisymmetric drop shape analysis

PET – Polyethylene terephthalate

WCA – Water contact angle, Degrees

CA – Contact angle, Degrees

mm – Millimeter

θ_a – Advancing angle, Degrees

θ_r - Receding angle, Degrees

1. Literature review

1.1. Biosensors

The Biosensor term generally identify as the fusion of the biology and the mechanical or electronic combination to get an appropriate output of applied chemical or biological input. The biosensors are usually applied to observe and identify chemical constituents of the substances. The biosensors are combined together with analytical devices the biological elements of a device which is adopt for exploration of the physical and chemical composition. First biosensor device was developed and demonstrated by Clark and coworkers in 1960s [1]. The components for example, tissue, smaller scale life forms, antibodies, nucleic acids are investigating the examination perceive and co-operate with the detecting and natural components of biometric segments. The signal is transformed by detector element from the combination of the chemical or biological with the help of biochemical element to another signal transducer. It was evaluated easier and efficiently. Biosensor devices are usually a combine together electronic component and signal transducers or processors and they are culpable for the demonstration of result data and also this device is user-friendly. The biosensor look into has a representative application in the outline and advancement of modern electronics with a variety of material composition for a many field of applications. In this thesis, various biosensors are presented with their variety of application in the different field.

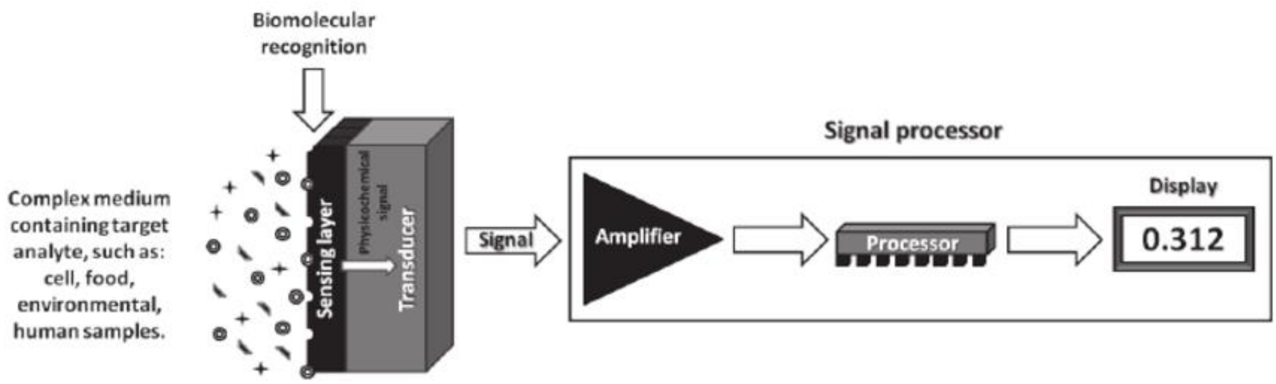


Figure 1. Schematic diagram of Biosensor [2]

The conceptual scheme of the biosensor is split into main three segments as shown in Figure 1. Consist of first sensing layer is called as Bio-receptors work as a sensing element. It is totally depending on the idea of the biological element and the transducer used to recognize the response. Then identified information sends to signal processor consist of amplifier, processor and screen display to show the desire measured output.

It is an independent incorporated device which is equipped for giving specific calculable investigative data utilizing an organic affirmation segment (biochemical receptor) which is in organize spatial contact with a transducer segment. A biosensor should be unmistakably recognized from a bioanalytical framework, which requires extra handling advances, for example, reagent expansion. Moreover, a biosensor should be recognized from a bio probe which is either expendable after one estimation, i.e. single utilize, or unfit to constantly screen the analyte fixation". Biosensors that incorporate transducers in light of coordinated circuit microchips are known as biochips. Specificity and affectability should be the fundamental properties of any proposed biosensor.

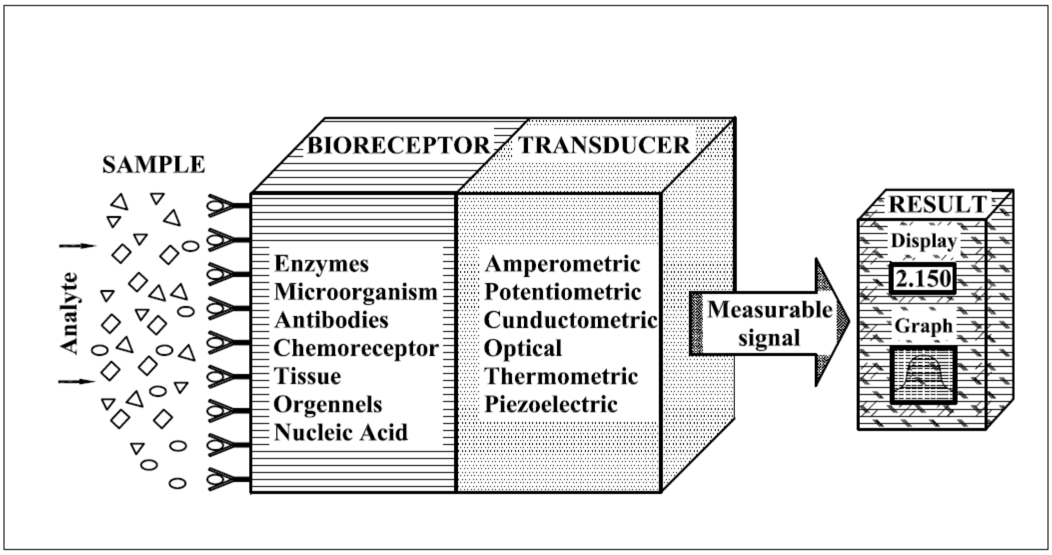


Figure 2. Key components of biosensor showing biological receptor, transducer and the signal display

Figure 2 illustrates the main elements of the biosensors. Also, commonly used bioreceptors and transducers are listed. Generally, Enzyme most favorable bio-recognition substrate for biosensors. Moreover, microorganism, antibodies, chemoreceptors, tissues, organelles and nucleic acid applied as a bio-receptor to recognize the chemical signal from outside cell. As described above transducers are converts measured variation in physical quantity. The transducer used for biosensor are electrochemical, it is briefly classified as an amperometric, potentiometric and conductometric. Also, optical, thermal and piezoelectric transducers are used for the various application.

1.1.1. General characteristics of the Bio-sensors

There are static and dynamic qualities such as selectivity, reproducibility, stability, sensitivity, and linearity that each biosensor acquires. The improvement of mentioned properties is emulated about the execution of the biosensor [3].

Figure 3 shows the characteristics and qualities of the biosensor. Any kind of sensor should satisfy the terms. For example, selectivity, reproducibility, stability, and sensitivity. Also, it should be good are lifespan of working, accuracy and precision, working range and response. Those important terms are described below.

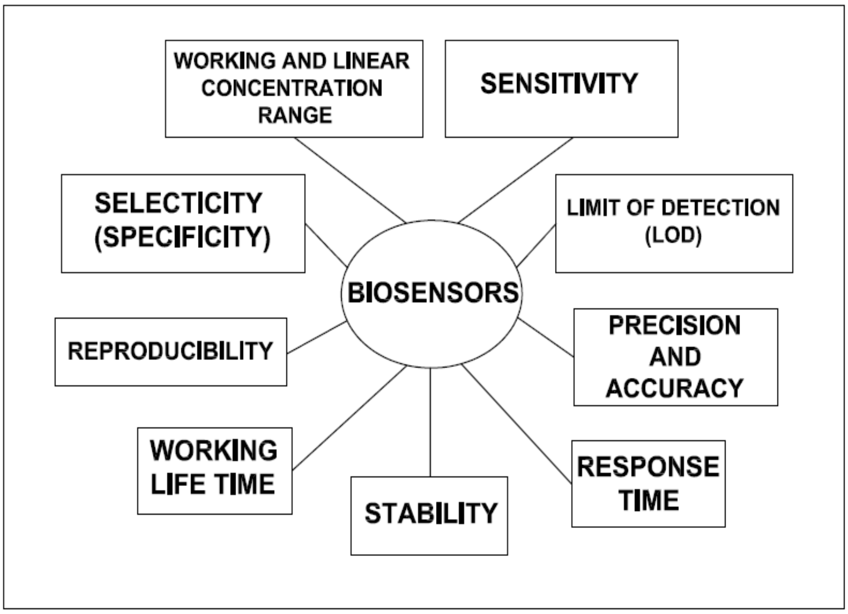


Figure 3. Characteristics of Biosensors

- **Selectivity**

Selectivity is the most critical element of a biosensor. Selectivity is the intelligence of a bioreceptor to determine a particular analyte in an example which contains various admixtures and contaminants. The first case of selectivity is delineated by the cooperation of an antigen with the antibody. Traditionally, antibodies acting as bioreceptors and they are immobilized on the whole face of the transducer. A solution containing the antigen and then presented to the transducer where antibodies connect just with the antigens. To design and develop a biosensor, characteristic like selectivity has a crucial and the primary thought while selecting bioreceptors.

- **Reproducibility**

Reproducibility is the capability of the biosensor to produce an exact reaction for a replicated set-up of the experiment. The reproducibility is briefly describing as the exactness and precision of the transducer and electronic parts which are used in a biosensor. Preciseness is the capability of the sensing element to give alike outcomes each time a sample is estimated and precision shows the sensor's ability to give a mean esteem near the genuine esteem when a sample is estimated more than once. Reproducible signs give a high amount of reliability to the surmising made on the feedback of a biosensor.

- **Stability**

Stability is that the constant condition to close aggravations in and all-around the biosensing framework. These interference impacts on the output signal which is produced by biosensor underestimated value. This can affect the accuracy level and performance. Stability is the most crucial perspective in applications where a biosensor requires advance or continuous monitoring. The output reaction of transducers and the electronic component can be temperature-touchy, which may cause the unstable effect in a biosensor device. In this manner, appropriate tuning of electronics is necessary to ensure an enduring the feedback of the sensor. Another factor that can affect the strength is the preferring of the bioreceptor, which is how much the analyte binds to the bioreceptor. Bioreceptor element with high affinity enables a strong electrostatic holding or covalent bond linkage of the analyte that maintains the accuracy level of the biosensor.

- **Sensitivity**

The measure of analyte recognized by a biosensor characterizes its breaking point of discovery or sensitivity of the biosensor. In various observation applications like medical and natural sources, a biosensor is required to identify analyte convergence of as lowest as ng/ml or even fg/ml to confirm the appearance of analytes in given sample. For example, a prostate-particular antigen (PSA) grouping in blood-related to the prostate disease for which specialists propose biopsy tests. Hence, sensitivity is the most important and basic property of a biosensor

- **Linearity**

Linearity is the characteristic that demonstrates the accuracy level of the calculated reaction (for an arrangement of estimations with various convergences of analyte). The linearity of the biosensor can be identified with the assurance of the biosensor and extent of analyte centers around the test. The determination of the biosensor is characterized as the low revision in the convergence of an analyte that needs to acquire a change the reaction of the biosensor. Dependent upon the application, an assurance is required as most biosensor applications require analyte area. Furthermore, the estimation of centralizations of the analyte over an extensive variety of working. General characteristics of Biosensors are mentioned below:

- Immobilized bioactive materials are utilized which is very less expensive or not so frequent so often possible to use more than one time, which beat the inadequacies of the examination strategy that has a higher consumption and unnecessary intricacy before.
- Biosensors are securing high command of accuracy level and specificity.
- In comparison with other sensors, biosensors have low cost with high speed in the monitoring analysis. So it provides the results within a fraction of minutes.
- Simple to deal with biosensors.
- Biosensors deliver simple experimental analysis.

- usually, don't require a tiresome & time-consuming sample pre-treatment and easy to implement automatic analysis.

1.1.2. Types of biosensors

Biosensor classification could be possible in many ways. Either it is possible according to nature of biorecognition component used or according to transducers used for the transduction process. So many possible ways to classify biosensor categories shown in Figure 4.

- According to the bio-recognition element, it could be classified into antibody biosensor, enzyme biosensor, DNA biosensor, protein biosensor and so on.

- According to the transducer, it is divided into main four divisions are electrochemical biosensors. It includes more briefly classification with an amperometric, Conductometric and potentiometric biosensors. Optical biosensors, thermal biosensor, and piezoelectric biosensor.

- Moreover, it is possible to split into the bases of association between sensitive biological material as affinity sensor and metabolic sensors.

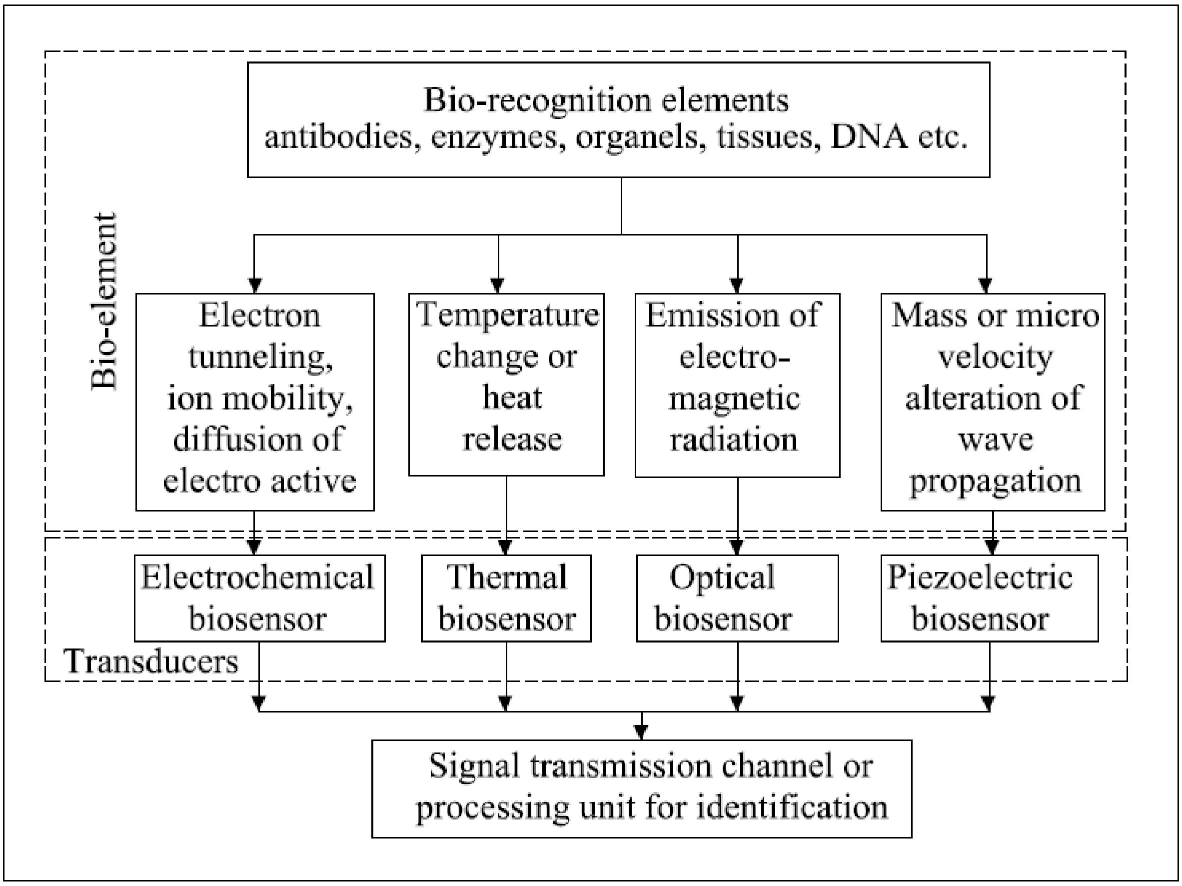


Figure 4. Classification of biosensor and their category

In this report, review of different types of the biosensor has been made according to their signal transformation from detecting to transforming and generating a various output signal according to their function.

1.1.3. Bio-recognition elements

Bio-recognition element or bio-receptors are applied to identify and interact with the analyte particularly and in this manner is an imperative part of any biosensor. For the design and produce substantial metal biosensors different categories of bio-recognition members as mentioned in Figure 5 have been utilized including whole cells, enzymes, non-enzymatic purified proteins, recombinant organisms and antibodies and many more [4].

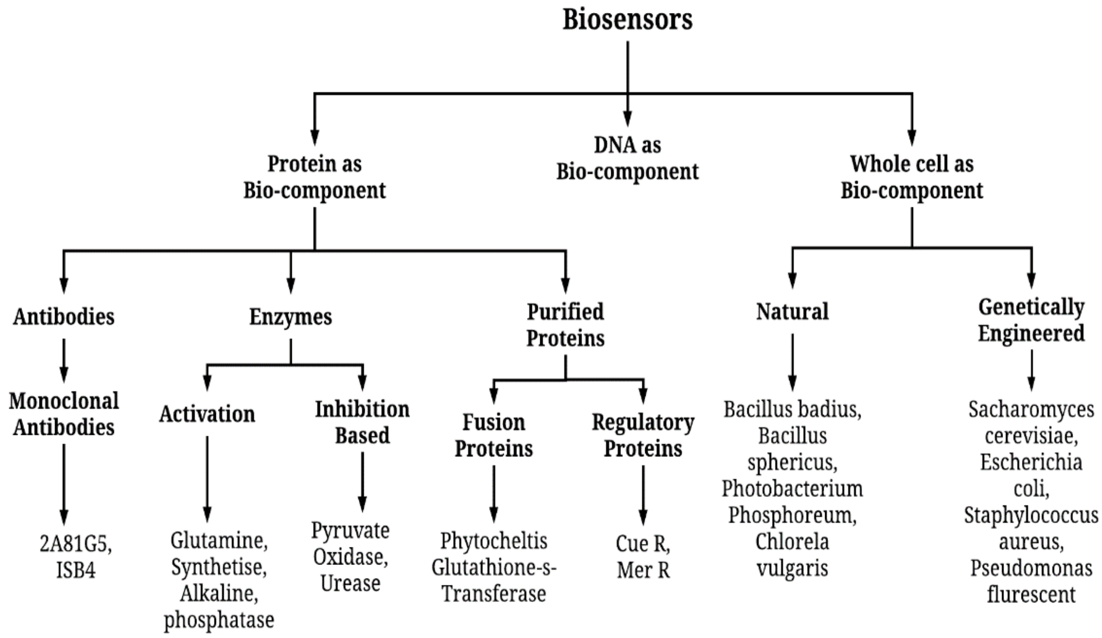


Figure 5. Classification of biosensors on the basis of bio-component [4]

Likely the primary challenge and the most imperative part in the development of biosensor is the determination of bio-receptors with solid metal-restricting capacities with specificity. The interaction of metal particles with biological particles, for example, proteins, antibodies, or nucleic acids delivers noteworthy favorable circumstances in this range as far as selectivity and points of confinement of identification. Different biological receptors are summarized in Table 1.

Table 1. Classification of Bio-receptors used for development of biosensors [5-8]

Type of Bio-receptor	**Substrates**	**Metal analyzed**
Antibody	2A81G5	Cd
	ISB4	Cd
	12F6	U
Enzyme	Alkaline Phosphatase	Zn
	Pyruvate Oxidase	Hg
	Urease	Cd
	Glucose Oxidase	Hg
	Trienzymatic (invertase, mutarotase and glucose oxidase)	Hg, Ag
Proteins	Glutathione S Transferase	Cd and Zn
	Mer R	Hg, Cu, Cd, Zn, Pb
	Cue R	Cu
	Metallothionein	Cd, Zn, and Ni
	Phytochelatin	Cd and Zn
DNA	T-T mismatch based	Hg
	DNAzyme based	Pb
	Metal-DNA interaction based	Pb, Cd, and Ni
Natural Whole cells	*P. phosphoreum*	Cr
	Chlorella Vulgaris	Cd and Zn
	Cardiac cells	Hg, Pb, Cd, Fe, Cu, Zn
Genetically Engineered	*E. coli* DH5α (pVLCD 1)	Pb, Cd
	Saccharomyces cerevisiae Y2805	Cd, As, Hg
	D. radiodurans (KDH081)	Cd
	E. coli DH5αn,(pMOL30)	Pb

1.1.4. Enzymes as Bio-recognition element

Enzyme-based biosensors for the examination of metal ions utilize either chemical hindrance or actuation as its bio-assay standard. Ordinarily, metal ion joins with thiol gatherings in the enzyme structures which provide information about conformational changes and consequently influence reactant action. For the implementation of hindrance based biosensor, numerous distinctive enzymes have been utilized, for example, glucose oxidase [5] urease, Glutathione-S-transferase, alkaline phosphatase, lactate dehydrogenase, and acid phosphatase [6] Soldatkin for the identification of different metals for example, cadmium, lead, copper, mercury and zinc and so on. Nevertheless, inhibition based biosensor experience the ill effects of a noteworthy downside of the absence of selectivity as a section of the compounds are hindered by a few metals, pesticides etc. Endeavors have been made by various scientists to help this entanglement [7]. Many types of the enzyme which is used for selective detection of their component substrate as analytes by the biosensor is summarized in Table 2 with their function of detection.

Table 2. Types of enzyme and function

Sr. no	Type of Enzyme	Functions
1	Oxidoreductases	Oxidation or reduction reactions
2	Transferases	Exchange of molecular groups one to another molecule
3	Hydrolases	hydrolysis
4	Lyases	Cleavage of C-C, C-O, C-N bonds by different means than oxidation or hydrolysis
5	Isomerases	Rearrangement of molecular
6	Ligase	For a combination of molecules

Immobilized glucose oxides in poly-o-phenulenefdiamine have been recognized Hg^{2+} based on inhibition of enzyme [5]. The ability of GST-Theta 2-2 biosensor is to detect Zn^{2+} from 1fM to 1mM and Cd^{2+} from 10 pM to 1Mm Simultaneously GST-(His)6 biosensor has the ability to detect Zn^{2+} and Cd^{2+} in between the range of 1fM to 10mM, and the range of detection for Hg^{2+} is 1fM to 10mM. A thermostable bacterial lactate dehydrogenase (LDH) was utilized to build a hindrance based electrochemical biosensor for mercury. The enzyme was purged and immobilized onto a gold sheet covered by PGA-pyrrole polymeric material [8].

Late advancement of the biosensor through immobilizing a battery of proteins incorporates invertase, mutarotase and glucose oxidase on the surface of the transducer. Three enzymes framework was utilized as bio selective component, bioassay standard depended on hindrance of invertase by the metal particles, invented biosensor showed the best affectability towards Hg2+ and Ag2+ [7].

These can be possibly used as the part of a refined shape or be available in a microorganism or in a cut of in place tissue. The systems of the task of these bio-receptors can include 1. transformation of the analyte into a sensor-perceivable item, 2. acknowledgment of an analyte that goes about as the enzyme inhibitor or activator, and 3. assessment of the adjustment of enzyme properties upon collaboration with the analyte.

1.1.5. Urease enzyme as Bio-recognition element

Urease enzyme has been oppressed by various scientists either single or fusion with different enzymes. The conductometric biosensor in context of urease to recognize overwhelming metals. Development of bi-enzymatic bioassay fundamental with urease and glutamic dehydrogenase (GLDH) to build up an electrochemical biosensor for the location of overwhelming metal in contaminated tests with a location point of confinement of 7.2µg/l, 8.5µg/l, 0.3mg/l and 0.3mg/l individually. Also, Screen printed dispensable urease based Potentiometric biosensor was found [9].

This kind of biosensor which could recognize metal particles such as silver particles and copper particles to sub-ppm levels. immobilized urease through sol-gel to establish a Conductometric biosensor for substantial metal particles assurance in the fluidic sample in the measuring range between 0.1mM to 10mM. Among the three metals utilized, the number of hindrances was found arranged by Cd > Cu > Pb. Establishment of an optical biosensor for Pb and Cd utilize urease and acetylcholine esterase (AchE) as bio-component, biosensor depends on urease performed better, could perceive up to 1ppb of Cd and Pb in the water test. immobilized rough urease separated from Dolichos uniflorus on non-woven cellulose swab to establish the biosensor for chromium [9].

1.1.6. Whole cell Biosensors

In whole-cell biosensors micro-organisms (for example, eukaryotic cells), plant tissues, or cell receptors are being utilized as biological identification component as shown in Figure 6. The utilization of micro-organisms as the detecting components of a biosensor has a few points of interest over the utilization of other detecting components, for example, enzymes, antibodies, or subcellular segments. There is commonly an assortment of micro-organisms are applicable for endowed principle, and they might be simply produced through basic development in generally economical media. Additionally, microorganisms are effective in explore a wide range of chemicals, they are amiable to hereditary alteration, and they can frequently adjust to a wide scope of addressing conditions [10-11].

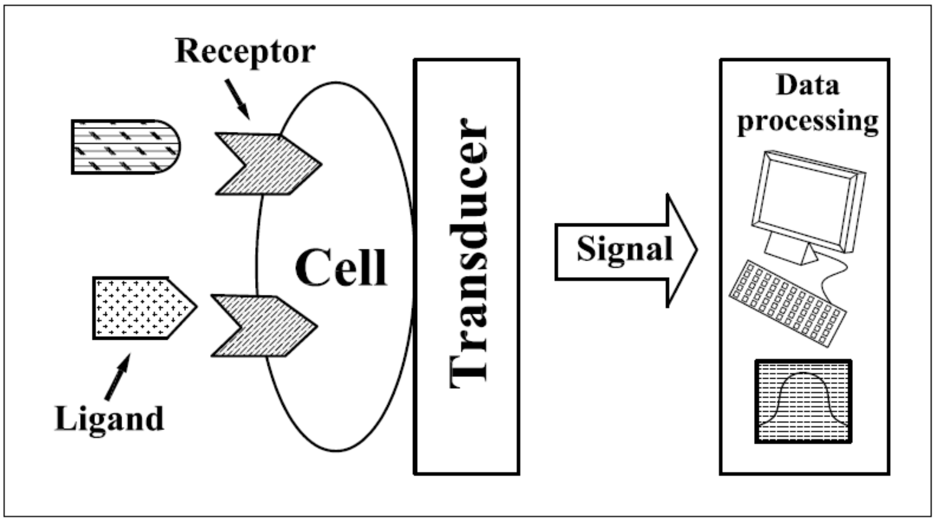


Figure 6. Scheme of cell-based Biosensor [11]

The application of whole cells as biological recognition components has numerous favorable circumstances:

I. Whole-cell biosensors are less expensive than chemical-based biosensor, the fact behind whole cells refined and producing is less demanding than segregation and decontamination of the enzyme.

II. Whole cells are more tolerant to a critical change in pH, temperature or ionic focus than sanitized enzymes.

III. A multi-step response is conceivable on the grounds that a solitary cell can contain entire enzymes and co-factors required for recognition of the analyte.

IV. Biosensors can without a lot of an extent be recouped or kept up by letting the cells re-fabricated while working in situ.

V. Extensive examine is not required.

Nonetheless, few disadvantages that breaking point the conceivable uses of whole cell biosensors, for instance:

I. They are defenseless to impedance by contaminants other than target analyte as contrasted with enzyme-based biosensors.

II. moderately slow reaction contrasted with different kinds of biosensors.

A bi-enzymatic Conductometric whole cell biosensor for pesticides and particles discovery in a water sample has been established in previous research. Whole cells of Chlorella vulgaris microalgae were immobilized inside the BSA films which were reticulated with glutaraldehyde vapors saved on interdigitated Conductometric cathodes. A neighborhood conductivity variety is made by algal enzymes; antacid phosphatase and acetylcholinesterase which could be distinguished electrochemically. Bioassay standard has been founded on the restraint of these enzymes by unmistakable groups of lethal compounds. heavy metal particles are known to suppress alkaline phosphatase while acetylcholinesterase is restrained via carbamates and organophosphorus (OP) pesticides. The sensitivity of developed biosensor towards Cd2+ and Zn2+ was observed to be 10ppb (10μg/l) for both. There was no noteworthy hindrance by Pb ions. To the extent, pesticides are concerned paraoxon-methyl was found to inhibit C [12].

Advancement of the cardiovascular cell-based biosensor for substantial metals by light addressable potentiometric sensors (LAPS). The particularly preferred standpoint of utilizing mammalian cell for the advancement of biosensor that offers knowledge into the physiological shock of the analyte. Particular changes regarding beating frequency, adequacy and time were noted under the introduction of various metal particles (Hg2+, Pb2+, Cd2+, Fe3+, Cu2+, Zn2+ in the grouping of 10μM) in under 15 min [13].

Discovery of substantial metal in fluid medium whole cell (Escherichia coli) based biosensor was discovered. As a bioreceptor, Escherichia coli were utilized. It is put on the acoustic approach of the sensor which is an overlay with a polyelectrolyte multilayer (PEM). A small scale stage was made for a quick investigation by embedding acoustic postponement line in an oscillation circle and connecting it with a Polydimethylsiloxane (PDMS) microfluidic chain. Fluctuation in frequency was observed when an example arrangement of Cd^{2+} and Hg^{2+} particles was pumped through the micro-channels. The framework could feedback of concentration up to 10^{-12} mol [14].

1.1.7. Immobilization of Biomaterials

As explained before biosensor is divided into three main segments i.e. bio-receptor, transducer and a display unit. Bio-receptor is placed closed of the transducer so that signal generated by contact of the analyte with bio-element could be exchanged to the transducer without any loss of signal strength. In this way, for the advancement of a whole cell biosensor, cells are either immobilized straightforwardly immobilized on transducers itself [15] or on some platform and after that acquired nearness of the transducer. Immobilization technique assumes an essential part in the reaction of biosensor, operational strength and utilizes for long-term in this manner decision of immobilization procedure is critical.

Hypothetically, Design of an interface must fulfill five conditions [16].

1.Stability of biological activity after immobilization.

2.Bio-element to transducer proximity should be maintained.

3.The bio-element should be stable and durable.

4.Recognizing specificity of the bio-element for its analyte.

5.Conceivable reuse of some biomaterial.

Therefore, immobilization of biomaterials (micro-organism) on support matrices or transducer possibly done by two methods wither chemical or physical.

- **Chemical methods**

Immobilization of microbial cell is conceivable through two chemical methods are covalent bonding and cross-linking. Various bonding methods are (Adsorption, covalent bonding, matrix entrapment, cross-linking, and encapsulation) illustrated in Figure 7.

In the first strategy, covalent bonding development of covalent is steady between the group of function and transducer. For instance, carboxyl, amine, epoxy, and tosyl with the same function of the micro-organism cell face components, for example, carboxyl, amine or sulphydryl. Covalent holding has a downside of uncovering the whole cells to destructive chemicals harsh response condition, which may cause the decrement in the biological action and harm the cell layer.

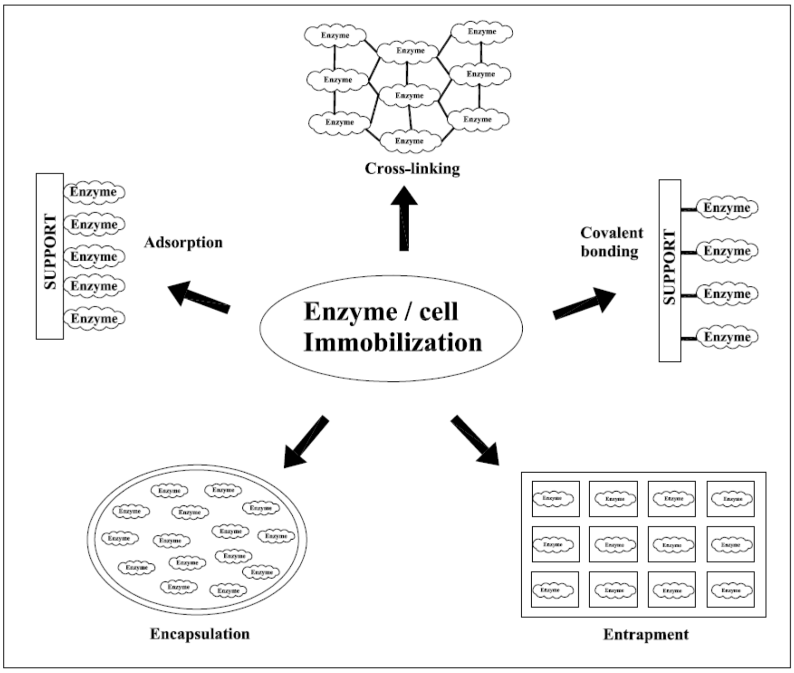


Figure 7. Methods for immobilization of enzymes and other bioreceptors in biosensors [17-19]

Another chemical technique for immobilization is cross-linking that include improvement of the system by influencing span between functional groups to show on the external layer of the cell utilizing multifunctional reagents e.g. glutaraldehyde and cyanuric chloride. Cross-linking is observed to be quick and straightforward strategy and in this way has wide acknowledgment for immobilization of microorganisms. Cross-linking of cells can be completed straightforwardly on the transducer faces or on a support membrane, which would then be put on the transducer [17].

Restoration of the membrane with the immobilized cells is favorable for the cross-linking approach. however, cross-linking specialists can influence the cell reasonability and the biomolecules of the cell membrane.

- **Physical methods**

Physical techniques for immobilization incorporate adsorption and entrapment. Physical techniques do not include a covalent bond arrangement with organisms in this manner do not have a bond with microorganism local structure and function. Also, these techniques are favored when feasible cells are required for improvement of the biosensor [18]. For the physical adsorption, a microbial suspension is brooded with an immobilization framework, for example, alumina and glass globule accordingly flushing with support to evacuate unabsorbed cells. Adsorptive communications incorporate hydrogen holding, ionic, polar and hydrophobic collaboration. Immobilization utilizing adsorption alone experiences the disadvantages of poor long haul strength because of desorption of organisms [17-18].

Physical ensnarement of microbial cells is completed by either utilizing channel or dialysis membrane or inorganic/synthetic polymers/gels, for example, polyacrylamide, polyvinyl alcohol, carrageenan, alginate, agarose, collagen, chitosan, polyethylene glycol, polyurethane, and so on. A noteworthy downside of immobilization through physical ensnarement is the extra dispersion protection by the entanglement material, which normally influences as far as possible and sensitivity of the produced biosensor [17].

The essential prerequisite of a biosensor is the natural material should bring the change in the physicochemical closeness of a transducer. Toward this path, immobilization innovation has assumed a noteworthy part. Immobilization not just aides in shaping the required closeness between the biomaterial and the transducer, yet additionally helps in balancing out it for reuse. The biological material has been immobilized specifically on the transducer or as a rule in films. Which can, therefore, be mounted on the transducer. It can be possibly immobilized either through adsorption, capture, covalent authoritative, cross-connecting or a mix of every one of these methods. Determination of a system, as well as help, would rely on the biomaterial, substrate, and setup of the transducer utilized. The decision of help and strategy for the planning of layers has regularly been managed by the low diffusional protection of the film [18].

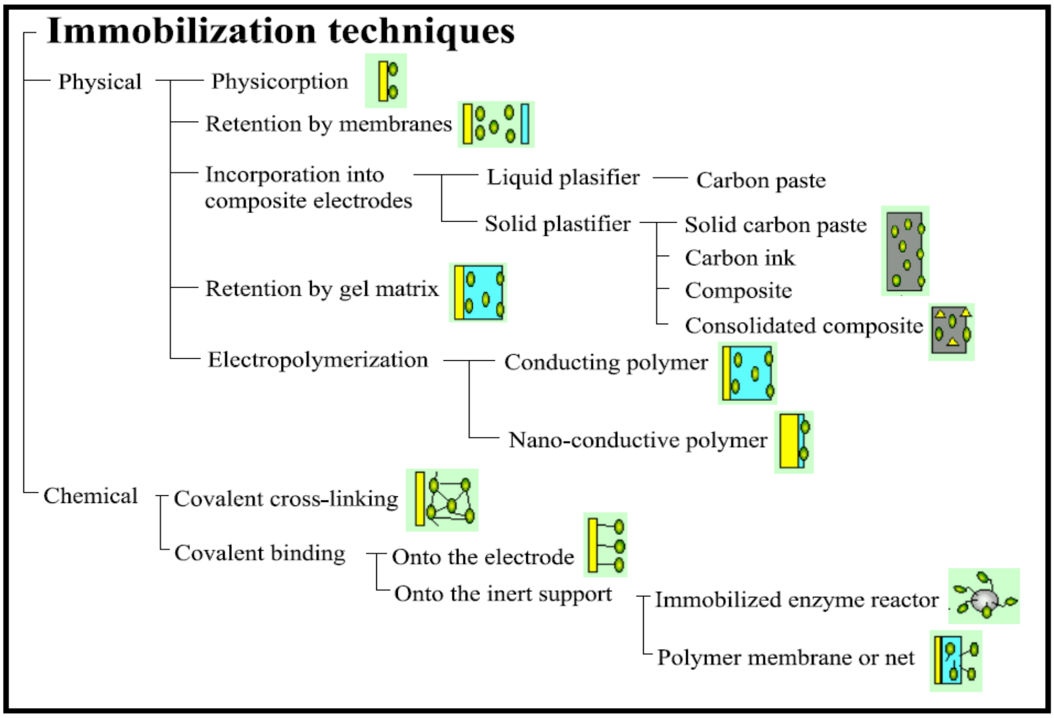


Figure 8. Immobilization techniques of biomaterials [20-21]

Delicate strategies should be connected when feasible cell arrangements are to be utilized. Covalent authoritative, a usually utilized procedure for the immobilization of proteins and antibodies, has not been utilized for the immobilization of cells. Ones of the general issues with covalent restricting are that the cells are presented to intense responsive gatherings and other unforgiving response conditions along these lines influencing their suitability. There may likewise be a misfortune in the basic respectability of the phone amid persistent utilize, promoting the loss of intracellular chemicals. Among others is the low cell stacking that is accomplished when contrasted with capture and different systems [20].

Cross-connecting utilizing functional reagents like glutaraldehyde has been effectively utilized for the immobilization of cells in different backings. These proteomic backings, for example, gelatin, egg whites have been broadly utilized. Despite the fact that this system blocks a portion of the confinements of covalent authoritative. The compound cross-connecting reagents utilized frequently influence the cell suitability. Therefore, the cross-connecting method will be valuable in acquiring immobilized non-practical cell arrangements containing dynamic intracellular enzymes. Stable microbial arrangements are frequently required for use under differed ecological variables. Cross-connecting has been widely applied for the adjustment of catalysts. It has additionally been used for the adjustment of cell organelles to osmotic stun, avoidance of lysis of to a great degree halophilic cells in low salt or salt-free situations and the counteractive action of lysis of microbial cells by lytic enzymes display in the preparing streams [20].

1.2. Transducers

The transducer is a most important Segment in a biosensor which gives an output related to the input from the bio-recognition element. Biosensors can be grouped concurring the transduction techniques they use Figure 9. Most types of transducers can be classified into five principal classes: electrochemical transducers, electrical transducers, optical transducers, piezoelectric transducers (mass recognition techniques) and thermal detection.

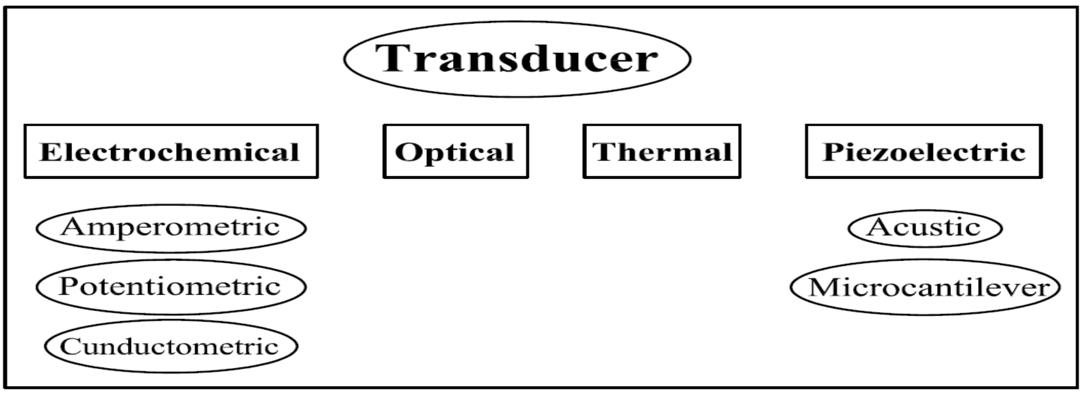


Figure 9. Classification of Biosensors according to Transducers

According to the signal transduced by the transducer from different detection in bio-recognition element and the identification of biosensor is presented in Table 3.

Table 3. Types of biosensors depending on transducer or signal transduced

Sr. no	Signal transduced		Name of Biosensor
	From	To	
1	Chemical signal	Electrical signal	Electrochemical biosensor
2	Optical signal	Electrical signal	Optical biosensor
3	Change in mass	Electrical signal	Piezoelectric biosensor
4	Temperature signal	Electrical signal	Thermal biosensor

1.2.1. Electrochemical Transducer

The basic main principle of this types of the biosensor is to highlight the chemical reaction between the immobilized biomolecules and target analytes produced or absorbed ions or electron, which has some effect on the measurable dynamic properties of the solution, such as electrical current or potentials. Characteristics for different electrochemical sensing is summarized in Table 4.

Table 4. Different electrochemical sensing characteristics [22-24]

Sr. no.	Electromechanical sensing			
	Quantity	**Amperometric**	**Potentiometric**	**Conductometric**
1	Measured parameter	Current	Potential / Voltage	Conductance / resistance
2	Applied voltage	Constant potential (DC)	Ramp voltage	Sinusoidal (AC)
3	Sensitivity	High	Poor	Low
4	Governing equation	Cottrell equation	Nerst equation	Incremental resistance
5	Fabrication	FET + Enzyme (2 electrodes)	FET + Enzyme (oxide electrodes)	FET + Enzyme

- **Amperometric**

A substantial extent of electrochemical sensors depends on the amperometric principle. In the amperometric compose, the bimolecular perception of the analyte is coupled to an oxidation or process of reduction that offers an ascend to an electrical current. In this kind of sensor, proteins can satisfy two distinctive utilitarian parts, specifically the particular knowledge of the analyte atom and the transduction of the acknowledgment event in the form of an electrochemical signal [22].

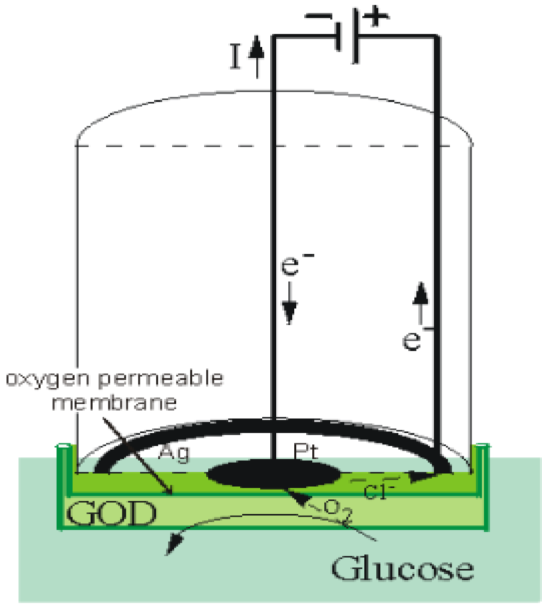


Figure 10. Schematic diagram of an amperometric biosensor [22]

A scheme of the amperometric biosensor shown in Figure 10. A potential is connected between the platinum cathode and the annular silver anode. This creates an electric current (I) which is conveyed between the cathodes by methods for an immersed solution of KCl. This anode compartment is isolated from the biocatalyst (here indicated glucose oxidase, GOD) by a thin plastic film, porous just to oxygen. The analyte arrangement is isolated from the biocatalyst by another film, porous to the substrate(s) and product(s). This biosensor is ordinarily around 1 cm in width yet has been downsized to 0.25 mm measurement utilizing a Pt wire cathode inside a silver plated steel needle anode and using plunge covered layers.

Amperometric biosensors are exceptionally delicate and more reasonable for mass manufacturing than the potentiometric. The working anode is typically screen-printed layer secured by the bio-component. Now a day's choice is to utilize carbon nanotubes [23]. As per previous research, they might be characterized as a low sub-atomic weight redox couple, which transports electrons from the redox focus of the enzyme to the surface of the indicator electrode. An ideal mediator should be steady, ready to respond quickly with target atom, display reversible heterogeneous energy. The overpotential for the recovery of the oxidized mediator between should be low pH autonomous, and decreased shape not respond with oxygen [24].

- **Potentiometric**

It measures contrast in the potential which is produced over an ion-particular element isolating two solutions for all intents and purposes zero flow of current. Almost all potentiometric sensors including glass anodes, metal oxide based sensors are financially accessible. In addition, it could be effectively mass-fabricated in the smaller than normal arrangements utilizing propelled modern silicon or thick-film advancements [25].

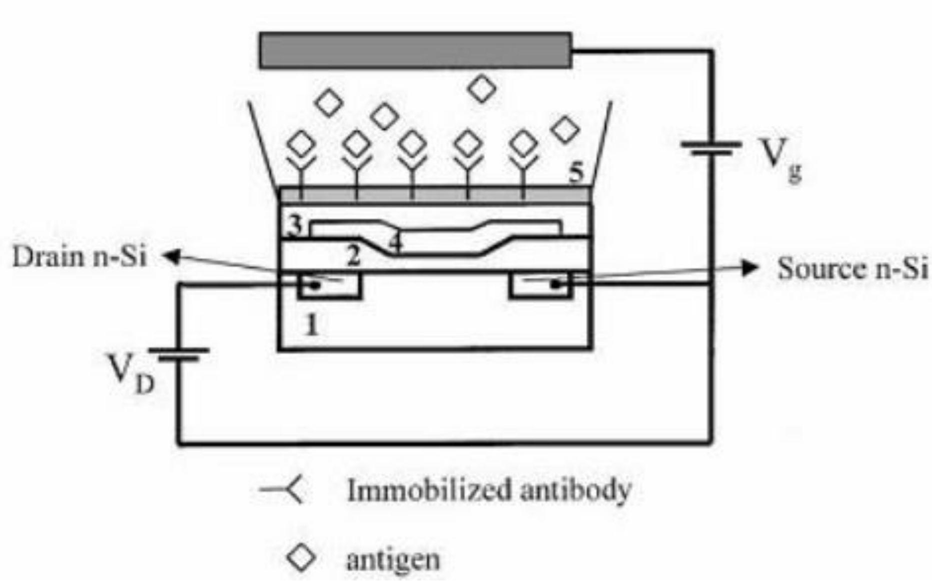


Figure 11. Schematic diagram of semiconductor immunosensor [26]

Figure 11 demonstrates a semiconductor immunosensor consists of 1. Substrate p-Si, 2. insulator SiO2/Si3N4, 3. Furrow metals, 4. Lacune (gap), 5. Selective coating and Vg and VD are gate voltage and drain voltage. It is generally used to recognize potential changes with the structure of an immunizer antigen complex in a couple of minutes. The conductivity of the n-channel district in the p-type silicon is controlled by the quality of the electrical field at the surface of the membrane. It is estimated by utilization of a voltage between the source and deplete electrodes. For appropriate working, the solution membrane interface required to remain in a perfect polarized and in this manner impermeable to the section of charge. Inability to meet this benchmark brings to the poor sensation [26].

- **Conductometric**

Conductometric biosensors act on the change in electricity of the medium when microorganisms process uncharged substrates, for example, sugars and lactic acid. This quantifiable change to identify little difference in the electricity of the medium between two electrical terminals. The amount of change of metabolites is specifically comparable to the development rate of the organism and it is effectively quantifiable. Numerous biological receptors might be checked by particle Conductometric devices utilizing interdigitated microelectrodes. Conductometric biosensors are generally non-specific particular and it has a low signal strength and noise ratio by reason of the poor characteristics this is not more in use [27].

1.2.2. Optical Transducer

Optical transducers have its own special properties and pros over electrochemical transducers, for example, it has no electromagnetic impedance, extreme powerful range and etc. Biosensor with the optical transducer is getting impressive consideration now a day, with advancement in optical filaments and laser innovation. These sensors had broadened the breaking points of applications of the spectrophotometric techniques in explanatory science, exceptionally, miniaturized systems.

The optical biosensor is mostly based on the fluorescence or optical diffraction. It is regularly utilized for biosensing because of its selectivity and affectability. These devices are identifying the optimal change occurrence of electromagnetic radiation outflow which is induced by the past absorption of radiation and furthermore by the generation of an energized state going for a short time. Single particles might be exciting more than one time to produce a high strength signal which could be obtained even at the level of the cell is single [28].

SPR optical sensor joins an optical structure. A transducing medium which interrelates the optical and bio-chemical regions. The most SPR sensor framework measure contrast in degree purpose of angular frequency. A SPR-based biosensor test is taken a stab at its adsorption to a covalently immobilized particle by surface delicate optical methods [26-28].

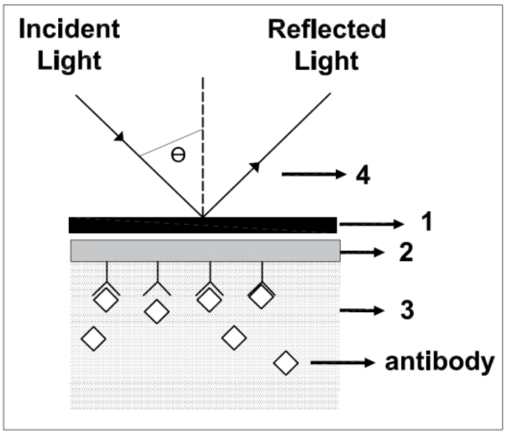


Figure 12. Schematic diagram of optical biosensor based on SPR principle [28]

Figure 12 demonstrated SPR immunosensor. In which, 1. Gold metal, 2. Sensing layer, 3. Antigen (Solution), 4. Optical substrate. It comprises of a crystal on a glass slide conveying to the very thin layer of metal. The sharpening counter acting membrane coordinate contact with the antigen to be resolved. Adjustments in the refractive list checked as a move in point of aggregate ingestion of occurrence light on the layer of metal conveying the counteracting membrane. In this kind of sensor, no need of earlier hatching and partition step [26].

1.2.3. Thermal Transducer

The sensors like optical and electrochemical are dominated the variety of transducer and transduction or detection, for example thermal and piezoelectric are utilized. It can be efficiently compelling in numerical analysis applications. Although the absence of selectivity, it is a trademark issue of these types of transducers, they introduce the construction possibility and development of varieties of sensors devices for synchronous assurance of many compounds.

These nature of biosensors are developing by biomolecules immobilization on the temperature sensors. At the point when analytes come in contact with the bio parts, the response of heat is relative to estimated analyte focus. The proportion of absolute heat delivered or retained to the sub-atomic enthalpy and the aggregate number of particles in response. The calculation of heat is regulated by the thermistor and this called as protein transistor since it is immobilizing with biocomponents. The biosensor like thermal are not required occasional recalibration and these kinds of the biosensor are not detecting the electrochemical and optical properties of the testing test [29]. Calorimetric biosensors were useful in food industries, cosmetic industries, pharmaceutical medical industries and other part examination [30].

1.2.4. Piezoelectric Transducer

Piezoelectric biosensors mostly based on the combination of bio-element with a piezoelectric element. Commonly coating of a gold electrode made of the quartz crystal. There are many types of material which are operated under piezoelectric effect which is (quartz, tourmaline, ceramics, lithium niobate or tantalite, oriented zinc oxide and aluminum nitride). Most common use and application of quartz are in the analytical solution [31] these crystals especially produce a vibration at specific values of frequency. Oscillation is depending on the amplitude of the applied electrical frequency to the quartz crystal or past material. With the change in mass of the binding material, the oscillation frequency also changed and measurement of resultant change has been done.

The piezoelectric transducers or PZT crystal transducers are more applicable in immunosensor. This transducer is also well known as (surface acoustic wave). In these type of device, a counteracting agent is immobilized on the whole surface of a crystal structure. The interrelationship of those components with the analyte very particular can be monitor by the oscillation of the inundated crystal in a fluid, it is supposed to create a change of mass in the crystal structure, recognizable by methods for its recurrence of frequency. The immunosensor with principle of wave acoustics among others, could be useful to detect microorganisms, varies gases, smells, pesticides, hormones and another field of research related to better improvement in the sensitivity and selectivity of transducers [31-32].

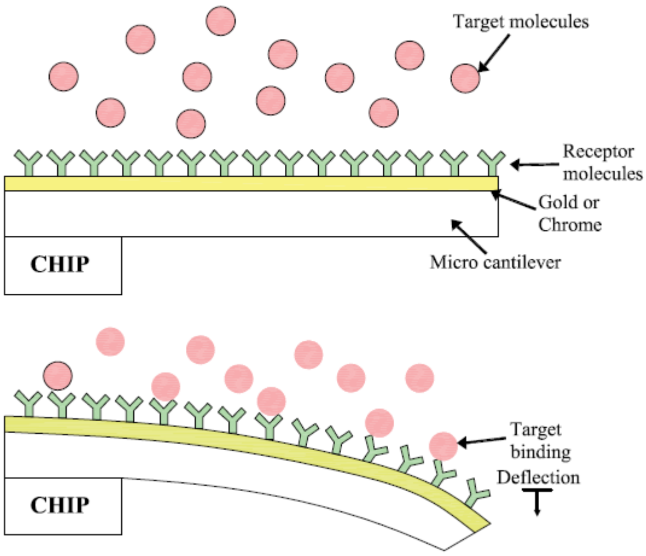


Figure 13. PZT Cantilever biosensor

Microcantilever types of piezoelectric sensor as shown in Figure 13. This type of sensor is chemical, physical or biological they are willing to measure bending in cantilever beam or frequency of vibration. The phenomenon of identification depends on the organic action of molecular adsorption and particular atomic interaction on the surface of cantilever into the mechanical reaction (Deflection) change of a cantilever beam. Properties such as, viscosity, density, and rate of flow can be examined by recognizing the diversity of vibrating frequency [32].

- **Merits demerits of transducers**

Different types of transduction method and their advantage and disadvantage are listed below in Table 5.

Table 5. Merits and demerits of different transducers [33]

Type of transducer	**Method of transduction**	**Merits**	**Demerits**
Electrochemical	• Amperometric • Surface conductivity • Potentiometric • Voltammetry • Conductivity	• More Sensitive. • Simplicity to good with current microfabrication. • Small in size. • Less power for disposing.	• Their utilization is limited to a fluid, for the most part watery, condition Interferences. • Fouling.

Optical	• Surface Plasmon • resonance (SPR) • Absorption • Reflection • Fluorescence • Luminescence	• Simple and Flexible with low cost. • Perform Multichannel • Sensing. • Remote sensing is possible. • Free from electromagnetic impedance. • Electrically inactive. • Wide powerful Range.	• Light obstruction (require a dull domain). • Irreversibility of immobilized bio-reporter atoms. • A need exists for more particular pointers and more immobilization steps.
Thermal	• Heat changes during bioreporter activity Calorimetry	• Possible multichannel • Sensing. • More stable for • continuously • monitoring. • Flexibility in shape and Size.	• Losses of heat during signal measurement due to the irradiation. • external temperature can effect system. • Poor specificity.
Piezoelectric	• Acoustic wave • Piezoelectric • Quartz crystal • microbalance	• Real-time output. • Simple in use. • Wider working pH range. • Cost-effectiveness.	• Effect of liquid medium on analysis. • Delicate to ecological change in temperature..

1.3. Biosensor application

Biosensors have many applications in the various area according to nature of detection, recognition element and transduction element used. The reason behind major areas of application is it has an ability to perform the continuous measurement, fast response and better stabilization. Also, these devices are reliable to produce real-time output, smaller in size, quick and easy to use and the cost is low. Some of the biosensors have drawback and restriction for work in specific environmental conditions but still, in the field of microdevice development, there are various devices are invented which are being used for daily life.

Major application area of the biosensor is food analysis, drug development, crime detection, medical diagnosis (for clinical and laboratory use), environmental field monitoring, quality control, industrial process control, a detection system for a biological agent, military, and manufacturing of pharmaceutical and replacement of organs [33]. According to field biosensor has a wide range of applications summarized in Table 6-7.

Table 6. Application of biosensor in various field [30-35]

Sr. No	Field	Application
1	Clinical use	Research laboratories, Central pathology laboratories, Point of care, Doctor's surgery, Emergency Room.
2	Pharmaceutical use	Drug and Natural product screening.
3	Defense	Detection of Biological weapons, Chemical weapons. To check the quality of water.
4	Food and safety	To detect Regulation.
5	Environmental	Treatment for wastewater and sewage and detect toxins in the water.
6	Processing and controlling	To check the quality of water.
7	veterinary	In the laboratory of animal disability benefits.

Table 7. Application of biosensor based on analytes [26-30]

Biosensor	Analytes	Application
Electrochemical	Amino acids, carbohydrates, alcohols and inorganic ions, Glucose, galactose, biological oxygen demand, cadaverine, histamine, and so on.	Clinical diagnosis, laboratories, detection of drug and glucose concentration on DNA.
Optical	Carbohydrates, alcohols, pesticide, and bacteria, and so on.	Monitoring wavelength, detection of multiple analytes.
Thermal	Carbohydrates, sucrose, alcohols, liquids, amines	Detection of pesticides and pathogenic bacteria.
Piezoelectric	Carbohydrates, vitamins, pathogenic microorganisms, and contaminants. Also to identify toxic as bacterial toxins.	pH detection, detecting organophosphate and carbamate.

1.4. Biosensors in the liquid environment

In the field of biosensors, many remarkable milestones are achieved in last few decades. Biosensors are generally worked in any working environment (gaseous, air or viscous). Mostly the problem was found in lack of sensitivity and response in a biosensor which is working in the liquid environment. Current researchers are going on to solve this problem to improve sensitivity and response of biosensor specially made from PZT material. Biosensor based on piezoelectric material established a great promise to serve a real-time output and high sensitivity and selectivity. Quartz crystal is the most applicable PZT material for biosensors and microchannel experiments. Several new techniques are developed for improvement in quality factor (Q- factor) and response of the sensor in a liquid environment by using microcantilever beams with a coating of PZT layer on the top surface of the membrane. PZT crystal becomes an electrically polarized when it is subjected to mechanical stress. Several materials have been used as transducing and sensing elements from the piezoceramics and composite polymer. For example, lead zirconate Titanate or PZT ($PbZr_{0.52}Ti_{0.48}O_3$), quartz (SiO_2), AT-quartz cut, BT-quartz cut, lithium niobiate ($LiNbO_3$), and lithium tantalate ($LiTaO_3$) as piezoceramics, polymers PVDF, rubbers (polydimethylsiloxane (PDMS) stamps or soluble glues and PZT composites (PZT/PVDF, PBLG/PMMA, etc.) [34]. In this situ piezoceramic materials are demonstrating excellent chemical stability and can withstand at high temperature. Quartz crystal showed a high amount of piezoelectric effect in its prism axis. A single crystal structure material such as PZT is a high-efficiency class of the piezoelectric energy transformation materials. Piezoceramics and crystal materials are firm or rigid so it has low stiffness and high acoustic impedance [34-35].

On the other side piezo films are made from polymers or composite materials. The piezo-composite films have a quality of more flexible, light in weight and tough. It has a large working area as compare to piezoceramic materials because it is available in many varieties of thickness. The major advantage of piezo films rather than ceramics is it has low acoustic impedance. It is very close to human tissue, organic materials, and water. Because of this advantage, it is able to offer cost effectiveness, real-time output, and practical simplicity [34]. Piezo-ceramic materials are fragile in nature that’s why it can be replaced by the piezo-composite material. Because it is more flexible in nature. However piezoelectric composite biosensor has some drawback but it can be resolved. For example, the piezoelectric coefficient of a ceramic material is higher than PVDF piezo-composite material but performance is higher than ceramics. Only the problem with a piezopolymer or composite material could be degraded easily in the air or gaseous environment [35].

Poly-piezoelectric materials display novel electronic execution practically identical to those of silicon-based materials. Natural field-impact transistors are of extraordinary enthusiasm for applications in expendable electronic gadgets, for example, radio-recurrence identification labels, sensor applications and in addition in flexible display devices. Superior hydrophobic surfaces, particularly predominant hydrophobic piezoelectric solids, have pulled in significant enthusiasm from both for example, field-impact transistors, electric security, transducers and biosensors. The hydrophobicity gives less obstruction from water, which is a noteworthy purpose behind the disappointment of electrical devices, particularly for piezoelectric polymers. The very hydrophobic surface could make a semiconductor material more steady and prompt broad applications of this material. There are two essential techniques to build the contact point and development of a more hydrophobic surface. One is to build the surface unpleasantness, which is known as the geometrical miniaturized scale/nanostructure technique. Another is the modification of surface organization to bring down the surface energy. As of late, numerous hydrophobic materials have been produced utilizing different segments, for example, polypropylene surfaces, poly (methyl methacrylate) and polystyrene, polyurethane with various procedures [56]. It was found that contact angle measurement is an attractive method to observe behavior of the liquid on polymer surface. A superior hydrophobic piezoelectric solid for widespread applications has barely been reported [56].

In order to develop a piezoelectric biosensor based on the PZT composite material, it is important to identify the mechanical properties of the material. Also, surface properties of material like hydrophobicity it has a significant role in the development of the PZT biosensor work in the viscous environment. Because of the viscous environmental condition capability of sensing and signal transmission is not so accurate for the polymer or PZT composite sensors. Because of the hydrophobic property of PZT composite, there has been less application reported. In a present number of research are going on to control or to develop PZT composite materials which are easy to apply for biosensor application in a liquid environment. The piezoelectric biosensor elements used in previous research is presented in Table 8.

Table 8. PZT material used for development of a biosensor in previous decade [34-35]

Sr. no.	**Recognition element**	**Type of Piezoelectric element**	**Analyte**	**Detection limits**
1	Immunoglobulin	Quartz crystal membrane	Albumin	0.1 µg/mL
2	Immunoglobulin	Quartz crystal membrane	Francisella becteria	10^5 CFU/mL
3	Antibody on the sensor surface and gold nanoparticles covered by antibodies	Quartz crystal membrane	Shiga toxins	10 CFU/mL
4	Molecularly Imprinted Polymer from electro polymerized 3-thiophene acetic acid	Quartz crystal membrane	Melphalan	5.40 ng/mL
5	Electrochemically polymerized I-methionine with molecularly imprinted taurine	Quartz crystal membrane	Taurine $C_2H_7NO_3S$	0.12 µmol/L
6	Single-stranded DNA	PZT plates 8 µm thick	DNA	10−19 mol/L in urine samples
7	DNA particular to stx2 quality from Escherichia coli O157:H7	PZT cantilever sensor	Escherichia coli O157:H7	700 cells/mL
8	Oligonucleotide functionalized gold nanoparticles	Quartz crystal membrane	Viruses related to dengue	2 PFU/mL
9	Immunoglobulin	PZT glass micro cantilever sensor	Cancer markers	0.06 nmol/L
10	Various type of peptides	Quartz crystal membrane	VOCs	------

1.4.1. Hydrophobicity

Hydrophobicity is the property of the liquid molecule that is intermolecular force from mass of water. From past two decades in the field of the biosensors and its application received a tremendous interest in fundamentals and applied point of view from the research and its measurement methods. It plays the significant role in applications in the industrial level as well as medial. Also, it is an important term in the field of bioscience and microhydraulic device development [36]. It has significance in oil recuperation, grease, fluid covering, paint enterprises, and printing. [37-38]. In exhibit years, there has been intrigue is in the investigation of surfaces incorporates superhydrophobic and superhydrophilic. Because of their possible application in self-cleaning, nanofluidics, electrowetting and to determine the properties of the liquid. To identify its reaction on the different surface which is used for development in biosensors and biomedical application [39-40]. Study of wettability and hydrophobicity involves primary data as contact angle study which is useful to know the degree of wetting while the liquid is intract with solid surfaces. There will be three possible conditions happened small contact angle less than (<<90°) indicates high wettability hydrophilic surface while the angle is large more than (>>90°) indicates low wettability it means hydrophobic surface [41]. Illustration of contact angle which is compose by liquid drop on the homogeneous surface is presented in Figure 14.

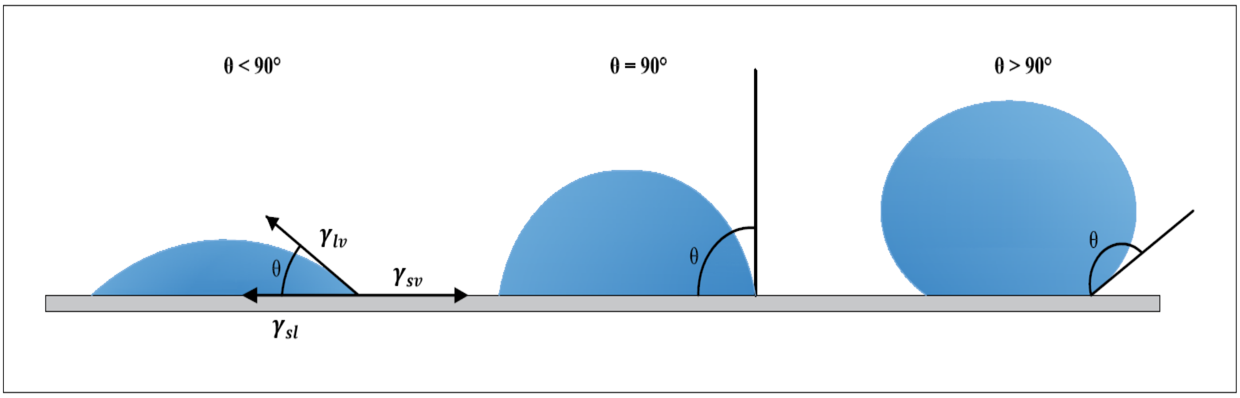


Figure 14. Contact angles formed by liquid drop on the solid surface [41]

To determine the hydrophobic and hydrophilic nature of the surface first it is very important to understand what is wetting and role of surface energy behind it. Term wetting describes the ability of the liquid substance to hold the interaction contact with the solid surface, originate from intermolecular communication when two are united. The intensity of wetting is identifying the balance of the force between adhesion and cohesion. Wetting of any liquid on the solid surface deals with three phase of the material gas, solid and liquid [42]. It is a new attention in the field of nanotechnology, sensors which are working on liquid environment studies and nanoscience development because of the occurrence of many materials in last few years. (e.g. graphene, carbon nanotube, boron nitride Nano mash etc.) [43]. Role of wetting is also more important in the bonding of more than one material (for example, PZT composite materials). Wetting and surface forces that control it also causes the other relevant effects, along with capillary effects. It can be described in another way when a liquid comes in contact with solid surface there is a certain reaction. Deformation of liquid is always depending on the surface tension and surface energy and environmental condition of the place of experiment. There will be a possibility of two conditions while drop of the liquid forms in a similar shape like a round ball it means a less contact with solid surface then drop of liquid has a higher surface tension than surface energy of the solid surface and if drop of the liquid looks flatter on the solid surface then surface tension of the liquid is less than the surface energy of the solid surface. This is called wetting. Less wetting indicates higher the difference and more wetting is lower the difference [44].

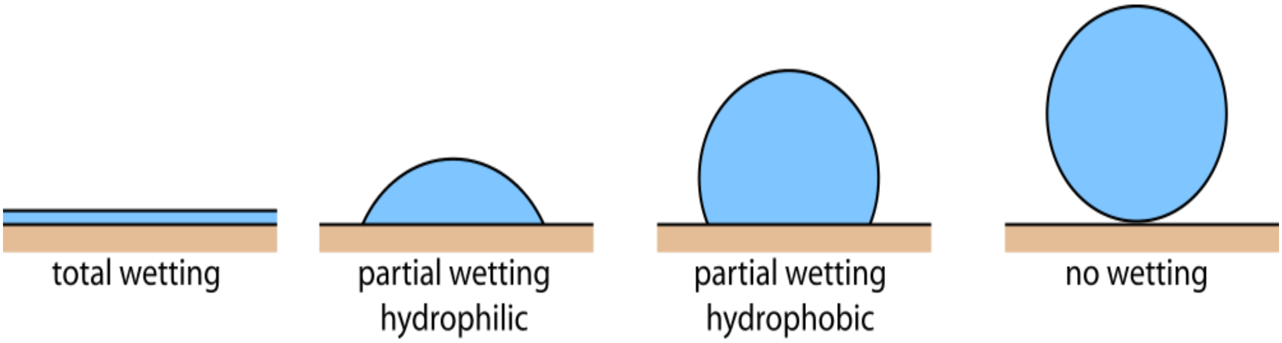


Figure 15. Wetting phenomenon [44]

In Figure 15 idea presented about the wetting phenomenon and surface properties such as hydrophobicity and hydrophilic. So the deformation of the drop depends on the surface tension of the fluid and surface energy of the solid substrate. It helps to determine the contact angle and the behavior of fluid to the solid surface. It has a significant role to develop biosensors working in the liquid environment [46].

Table 9. Wetting of fluid angle and their interaction [45]

Sr.no	**Angle formation**	**Wetting identification**	**The quality of Solid to fluid synergy**	**Liquid to Liquid synergy**
1	θ = 0°	Perfect wetting	Strong	Weak
2	θ = 0 - 90°	High Wettability	Strong or weak	Strong or weak
3	90° ≤ θ 180°	Low wettability	Weak	Strong
4	θ = 180°	Perfectly non-wetting	Weak	Strong

Table 9 contains the data about the connection between the contact edge arrangement of the drop on the strong surface and the quality of collaboration between solid to fluid and cooperation between the fluid to fluid is exhibited. Also, an illustration of the non-wetting, high-wetting, low-wetting and perfect wetting is clearly presented in Figure 16. So the contact angle form after drop formation is

180 deg., > 90 deg., < 90 deg. And 0 deg. Indicates non-wetting, high-wetting, low-wetting and perfect wetting respectively.

The contact angle is (less than 90°) mostly specify that solid substrate is very favorable for wetting, and liquid will spread out and covers more surface. Simultaneously high contact angle (more than 90°) mostly specify that solid substrate is unfavorable, so in this case, fluid will not spread out minimize contact with the solid substrate and it will be formed in a small liquid droplet. So in case of water, term hydrophilic means surface is wettable and non-wettable termed as hydrophobic. Also in case of the superhydrophobic surface have contact angle more than 150° it indicates almost less or no any contact between the surface and liquid drop. This is occasionally called the "Lotus effect". [45]. For the liquids which are not containing water, the term use lyophilic for contact angle condition is very low and for higher contact angle result term used is lyophobic. Furthermore, for polar and a-polar liquid term Omni-phobic and Omni-philic apply respectively. Also, it was derived from previous research that surface morphology and surface preparation does not effect on the contact angle of water or water containing fluids [54].

Fluids can interface with two fundamental of solid surfaces. Customarily, solid surfaces have been isolated into high-energy and low-energy composes.

- **Wetting of high energy surfaces and low-energy surface**

The relative strength of a solid needs to do with the mass nature of the solid itself. Solids, for example, metals, glasses, and ceramics are hard solids on account of the synthetic bindings that helps to hold them (for example, covalent, ionic, or metallic) are extremely solid bonding. Accordingly, it takes a vast contribution of energy to break those solids. So it called as "high energy". Most atomic fluids accomplish finish wetting with high-energy surfaces [47].

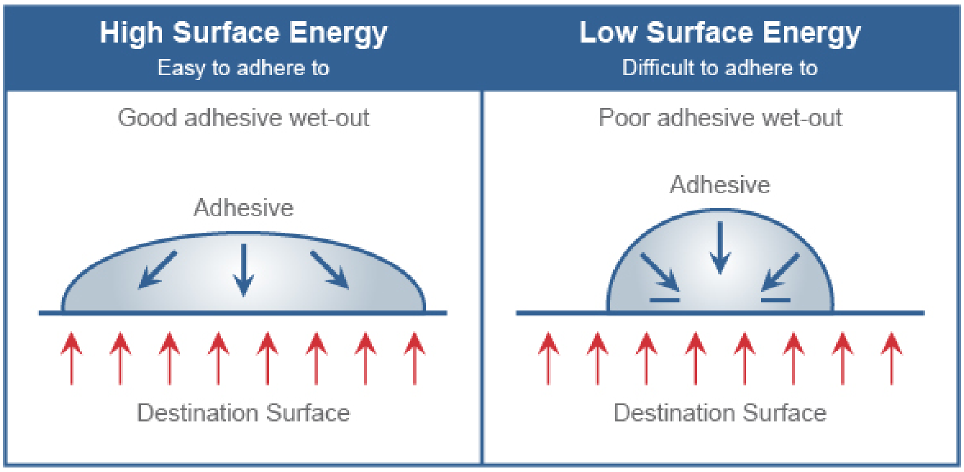


Figure 16. Illustration of surface energy and wetting [48]

Another type of solids is weak molecular crystal (e.g., fluorocarbons, hydrocarbons, etc.) in this type of the solids all the particles are combined with each other virtually by physical force, for example, Van der Waals and hydrogen bonds. Since these types of solids which are combined with the weak force, they can easily break with very low energy. Thus, the tram use for indicating this kind of material is “low energy”. It is fully depending on the type of chosen liquid surface is allowing it to penetrate the drop inside the surface and it indicates partial or complete wetting phenomenon [47-48].

Low-energy surfaces are coming in touch with liquids through dispersion (van der Waals) forces. In previous decades William Zisman has found many keys in the research he executed. He observed that value of cos theta increasing linearly then surface tension (γ) of the liquid will be decreasing. Thus, he was capable to build a linear function between cos theta and surface tension (γ) for different essential liquids. When the value of γ and θ are low the solid surface indicates more wetting. William Zisman called it as a critical surface tension when the line is intercepted $\cos\theta = 1$ of the surface. Critical surface tension is the important term for solid surface because it is the properties of the solid surface [49].

The critical surface tension of the solid is known then it is easy to achieve surface wettability [49]. It is also determining a chemical group of solid. Wettability also depends on the structure and the binding of the internal atoms. It is not depending on the same material characteristic.

1.4.2. Contact angle hysteresis

There are several techniques developed to obtain contact angle. The contact angle is nothing but the angle formed by the liquid drop to the solid surface. After releasing the drop from the dispenser or any kind of automatic syringe towards the solid surface it will form in shape because of the surface tension and the interference cohesion force between molecules. Also, it depends on the solid surface tension of the surface. From the review of research number of instruments and automatic devices were developed for industrial and laboratory purpose for the development in a variety of field. The surface tension of the liquid is played a significant role in contact angle measurement.

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv}\, cos\theta \qquad 1.1$$

where γlv, γsv, and γsl represent the liquid to vapor, solid to vapor, and solid to liquid interfacial tensions respectively. θ is the contact angle is usually referred to as Young’s equation.

From Young's condition to a particular liquid to solid framework, three thermodynamic parameters γlv, γsv, and γsl decide contact angle θ. The circumstance of wetting is other than a static state. The liquid moves to uncover its non- wet surface and to wet the new solid surface. In the event three-phase contact line is in real movement, the contact angle formation by this effect is known as a "dynamic" contact angle. Specifically, the contact angle framed by increment and decrement the liquid is called as the advancing contact angle θa and the receding contact angle θr, illustration is presented individually in Figure 17. The difference of the θa and θr angle known as the contact angle hysteresis (H) as shown in equation 1.2. [50]

$$H = \theta a - \theta r \qquad 1.2$$

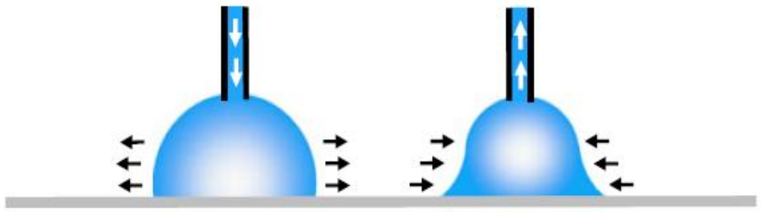


Figure 17. Advancing and receding contact angles phenomenon [50]

1.5. Thesis objectives

Biosensor device exposure is unique amongst the categories of sensors according to market survey and overview of the scientific literature. It has a significant role in the various field of application. The approach of the piezoelectric biosensors working in viscous (liquid) environment has had to be implemented by developing an innovative piezocomposite material to enhance the application in a variety of areas. There have been many piezo-composite material techniques used to develop new composite material for biosensor application. Also, it is possible to develop a piezoelectric composite material which is a help to obtain the main characteristics of sensitivity, stability, repeatability. It helps to obtain the real-time output. Mechanical properties of materials were determined by dynamic investigation (Impact test). Hydrophobicity term which is important for biosensor works in the viscous environment was determined by new designed experimental setup to measure contact angle for different fluid and solid surfaces.

The aim of the research is to investigate the mechanical properties as Young's modulus and design new experimental setup to identify the hydrophobicity of piezo composite materials for biosensor application.

In order to achieve this aim, the following objectives were determined:

1. To identify Young's modulus and density of the PZT composite materials.

2. To design experimental setup for contact angle measurement of PZT composite materials.

3. To investigate hydrophobicity of PZT composite materials.

2. Experimental setup

Miniaturization of analytical instruments utilizing microfabrication technology has increased the broad usage of analytical chemistry. The main focus was an increasing demand for low-cost instruments able to analyze compounds of very small volume with a high level of automation and precision. The terms ‘Micro-Total Analysis Systems (μ-TAS)’, and also ‘Lab-on-a-chip’, aims to develop integrated micro-analytical systems. which could complete analysis cycles (e.g. sample pre-treatment, chemical reactions, analytical separation, detection, and data handling) on the same microdevice. In addition to multi-functional abilities, microfabricated analytical systems should enhance performance, like fast response and increased analysis speed. One of the recent new applications in this area is related to biosensing elements based on cantilever or membrane type sensing platforms. These platforms are able to convert biological responses into electrical signals using the piezo effect created by the piezoelectric material, on which the active element of the system is based. Many various materials are used as an active element in the sensing platforms, whether it is a micro-pump, a biosensor or a drug delivery device. PDMS (Polydimethylsiloxane), PU (Polyurethane), PZT (Lead zirconate titanate), Si (Silicon), and SiO (Silicon oxide) have shown a great promise, but all of them lack of flexibility and abilities to change their parameters on-demand. To increase the sensitivity and ability to change parameters of the material a high Q factor is needed. In this experiment a piezoelectric composite material displaying its mechanical properties such as resonant frequencies, Young’s modulus, and density. Nanocomposite polymer highlights the property of piezo effect and is suitable for formation of periodic microscale patterns on it. These micropatterns are intended to be used as innovative functional elements in biomedical micro hydro-mechanical systems such as microchannels and biosensors. Thus by controlling surface configuration and the shape of active deformable polymer, the pressure in microfluidic vessels can be changed and mobility of the transported bioparticles can be ensured. In order to investigate Young's modulus of elasticity and hydrophobicity of PZT composite material for biosensor application, three different types of PZT composite was created. PZT composite was produced with PZT nanopowder and three different binding polymers polyvinyl butyral (PVB), polystyrene (PS) and polymethyl methacrylate (PMMA). Detailed about the synthesis of Piezocomposite are described in below in section 2.1.

2.1. Piezocomposite synthesis

An oxalic acid-water ($C_2H_2O_4$) based Nanopowder of lead zirconate titanate (Pb (Zrx, Ti1–x) O3) with PZT (58/42) were utilized. The chemical compound of PZT (52/48) solution was lead (II) acetate [Pb(NO3)2], titanium butoxide [Ti(C4H9O)4], and zirconium butoxide [Zr(OC4H9)4]. Alternate reagents utilized were oxalic acid, deionized water, acetic acid, and ammonia solution. Lead (II) acetate [Pb(NO3)2] (8.26 g) was mixed with 100 mL of water. Then in the same mixture of liquid acetic acid were poured and the solution of the mixture was warmed at 50 °C and blended to dissolve. Thirty-two grams of oxalic acid was diffused in 500 mL of water, then blend with the titanium butoxide (5.1 g) and zirconium butoxide (7.65 g) at a combination of 80%. Thereafter, the solution of lead acetate was combined with the titanium butoxide and zirconium butoxide solution. The last solution was alkalized with 25% ammonia solution to pH 9–10 and mixed for 60 minutes. The precipitate of the solution was separated in a vacuum and during filtration, it was washed with water and acetone. The material was dried at 100 °C for half of the day after separation process. The powder was warmed at 1000 °C for 9 hours. At last, PZT powder was grinded and blended with 20% solution of polyvinyl butyral in benzyl alcohol blended under defined conditions: 80% of PZT and 20% of the binding material. Finally, the coating of paste was done on a copper foil using a screen printing technique [52].

Thus, three coatings of different thickness were screen printed and investigated: element 1 with a PZT + PVB coating of 50 μm thickness, element 2 with PZT + PMMA coating of 60 μm thickness, and element 3 with PZT + PS coating of 60 μm thickness. Afterward, a laminar microstructure of 4 μm period could be embedded into formed piezoelectric film using the thermal embossing technology. Moreover, it is possible to increase optical parameters (surface Plasmon resonance) and ensure antibacterial properties of the formed microstructures using silver nanoparticles. Silver nanoparticles were formed from the solution of 0.05 $AgNO3$ in deionized water and dip-coated on top of the periodic microstructure [53].

2.2. Specimen preparation

As mentioned above for the experiment three different specimens were prepared. The properties of binding materials are presented in Table 10.

Table 10. PVB, PMMA, and PS properties

Property	**PVB**	**PMMA**	**PS**
Tensile strength	≥20 MPa	48–76 MPa	32–44 MPa
Poisson's ratio	0.45–0.49	0.35–0.4	0.4–0.41
Density	1070 kg/m3	1170 kg/m3	1050 kg/m3
Young's Modulus	50 MPa	3.1 GPa	2.7 GPa

Using these properties, the density of every used composite material was calculated. The density of different PZT composite materials is presented in Table 11.

Table 11. Density of the composite material

Sr. no.	**Composite material**	**Density**
1	80% PZT+ 20% PVB	6294 kg/m3
2	80%PZT+ 20% PMMA	6314 kg/m3
3	80PZT + 20% PS	6290 kg/m3

The bottom layer of the multilayer structure is a copper foil made from UNS C10100 copper alloy. Mechanical properties of the used copper alloy are presented in Table 12.

Table 12. Mechanical properties of UNS C10100 copper alloy

Property	**Value**
Hardness, Vickers (1/2)	75.0 – 90.0
Tensile strength	221- 455 MPa
Elongation at break	55%
Modulus of elasticity	115 GPa
Poisson’s ratio	0.31
Machinability	20%
Shear modulus	44.0 GPa

- **Multilayer structure**

A cantilever type multilayer specimens were prepared. The proposed specimen consisted of two layers: copper foil and composite piezoelectric material. Geometrical representation of multilayer structures is illustrated in Figure 18-20.

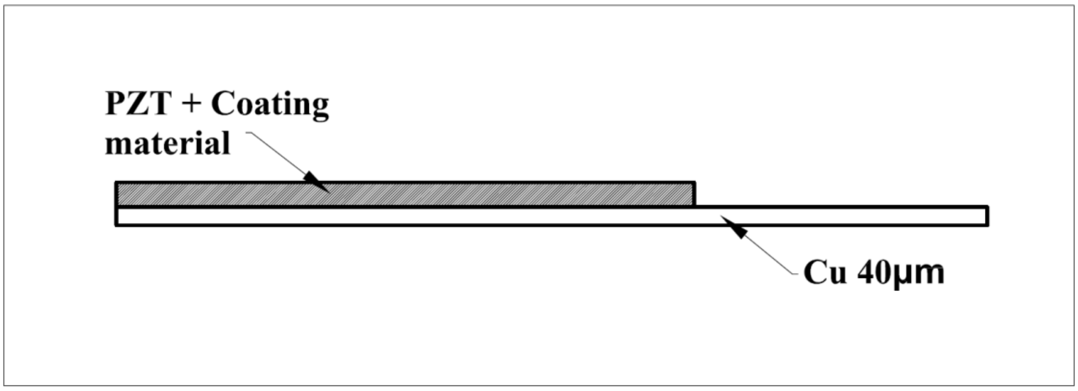


Figure 18. Schematic view of multilayer specimen

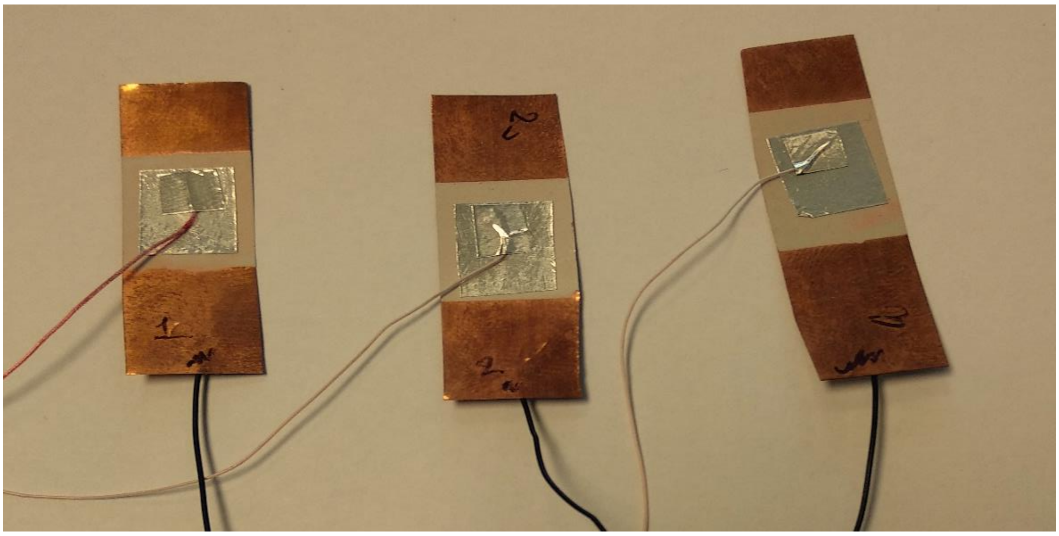


Figure 19. Multilayer specimen

As shown in Figure 18 the aluminum tape glued on top layer of the multi-structure with epoxy for gluing the wires on the piezoelectric material. But the influence of aluminum tape on the experiment was negligible.

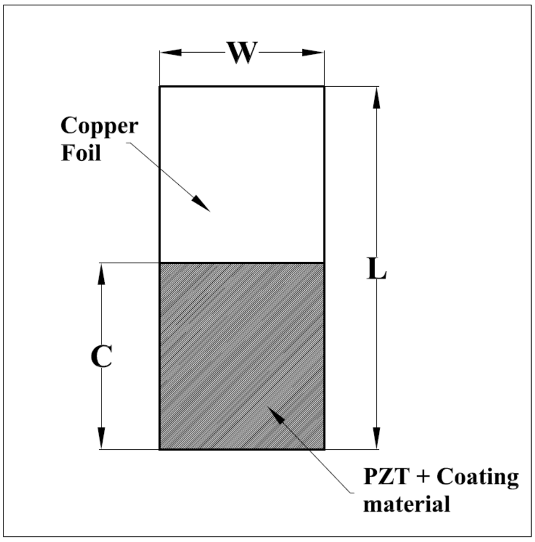


Figure 20. Geometric dimension of the multilayer structure

Geometrical dimensions of these three elements are given in Table 13.

Table 13 Specimen dimensions

Specimen	**Coating length (C), mm**	**Clamped length (L), mm**	**Width (W), mm**	**Total thickness, µm**	**Coating thickness, µm**
PVB	15	32	18	90	50
PMMA	17	30	15	100	60
PS	20	33	21	100	60

2.3. Dynamic Investigation of Composite Piezoelectric Material

The experiment was performed in Institute of mechatronics and Department of mechanical engineering, Kaunas University of Technology. Determination of damping ratio and modulus of elasticity of the piezocomposite materials were done using the hybrid experimental-numerical method. Dynamical response of the multilayer cantilever in to impulse excitation were measured using laser triangular displacement sensor LK-G3000 series together with sensor head of LK-G82 and control block LK-G3001PV made by Keyence from Illinois, US. The signal from control block and the generated voltage were collected with data acquisition system, a 4 channel USB oscilloscope PICO 3424, and collected in a computer. Data which was obtained it was analyzed with PicoScope 6 software. Experimental setup scheme used to measure dynamic characteristics of piezoelectric elements is presented in Figure 21-22.

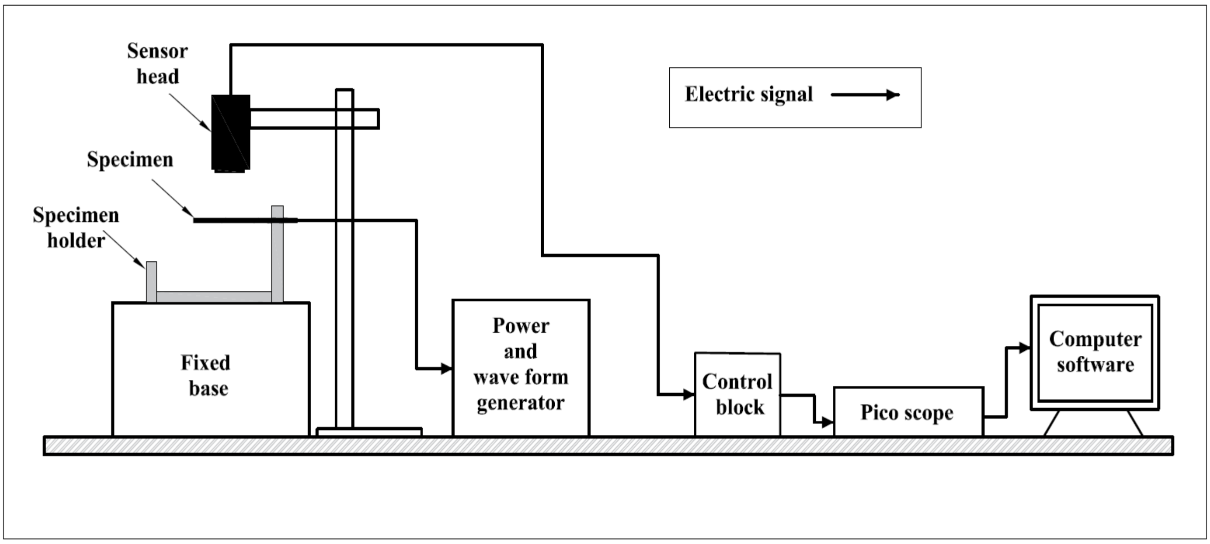


Figure 21. Experimental scheme for response characteristics measurements of multilayer piezoelectric element

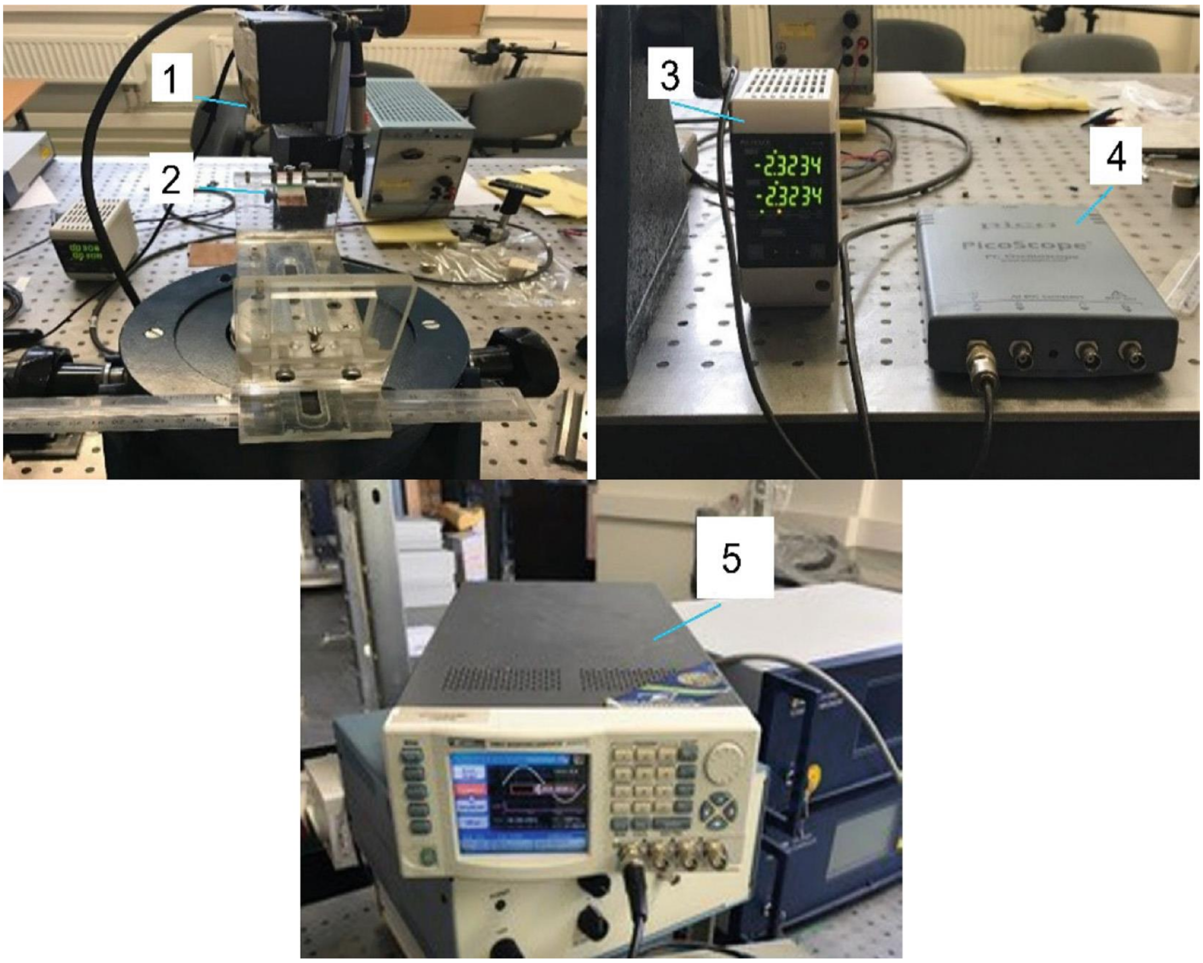


Figure 22. Experimental setup for response characteristic measurements of multilayer piezoelectric element consisting of (1) sensor head LK-G82, (2) horizontally clamped specimen, (3) control block LK-G3001 PV, (4) Pico Scope oscilloscope, (5) power and a waveform generator

As shown in Figure 22 The multi-layer sample of PZT is clamped firmly using custom fixture over the clamped length. The sample is displaced to a certain degree and left to vibrate freely. The triangulation position sensor LKG82 records the logarithmic decrement with the accuracy of 0.2mm. The data is recorded using Pico data acquisition system. The recorded data is plotted against elapsed time and studied to determine natural and damped frequency response.

- **Theoretical calculation**

In order to compare the experimental and theoretical models, the natural frequency of proposed multilayer specimen had to be calculated by the following formulae 2.1 - 2.3. Logarithmic decrement δ from experimental data for n observed periods was calculated according to the formula:

$$\delta = \frac{1}{n}\ln\frac{x(t)}{x(t+nT)} \qquad 2.1$$

where, x(t) – amplitude at time t and x(t + nT) – amplitude at time t + nT (after n periods). Then damping ration ξ was calculated from formula:

$$\xi = \frac{1}{1+(\frac{2\pi}{\delta})^2} \qquad 2.2$$

Finally, the natural frequency wn of the multilayer structure was calculated according to formula:

$$wd = wn\sqrt{1-\xi^2} \qquad 2.3$$

Where wd the damped natural frequency calculated from experimental data.

2.4. Contact angle measurement

Contact angle measurement setup was designed independently by myself. The experiment was performed in Institute of mechatronics and Department of mechanical engineering, Kaunas University of Technology. Purpose of this experiment was to understand the hydrophobic properties of different PZT composite material because liquid behavior differs on the various surface, so the easiest way to determine the behavior of the liquid to the solid surface was done by contact angle measurement. There are various types of methods are used in previous research. There are several instruments developed on industrial basis also available in the market. But the purpose of this experiment is to develop an inexpensive method which can be used for research laboratory and research purpose in order to identify hydrophobic properties of the liquid to the solid surface. In this experiment manual method using digital image analysis software has been applied. There is a range of image analysis programs available for example, Corel Draw, Adobe Photoshop and so on. In this experiment freely available image analysis software platform ImageJ used for its ever-expanding flexibility. This software has three plugins to measure contact angle includes contact angle analysis, low bond axisymmetric drop shape analysis (LB-ADSA) and DropSnake.

- **Specimen preparation**

For the contact angle measurement specimen was prepared according to using the same screen printing technique presented in section 2.1. For the experimental measurement of contact angle, three different PZT composite was produced with PZT nanopowder and three different binding polymers polyvinyl butyral (PVB), polystyrene (PS) and polymethyl methacrylate (PMMA). Properties and density of composite materials were presented in Table 9-10. Moreover, to observe the influence of different base materials on the contact angle two different types of base material was used. two base materials were Aluminum natural sheet and Polyethylene terephthalate (PET). Also, the thickness of coating for PZT binding polymer was different for each base materials in order to examine the influence of the different thickness of the coating on the contact angle measurement. Thus, different base materials are also used for the same purpose for observation of the different base material influence on the measurement. Four different liquids were used in the experiment in order to determine the hydrophobic properties of the surface with different liquids. As a liquid Distilled water, Spirit, glycerin, and Olive oil were used. The properties of the liquid materials are presented in Table. 16.

The bottom layer of the multilayer structure was aluminum sheet and Polyethylene terephthalate (PET). The properties of the base material are presented in Table14-15.

Table 14. Properties of Polyethylene terephthalate (PET)

Property	**Value**
Young’s Modulus	2800 - 3100 MPa
Tensile strength	55 - 75 MPa
Glass transition temperature	61 – 81 °C
Softening Temperature	82 °C
Density	1.370 g/cm^3

The properties of Aluminum are presented in Table 15.

Table 15. Properties of the aluminum sheet

Property	**Value**
Young’s Modulus	70 GPa
Shear modulus	26 GPa
Bulk Modulus	76 GPa
Poison’s ratio	0.35
Density	2.70 g/cm^3

Table 16. Properties of liquid material

Sr. no.	Liquids	Surface tension, dyne/cm	Density, Kg / m^3
1	Water	72.8	997
2	Glycerin	64.2	1260
3	Spirit	26.02	793
4	Olive oil	34.76	888.89

- **Multilayer structure**

The multilayer structure was prepared for contact angle measurement. The multilayer specimens are made with two different base materials and with different thickness of the PZT polymer coating with the base material of aluminum and Polyethylene terephthalate (PET). The size of the specimens is 65 X 75 mm and 30 X 18 mm for aluminum and Polyethylene terephthalate (PET) base materials respectively. The geometrical dimensions of the specimens are presented in Table 17.

Table 17. Specimen dimension

Specimen	Base material	
	Aluminum, Coating thickness (Total), µm	PET, Coating thickness (Total), µm
PZT + PVB	20 (310)	50 (140)
PZT + PS	20 (310)	50 (140)
PZT + PMMA	20 (310)	50 (140)

Specimens which used for contact angle measurement are presented in Figure below.

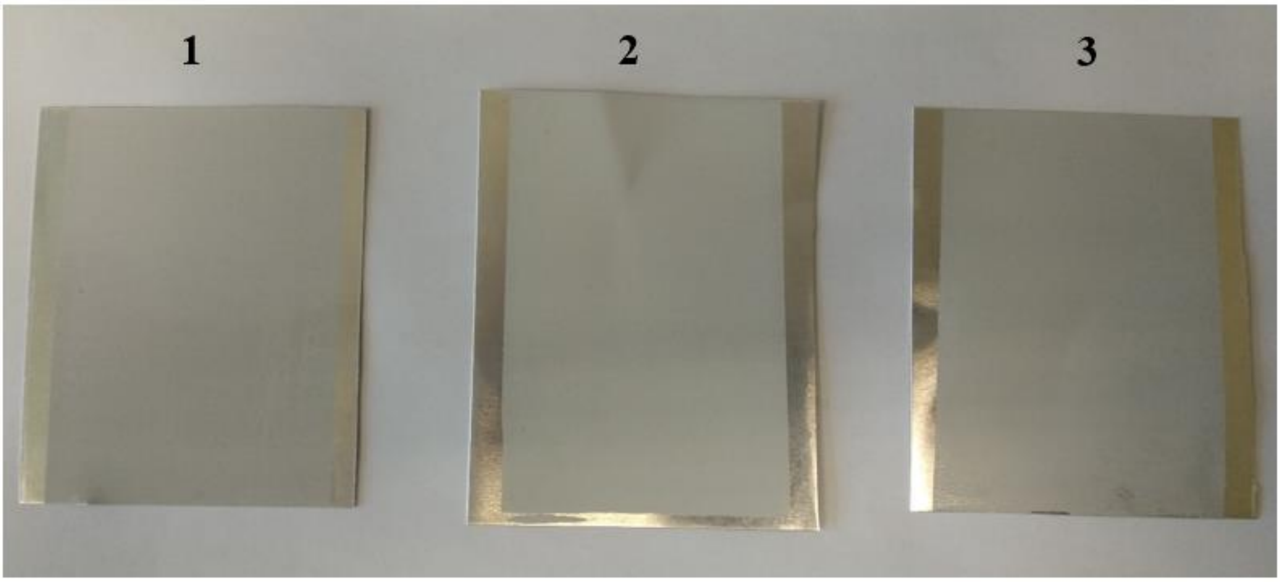


Figure 23. Specimen with aluminum base material

Specimen prepared with aluminum base material and PZT + 20 % polymer material are presented in Figure 23. Specimen 1 is prepared with composition of PZT nanopowder and polymers polyvinyl butyral (PVB) polymer binding material, specimen 2 with polymethyl methacrylate (PMMA), and specimen 3 with polystyrene (PS). The thickness of the composite layer is 20 µm and total thickness is 310 µm for all three for all specimen.

Also, Specimen prepared with Polyethylene terephthalate (PET) base material and PZT + 20 % polymer material are presented in Figure 24. Specimen 1 is prepared with composition of PZT nanopowder and polymers polyvinyl butyral (PVB) polymer binding material, specimen 2 with polymethyl methacrylate (PMMA), and specimen 3 with polystyrene (PS). The thickness of the composite layer is 50 µm and total thickness is 140 µm for all three for all specimen.

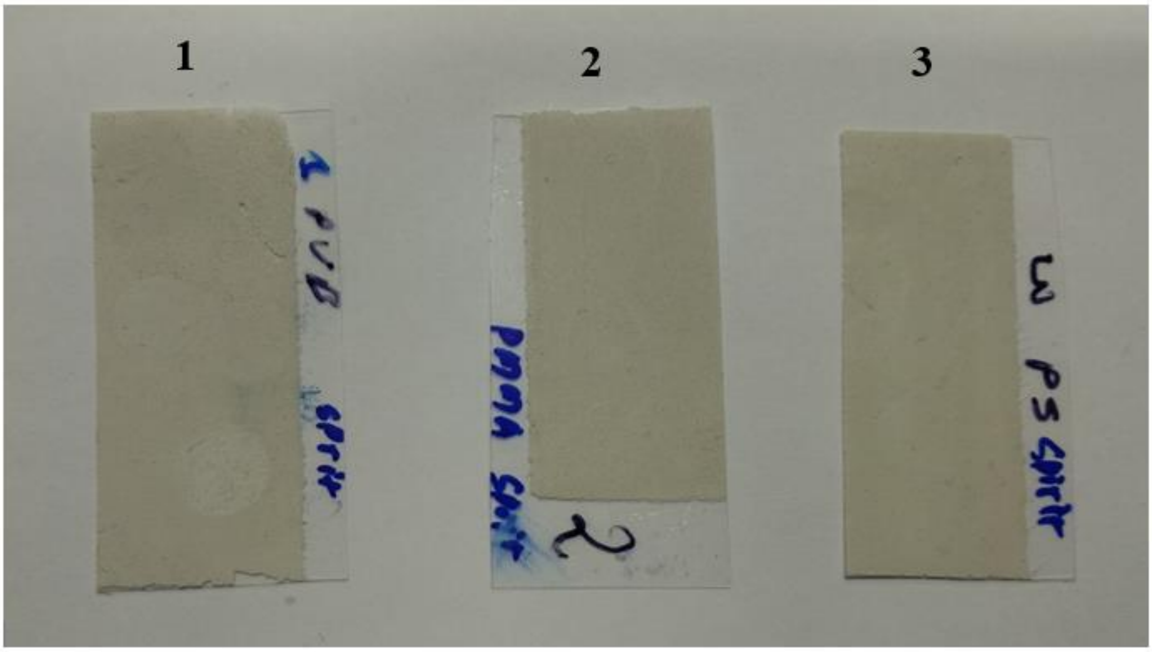


Figure 24. Specimen with Polyethylene terephthalate (PET) base material

2.5. Experimental setup for contact angle measurement

For the investigation of contact angle, a testing protocol has been designed and developed for the direct measurement of contact angle on three different specimens with different thickness and binding polymers. The three different types of solid surface composition with PZT Nanomaterial with Polyvinyl butyral (PVB), Poly (methyl methacrylate) (PMMA) and Polystyrene (PS). Also, all measurements were done with high purity probe liquids such as Distilled water, Glycerin, spirit and Olive oil.

Figure 25. Glass pipet class AS

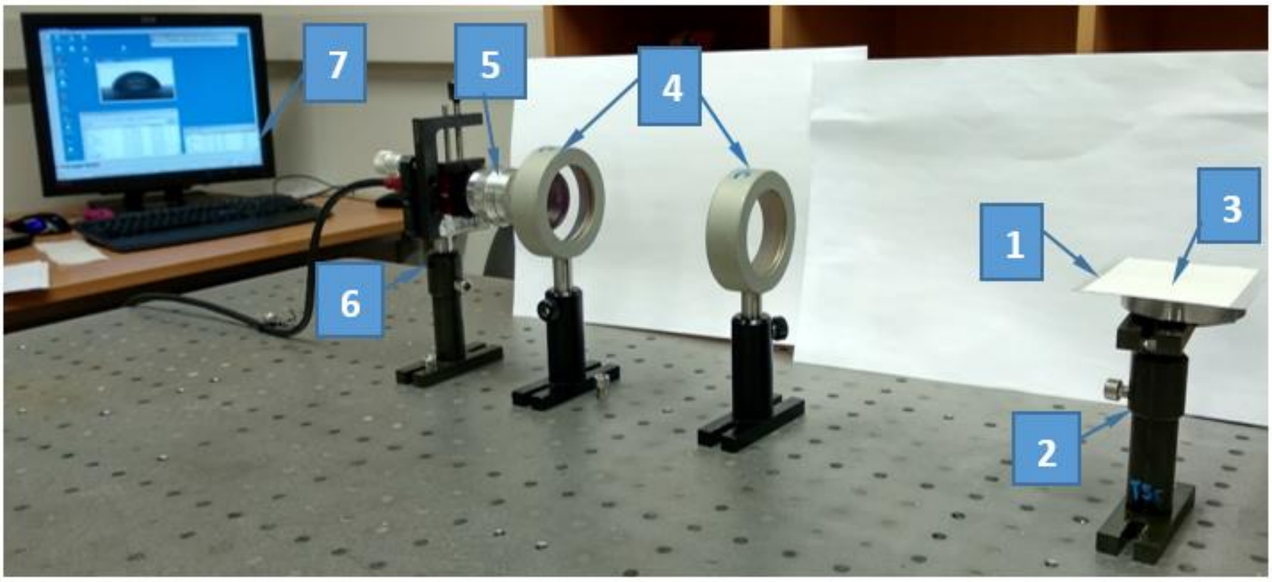


Figure 26. Experimental setup for contact angle measurement

Figure 26 shows the experimental setup for contact angle measurements. Experimental setup for Contact angle measurement for hydrophobic material consist of :(1) Specimen, (2) adjustable Specimen holder, (3) Drop on Specimen, (4) Double convex lenses, (5) Guppy F-503 B&W CMOS Camera, (6) Adjustable Camera Holder, (7) Computer system with live image of drop with image processing software to analyze captured image. The optical parts consist of camera (Guppy F-503 B&W CMOS camera specification of camera: pixel size: 5,038,848, Resolution: 2592(h) 1944 (v), sensor size: ½ Inch, Sensor type: CMOS, Connection: 1394A, Speed: 7.5 Frames/sec with 2592 X 1944 resolution and 60 frames/sec with 640X480 resolution) and optical lenses with a focal length of 600mm (holographic and optical measurement laboratory, double convex lenses with 50mm Diameter) that is placed between the camera and sample Figure 26. Pipet used for dispensing drop for different liquids is shown in Figure 25. Pipet capacity is 5 ml. each subdivision is divided into 0.1 ml with blue color code scale. Permitted drawing error for pipet is ± 0.030 ml.

The practical was performed in an ambient light source in dark laboratory room and lights setting are arranged for making the liquid drop to appear black, which is necessary for measurement accuracy as well as for image analysis. A system of measurement shown in Figure 28 the illustration of full system and equipment arrangement. Light conditions are fully arranged to avoid any reflection of light that spoils the measurement. Also, precautions were taken to prevent the drops polluted by air impurities such as dust and particles.

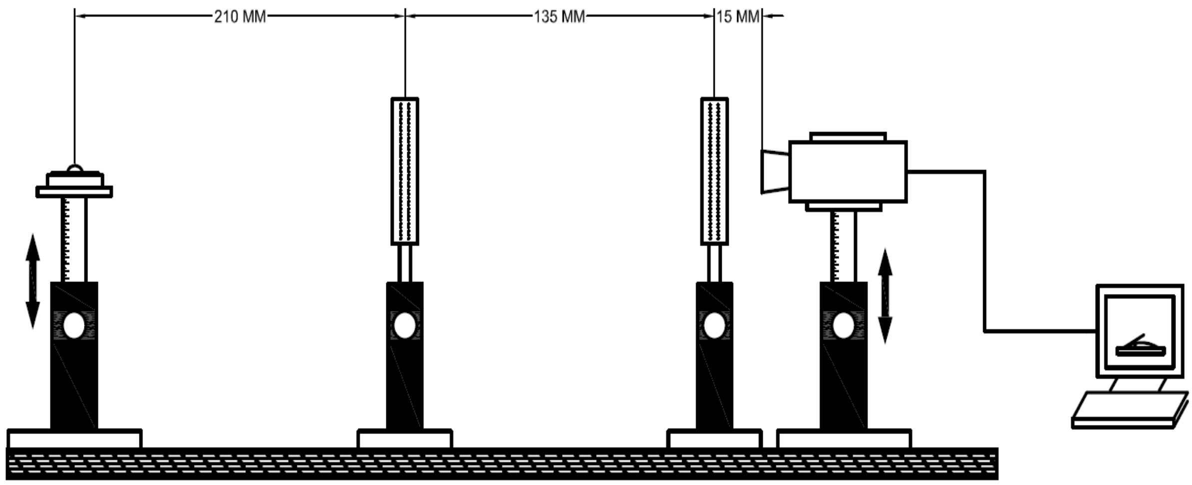


Figure 27. Illustration of critical distance between important parts. (camera, lens, and specimen with a drop)

Figure 27 illustrates the critical distance which is very important during the experiment to maintain the stability of image quality to obtain accurate results. All parts are placed on the stable surface table for the accurate measurement of contact angle. Thus, there are two optical lenses placed between camera and specimen for exact focus on the drop. Also, lenses are kept stable and fixed. camera fixed on the adjustable camera holder for adjusting height respect to the drop. While the specimen fixed on the adjustable specimen holder for the stable focus on the drop and to adjust the height of the specimen for excellent focused and good quality image for further measurements.

Firstly, before starting experiment testing of pipet and drop were held. For checking the drop sample from pipet was release on a normal surface. Then pipet was cleaned with water and wiped with the help of tissue. Then, the height of the specimen holder was adjusted according to parallel camera vision for the accurate position of drop image. Then drop of distilled water diffused from the pipet to the PVB surface from the height 15 mm and drop size is 20 µl. height for diffusing drop from pipet and quantity of diffused drop was kept constant for each drop of measurement and for all three solid surface and all different liquids. Then after few seconds of stabilization drop image was captured by camera application. The software used for image processing was ImageJ plugin DropSnake plugins and Contact angle measurement plugin freely available software provided by Wayne Rasband (retired from NIH). This software provides an easy interface for image processing and accurate result quality. This method is obtaining contact angle by using polynomial fit. In this method initially, the image should be in a 32bit format and grey scale so that can convertible into ImageJ software before start Drop snake plugin. A sample of the captured image is presented in Figure 28.

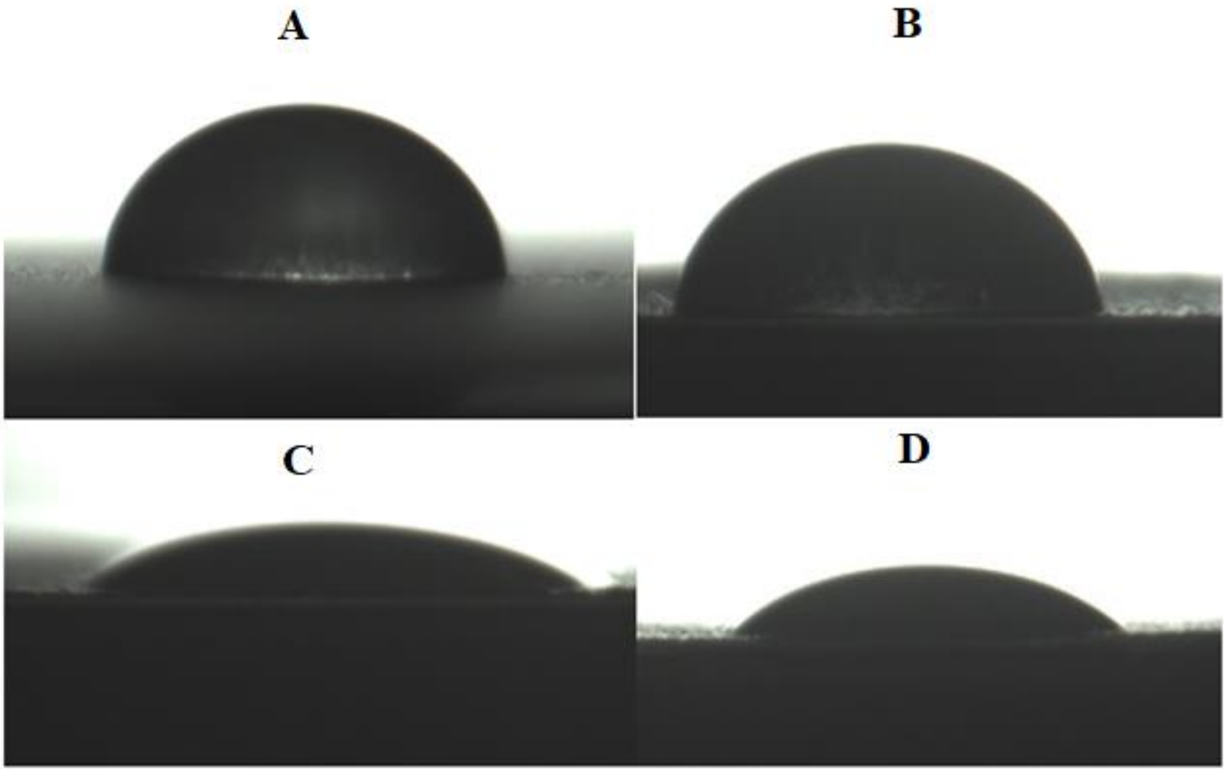


Figure 28. Illustration of image sample taken for measurement

Figure 28 illustrates the four different liquid drops dispensed on Polyvinyl butyral (PVB) surface with same consideration of releasing height (15 mm) and drop quantity (20 µl). A) shows the water drop B) Glycerin Drop, C) olive oil drop, and D) Spirit drop on PVB surface. Pictures were taken right after the drop formation it means the few seconds after drop dispensed from the pipet.

Then after by using ImageJ software and DropSnake plugin. Start to put 7 knots from left lower end to right lower end along the profile of drop should cover inside all knots. Then click twice on the image that will demonstrate the rough estimated value of angle left hand and right-hand side. Then adjust the knots to achieve exact profile then click on the fast snake button that will also show the red profile of drop and angles if its appear exact to the drop profile then click on the green play button for redefining the drop profile and accept it. It will indicate exact value of the left and right-hand values as well as the profile of drop as shown in Figure 29.

The DropSnake plugin with important tab tools is shown in the top of Figure 29. The contact edges are shown in the upper-left part of the picture (85°) and in the "final profile" result window. The underlying (blue) and refined (red) snakes drawn by the DropSnake plugins appear on the picture.

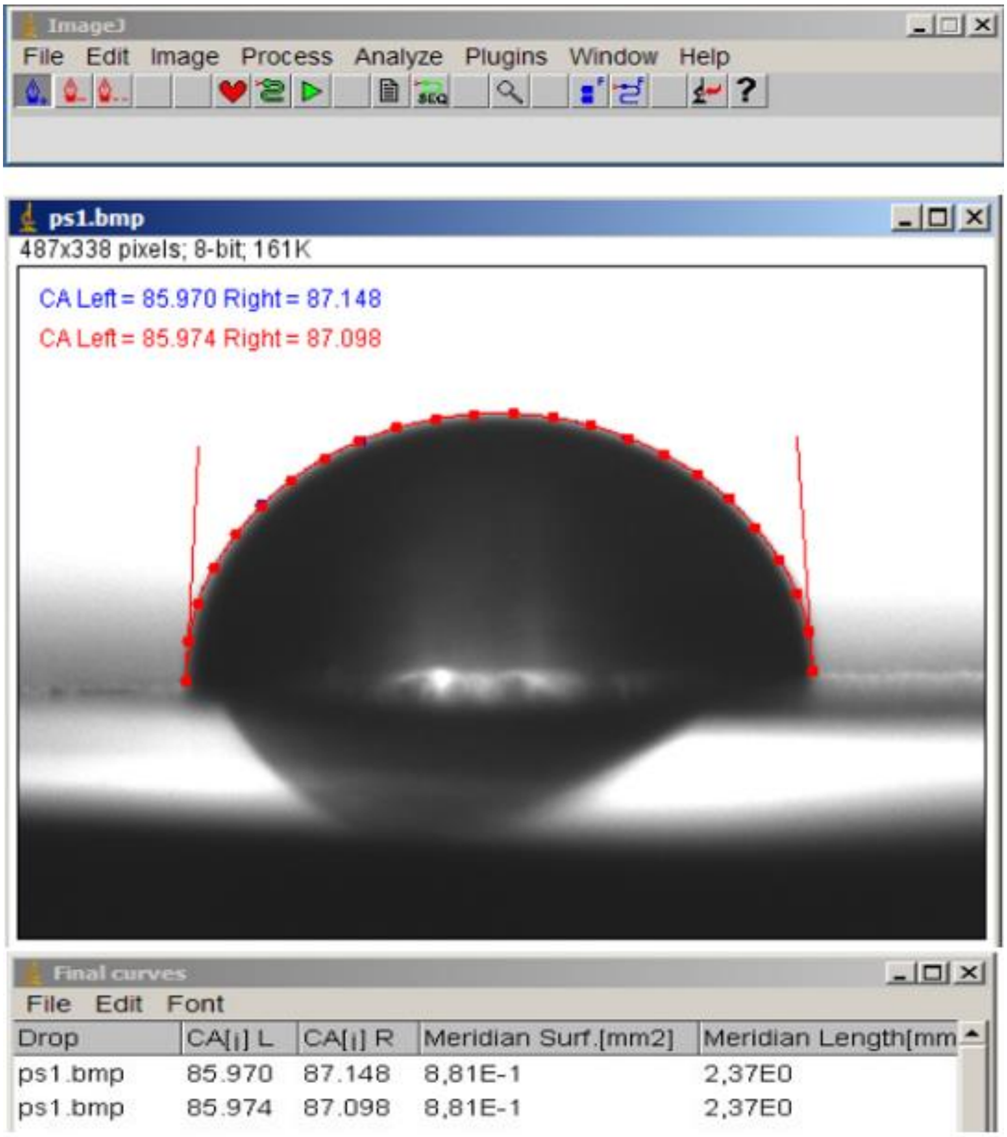


Figure 29. DropSnake plugin tool interface

Calculation of confidence level interval for error in measurement has been done using Formula 2.4. The value of Z is taken from normal distribution 1.96 because measured values are lies within the range of standard deviation of the mean.

$$Confidence\ interval = \bar{x} \pm Z \frac{s}{\sqrt{n}} \qquad 2.4$$

Where, $\bar{x}$ - average of measurement, Z = 1.960 constant for accuracy level 95%, s- standard deviation and n - number of measurement.

3. Results and discussion

Dynamic investigation of PZT composite material and hydrophobic properties measurement was done using the hybrid experimental method and contact angle measurement method respectively. The sample has been prepared for both the experiment according to PZT composite synthesis as described in chapter 2. For dynamic investigation of PZT composite material samples was prepared with copper base material with different thickness presented in Table 13. Also for contact angle measurement samples was prepared with two different base material and thickness of the coating was also different for both the base material (aluminum and PET). For both the experiment PVB, PMMA, and PS was used as the binding polymer.

3.1. Dynamic investigation of the multilayer structure

Pulse excitation response for PVB binding material microstructure membrane has been measured using experimental setup explained in chapter 2 and presented in Figure 30.

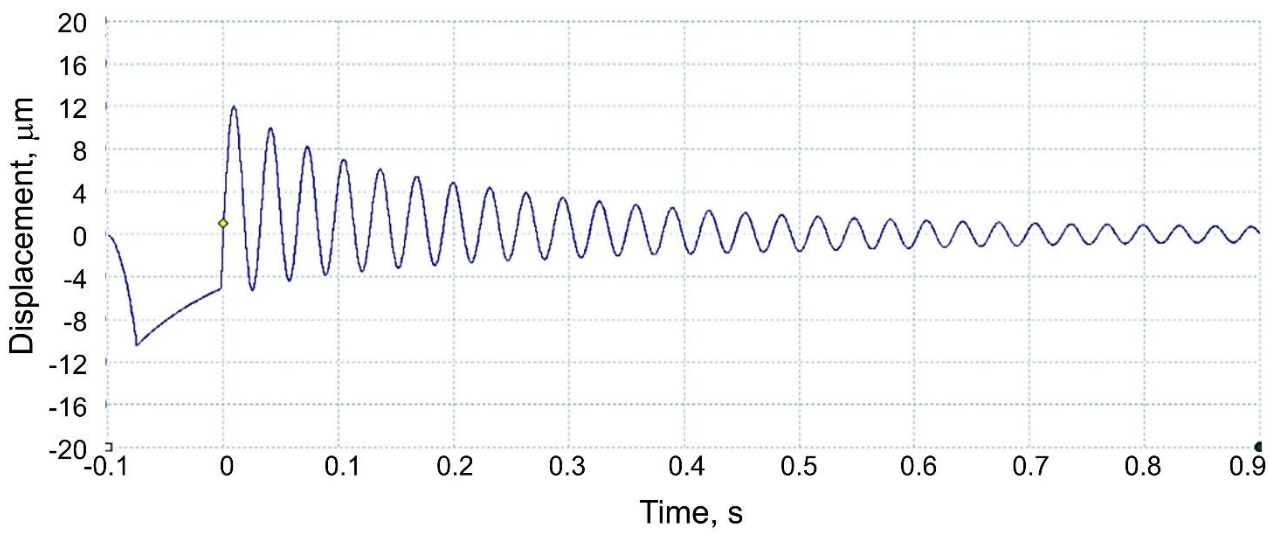


Figure 30. Graphical representation of the logarithmic decrement of the specimen with PVB binding material

Natural frequency has been measured for PVB microstructure membrane using formulas 2.1-2.3 and obtained value of frequency was 31.5 Hz. The obtained value of frequency has been used for numerical simulation by using Comsol Multiphysics 5.0 software to measure Young's modulus. So from the numerical simulation frequency were determined 32.1 Hz for multilayer structure with PVB binding material presented in Figure 31. When Young's modulus is 3.9 GPa. According to numerical simulation and calculated frequency, it shows difference only 1.5%. so it could be expressed that Young's modulus of PZT + PVB composite material is 3.9 GPa.

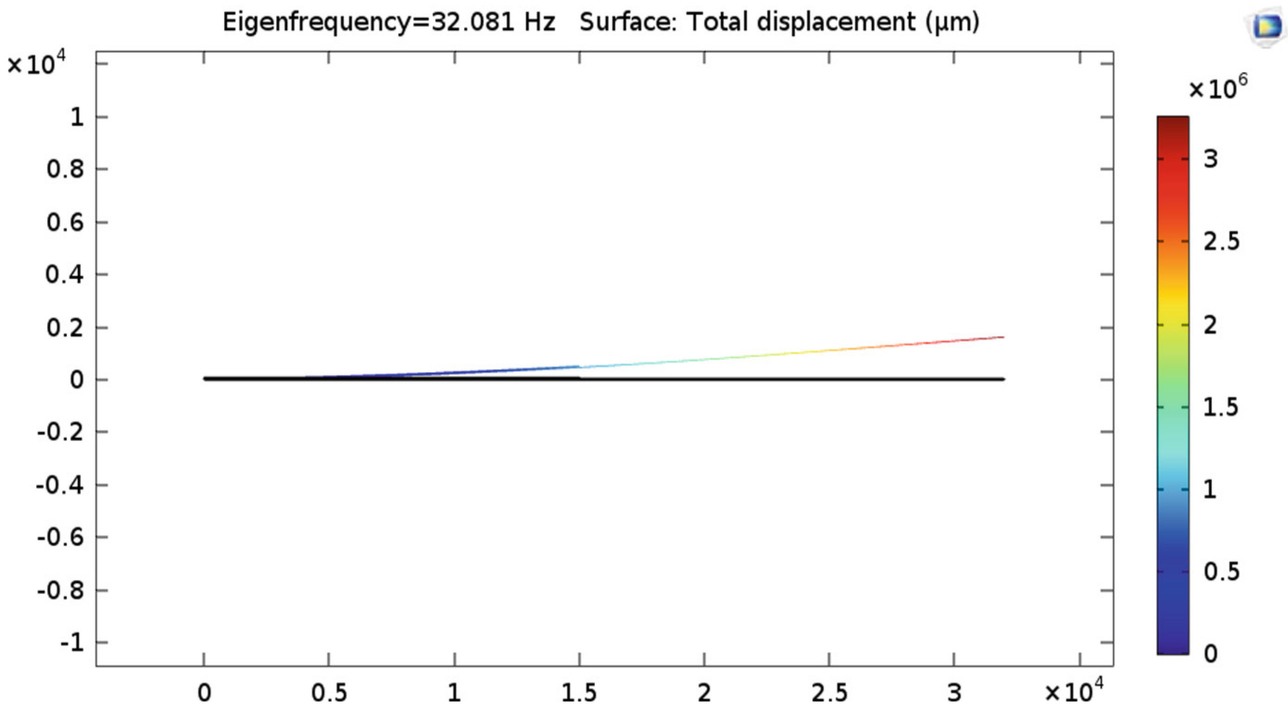


Figure 31. The resonant frequency of the specimen with PVB binding material

Similar way, pulse excitation response for PS binding microstructure membrane has been measured and presented in Figure 32.

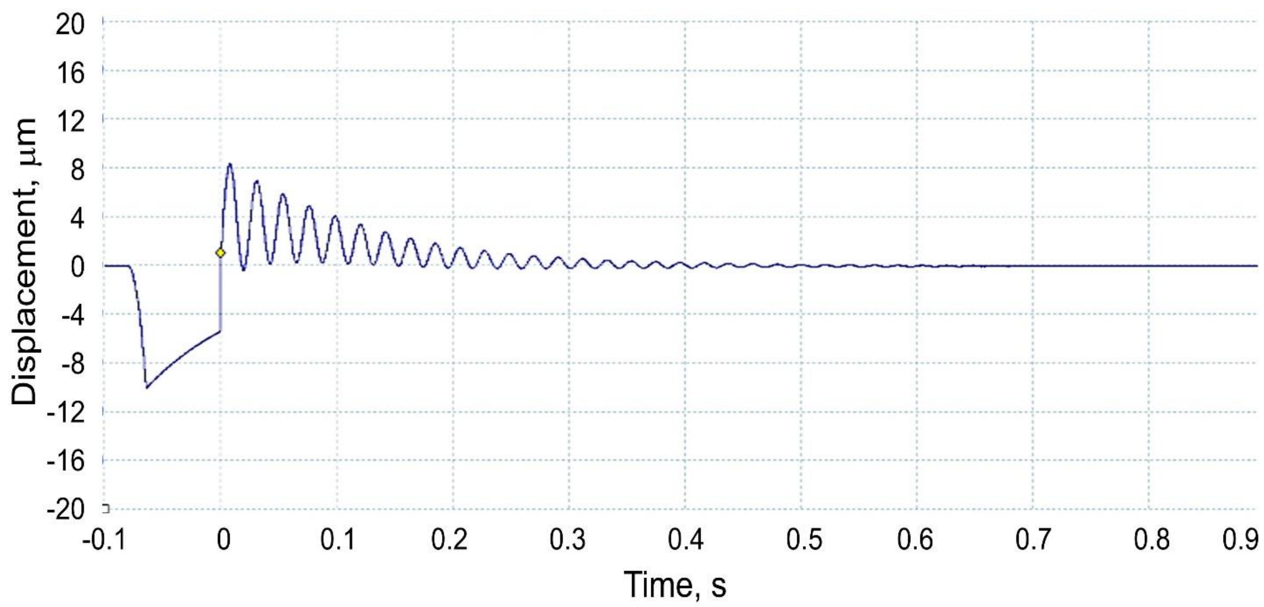


Figure 32. Graphical representation of the logarithmic decrement of the specimen with PS binding material

Similarly, the frequency was calculated using formulas 2.1 – 2.3. the measured experimental frequency was 43.15 Hz. Also, numerical simulation has been done using simulation software to verify the value of frequency obtained from microstructure membrane. Obtain value from the numerical simulation were 43.02 Hz presented in Figure 33. It shows almost 0.3% of accuracy level. This value of frequency has been obtained when Young’s modulus was 5.3GPa. so it is correct to say Young’s modulus of PZT + PS composite material is 5.3 GPa.

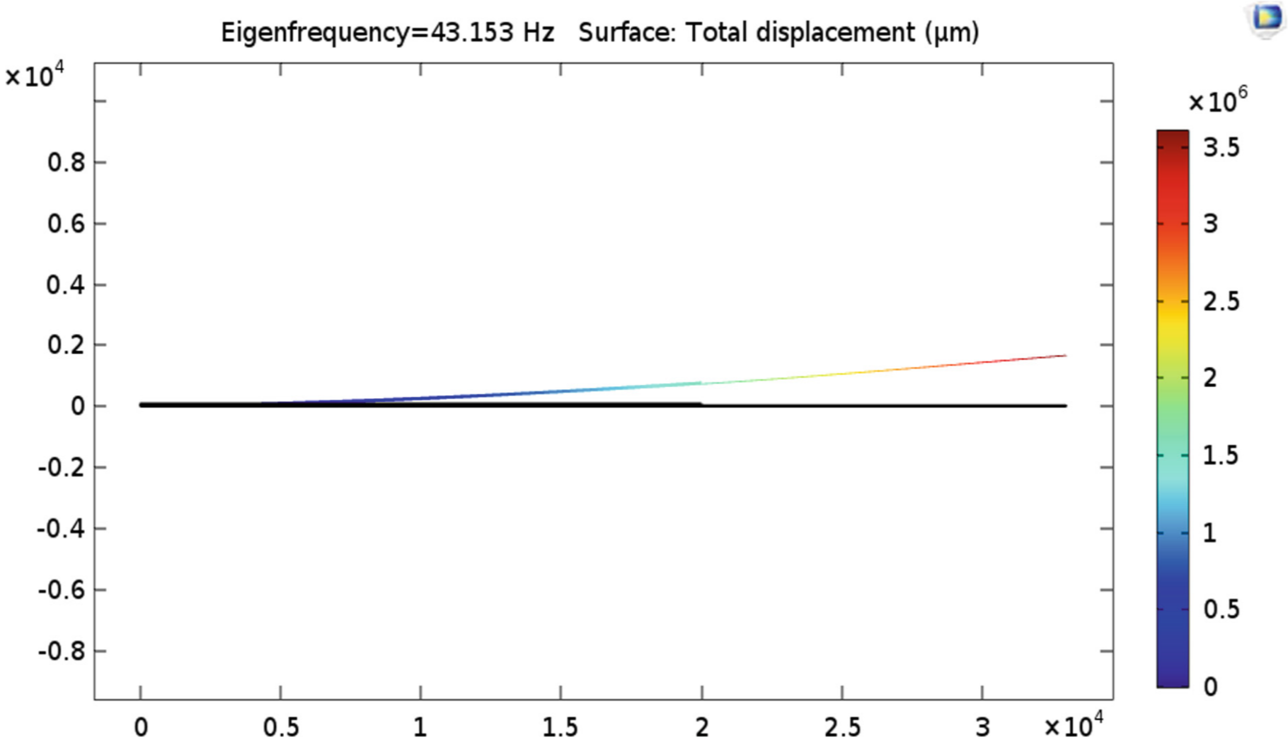


Figure 33. The resonant frequency of the specimen with PS binding material

For PMMA follows the same procedure and PZT + PMMA has shown the highest value of the amongst all three microstructure membrane. Calculation of natural frequency also has been done using formula and it was also compared with numerical simulation. The value of Young's modulus for PMMA is 6.3 GPa. The experimental result is shown in Figure 34.

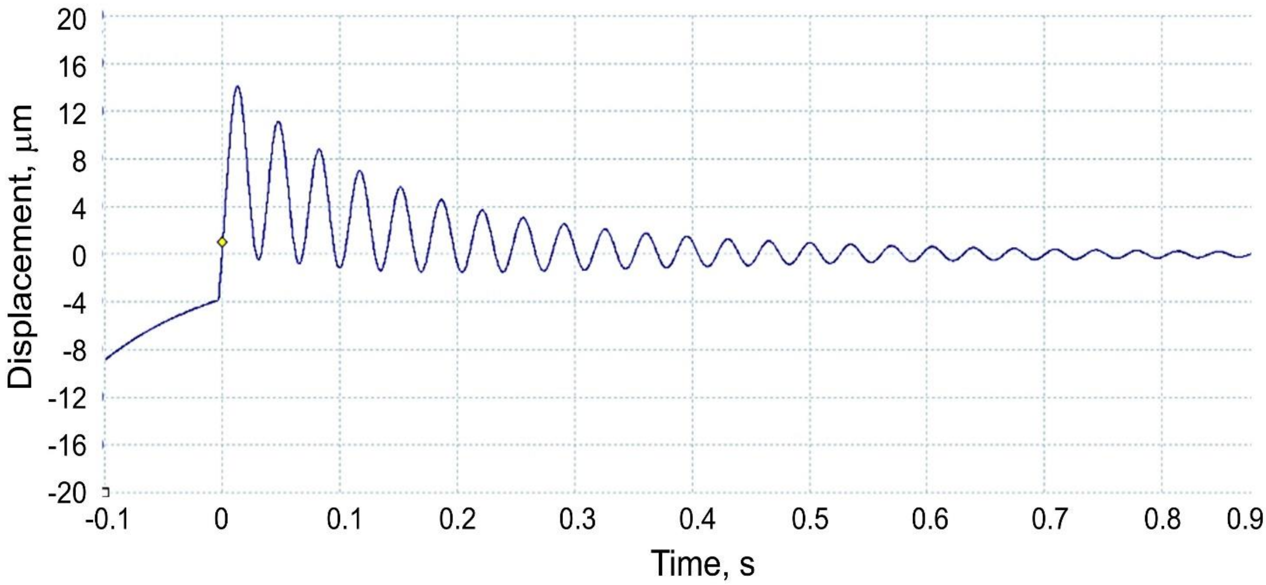


Figure 34. Graphical representation of the logarithmic decrement of the specimen with PMMA binding material

Theoretical natural frequency was obtained 46.99 Hz. hence, a frequency obtained from numerical simulation was 46.17 Hz it is presented in Figure 35. it shows only 1.8% difference.

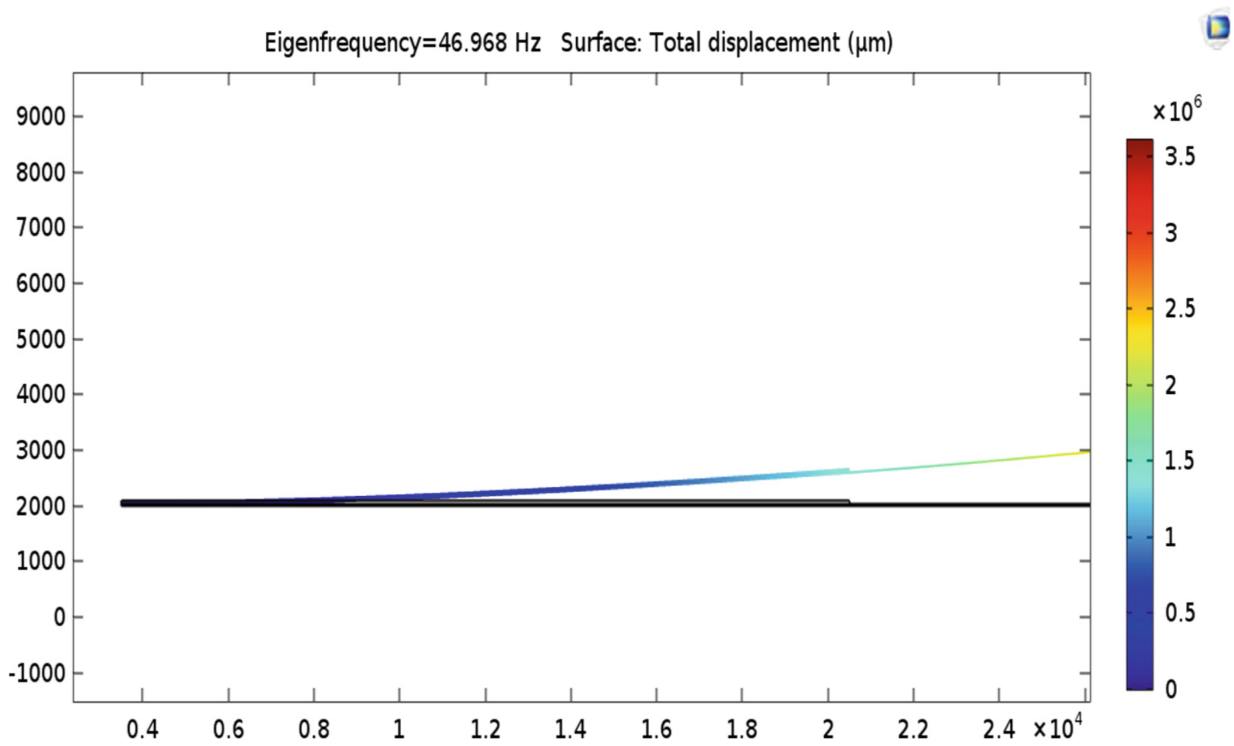


Figure 35. The resonant frequency of the specimen with PMMA binding material

From the result of experiment and numerical simulation, it reflects the very less amount of difference so it could be stated Young's modulus for PZT + PMMA is 6.3 GPa. Also, results are presented in Table 18.

Table 18. Results for dynamic investigation

Specimen	**Experimental results, Natural frequency (Hz)**	**Numerical simulation, Natural frequency (Hz)**	**The difference in results (%)**	**Young's Modulus (GPa)**
PZT + PVB	31.5	32.1	1.5	3.9
PZT + PS	43.15	43.02	0.3	5.3
PZT + PMMA	46.99	46.17	1.8	6.3

From Table 18, Newly developed composite piezoelectric material incorporated to an actuator was proposed. Natural frequencies obtained from experimental results for composite material PVB, PS, and PMMA are 32.1 Hz, 43.15 Hz, and 46.99 Hz respectively. There was a very less difference between experimental and theoretical values (maximum deviation 1.8%) so Young's modulus for this material can be clearly expressed. Young's modulus for PZT mixed with PVB is 3.9 GPa, PS – 5.3 GPa, and PMMA – 6.3 GPa.

3.2. Contact angle measurement

The contact angle measurement has been done with the help of newly designed experimental setup with the image analysis software platform. The volume of dispensing drop (20 μl) kept constant from pipet for each liquid to the PZT composite material solid surface. Also, the height of dispensing drop was maintained (15 mm) for each measurement to maintain result exactness for each measurement.

For a first liquid sample of water tested on three different PZT polymer composite Polyvinyl butyral (PVB), Poly (methyl methacrylate) (PMMA) and Polystyrene (PS). Each sample of drop measurement on polymer was done using same parameter a and the images were analyzed several times. The mean value of Contact angle (θ) measured for water liquid on with binding polymers of PZT + PVB, PZT + PS, and PZT + PMMA were 80.71 ± 1.16°, 88.88 ± 1.31°, and 92.94 ± 1.56° respectively.

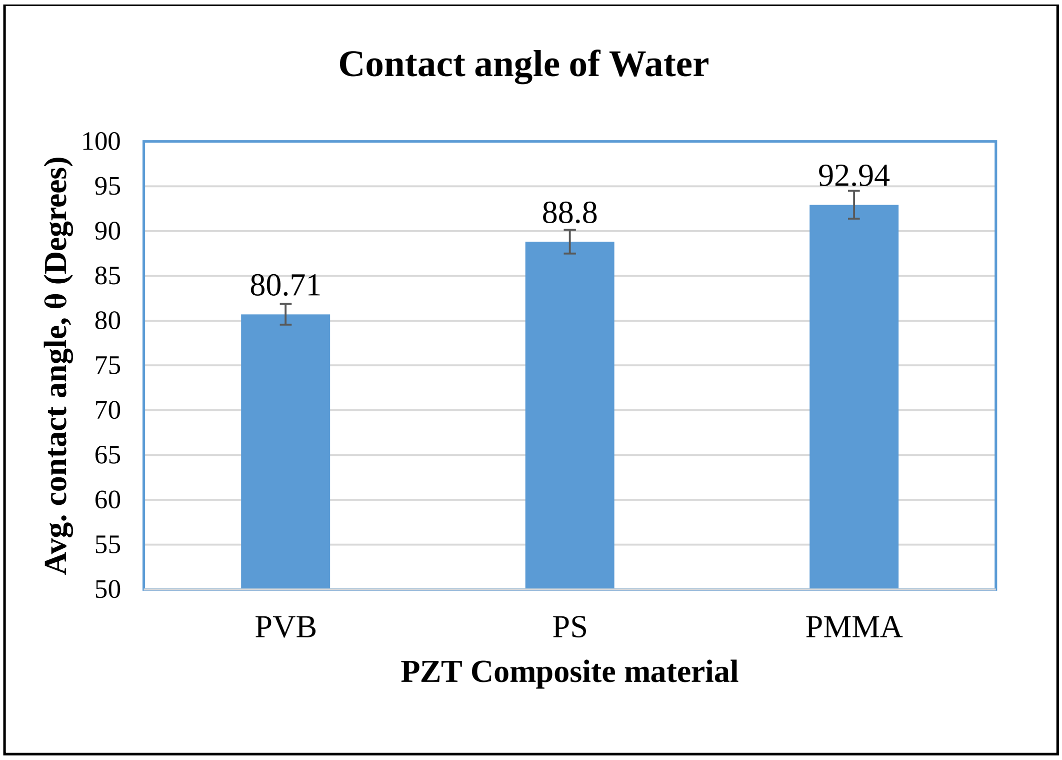


Figure 36. The contact angle of water on three different polymer surface

Graphical representation of measured contact angle (θ) values for different multilayer polymer specimens is shown in Figure 36. From the measured values of contact angle maximum angle was observed with PZT + PMMA is 92.94° with the error of ±1.56°. The statistical error for measurement has been calculated using formula 2.1. Also, the lowest contact angle was observed with PZT + PVB is 80.71° with measurement error ±1.16°. Measure value of contact angle for PZT + PS is 88.8° with an error of measurement ±1.31° it falls between measure value of PMMA and PVB. So according to the literature review, it could be stated that PMMA is almost fallen in the range of hydrophobic material with water. PVB and PS show contact angle less than 90° so it could be a hydrophilic surface with water.

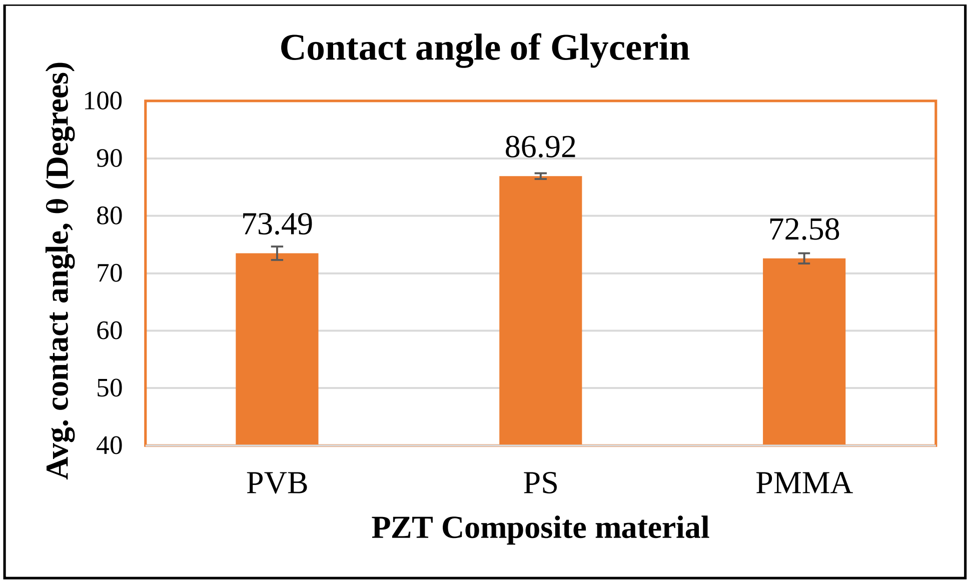


Figure 37. The contact angle of Glycerin on three different polymer surface

Similarly, the experiment was performed with glycerin on the same composite polymers binding surfaces PVB, PS, and PMMA. Graphical presentation of resulting contact angle is shown in Figure 37. The measured value of contact angle for PZT + PVB, PZT + PS and PZT + PMMA is 73.49 ± 1.19°, 86.90 ± 0.50°, and 72.49° ± 0.89° respectively. It shows the no significant measurement error between PVB and PMMA but there is a significant difference in statistical error between PS and PMMA, PVB. From the results, it is observed that amongst of three polymer binding material the highest contact angle value measured for PS (86.92°) and measured for PVB and PMMA is 73.49° and 72.58° respectively it is almost closest to each other. It shows the only difference of 1.24% in contact angle. From the measured value, it could be possible to say for glycerin none of any polymer shows contact angle more than 90° so it could be a hydrophilic surface for this type of liquid.

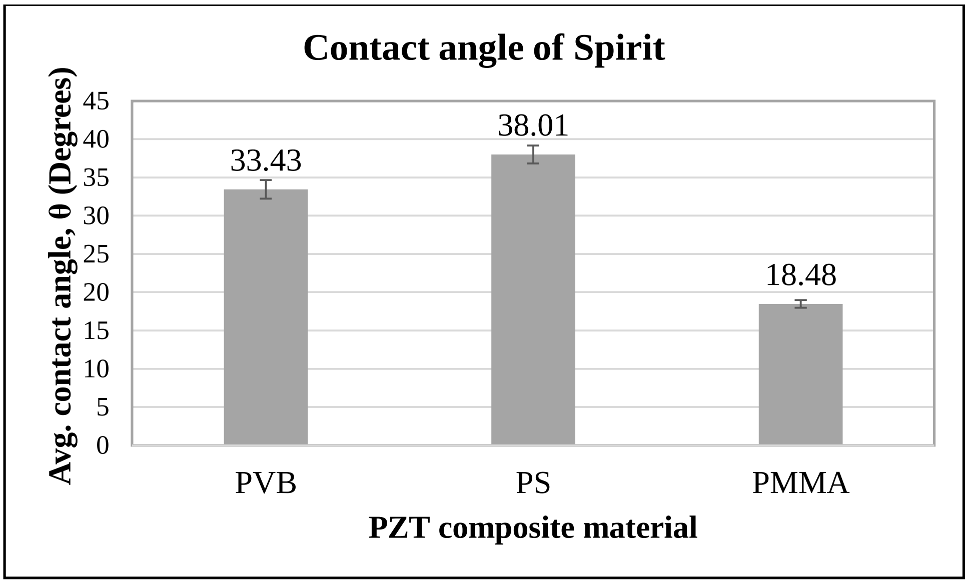


Figure 38. The contact angle of Spirit on three different polymer surface

The measured contact angle for the spirit drop on different polymer binding material is presented in Figure 38. The measured value of contact angle for PZT + PVB, PZT + PS, and PVB + PMMA are 33.43 ± 1.76°, 38.01 ± 1.89°, and 18.48 ± 0.5° respectively. It is significant statistical difference is observed. Measurement errors are calculated using formula 2.1. From the measured value of contact angle, it could be stated that all three polymer composite materials are hydrophilic with the spirit.

The measured contact angle for olive oil to different PZT polymer composite PVB, PS, and PMMA are presented in Figure 39. As seen in graph measured values of contact angle for PVB, PS, and PMMA are 23.18 ± 0.4°, 25.86 ± 1.14°, and 23.57 ± 0.7° respectively. There is no significant statistical difference between results.

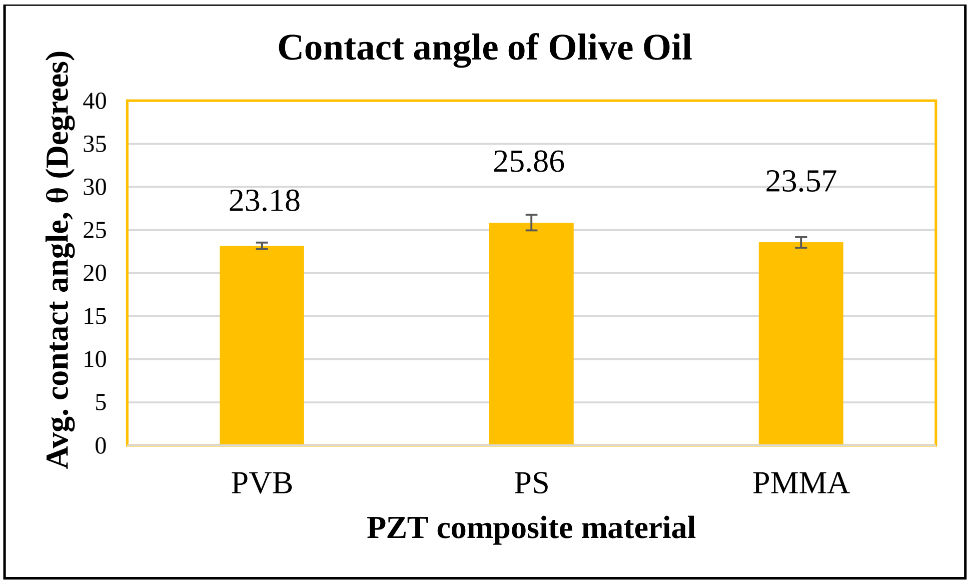


Figure 39. The contact angle of Olive oil on three different polymer surface

According to obtain values of measurement for PVB, PS, and PMMA composite materials it is found that the contact angle is less than 90°. So, it could be possible to state all of these polymer binding materials are belonging to the range of hydrophilic segment with olive oil.

The experiment has been performed on different PZT polymer composite material (PVB, PS, and PMMA) with different liquids (water, glycerin, spirit, and olive oil). Obtained results are separately presented in the form of the plot in figure 36-39. Comparison of the result of the measured contact angle of various liquid drops on different PZT composite solids surfaces is presented in Figure. 40. Mean value of measured contact angle with the statistical error in measurement for each composite material for each liquid presented in the form of a graph. Only PMMA shows the hydrophobic property with water. From the plot in Figure 40 contact angle for PMMA with Water (92.94°) is only more than 90°. All the other polymer composite materials show the contact angle less than 90° and lowest contact angle was found for olive oil (23.57°).

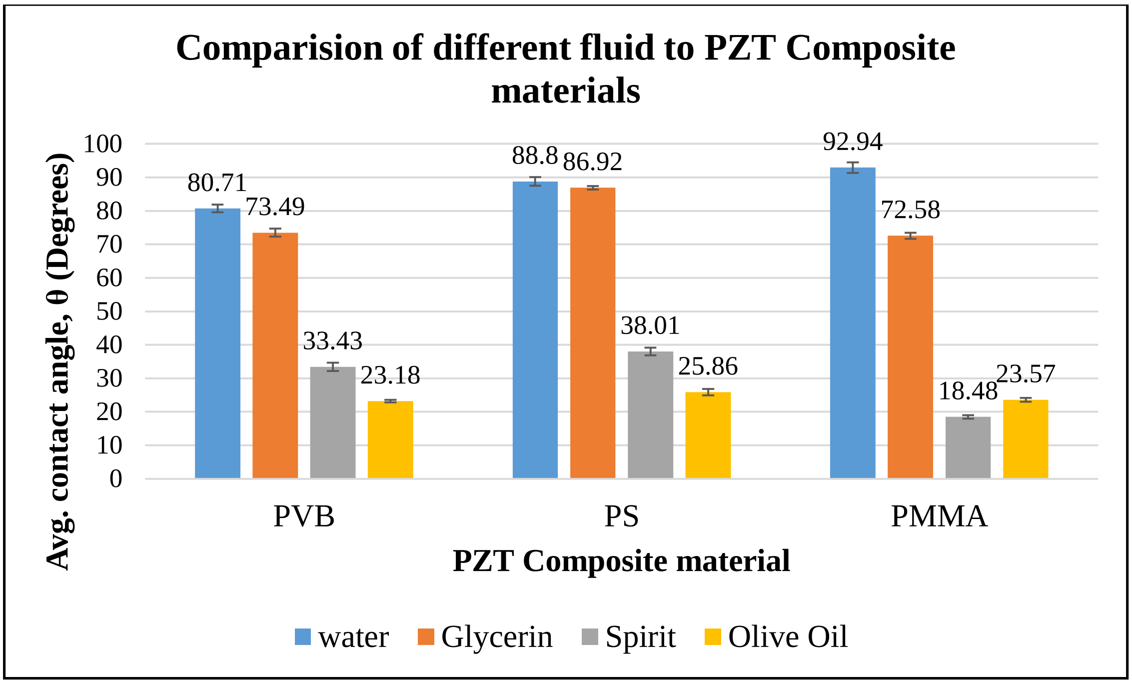


Figure 40. Contact angle Comparison of different fluid for three different PZT composite materials

Table 19. Contact angle illustration of different liquid for PVB, PS, and PMMA

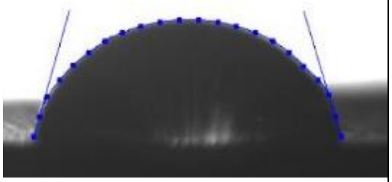
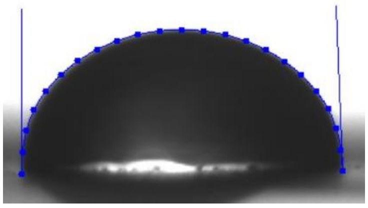
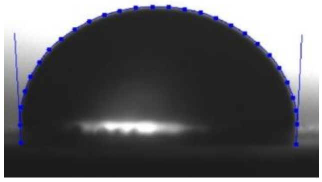
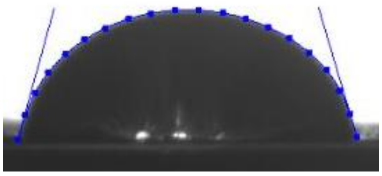
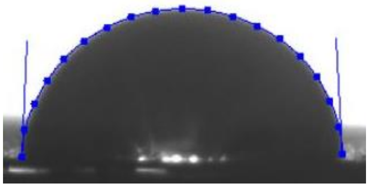
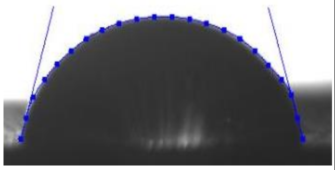
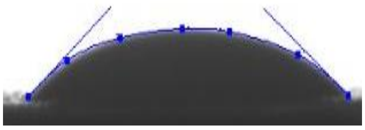
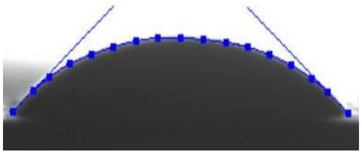
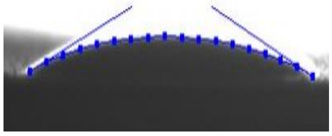
Liquid	**PZT composite binding materials**		
	PVB	**PS**	**PMMA**
Water	CA: L = 80.02 R= 80.06 	CA: L = 89.50 R= 88.38 	CA: L = 93.6 R= 92.3 
Glycerin	CA: L = 72.5 R= 72.8 	CA: L = 86.86 R= 87.13 	CA: L = 72.8 R= 71.7 
Spirit	CA: L = 37.7 R= 33.2 	CA: L = 38.7 R= 38.27 	CA: L = 18.5 R= 18.9 
Olive oil	CA: L = 23.2 R= 23.04 	CA: L = 24.78 R= 25.66 	CA: L = 24.81 R= 23.2 

Table 19, an illustration of measured contact angle by using image analysis software (ImageJ, DropSnake plugins) for different liquids (Distilled water, glycerin, spirit, and olive oil) on three different PZT binding polymer surfaces (PVB, PS, and PMMA). The idea of measurement for software is presented in chapter 2.

The result of all measurement is summarized in Table 20. To obtain reasonable precession for specific liquid on specific PZT polymer binding substrate image analysis performed many time for multiple drop images. The obtained mean value of the analyzed image is the contact angle for the specific liquid and specific solid surface. Several drop images were captured and analyzed and then average values of the measured contact angle, maximum value, minimum value, and standard deviation are presented.

Table 20. The measured contact angle for several test liquids

Liquid	**PZT Composite**	**min value, θ/deg.**	**Max value, θ/deg.**	**Mean value, θ/deg.**	**Standard deviation, θ/deg.**
WATER	PVB	78.98	82.68	80.01	± 2.22
	PS	85.97	90.70	88.80	± 2.12
	PMMA	91.60	95.25	92.94	± 2.99
GLYCERIN	PVB	72.82	75.20	73.49	± 1.92
	PS	85.66	87.83	86.92	± 0.7
	PMMA	71.06	73.85	72.52	± 1.44
SPIRIT	PVB	31.05	34.84	33.43	± 1.76
	PS	36.59	40.17	38.01	± 1.89
	PMMA	17.74	18.95	18.48	± 0.5
OLIVE OIL	PVB	22.45	23.88	23.18	± 0.4
	PS	24.79	26.97	25.86	± 1.14
	PMMA	22.59	24.43	23.57	± 0.7

As mentioned above in chapter 2, the experiment was performed with two different base materials (aluminum and PET). The coating thickness of PZT composite polymer binding (PVB, PS, and PMMA) is different for aluminum and PET. The coating thickness of composite polymer material on aluminum is 20 µm for PVB, PS, and PMMA. For PET base material thickness of the coating is 50 µm for PVB, PS, and PMMA. Then by using the same experimental setup and method contact angle was analyzed. Measured result for the aluminum base material is compared with PET base material is presented in Figure 41.

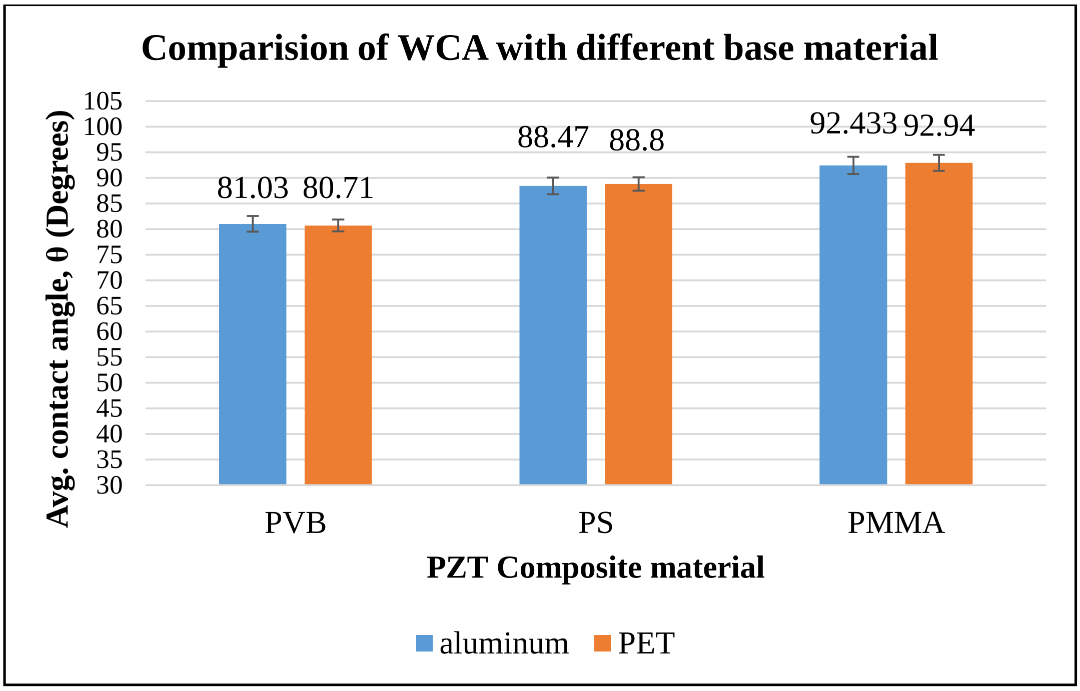


Figure 41. Comparison of WCA on Different base material

From the experiment results, it is observed that contact angle measured for a drop of water on PZT + PVB binding polymer with aluminum and PET base material is 81.03 ± 1.55° and 80.71 ± 1.65° respectively. It is only a difference of 0.4 %. For PZT + PS with aluminum and PET base material is 88.47 ± 1.62° and 88.8 ± 1.31° respectively. The difference in contact angle is just 0.37 %. PZT + PMMA has shown the highest contact angle among the three polymer binding materials. The observed values are 92.43 ± 1.67° and 92.94 ± 1.56° with aluminum and PET base material respectively. It is only a difference of 0.55 %.

Table 21. Measured data for different base material

Base material	**Aluminum**		**PET**		**WCA Difference between two base material, %**
PZT composite polymer	**WCA, θ°**	**Error**	**WCA, θ°**	**Error**	
PVB	80.03	± 1.55	80.71	± 1.16	0.4
PS	88.47	± 1.75	88.08	± 1.31	0.37
PMMA	92.43	± 1.67	92.94	± 1.56	0.55

According to measured values of water drop contact angle for two different base materials (aluminum and PET) with different polymer binding materials, it is observed the values of contact angle does not have more difference. So it could be possible to state that there is no influence of base material on the water contact angle measurement. As well as the thickness of PZT composite polymer coating was different for both base materials. Hence, WCA is also not depended on the thickness of the polymer binding specimen.

In order to understand the environmental effect (temperature, air/impurities) on the contact angle measurement time dependence study was performed for the liquid drop. It was done only with the distilled water drop sample. Images for a water drop on different PZT polymer composite material surface has been captured at a different time interval. The image was analyzed by using the same software and experimental platform. The evaluated contact angle for different time interval on the different surface is plotted in Figure 42.

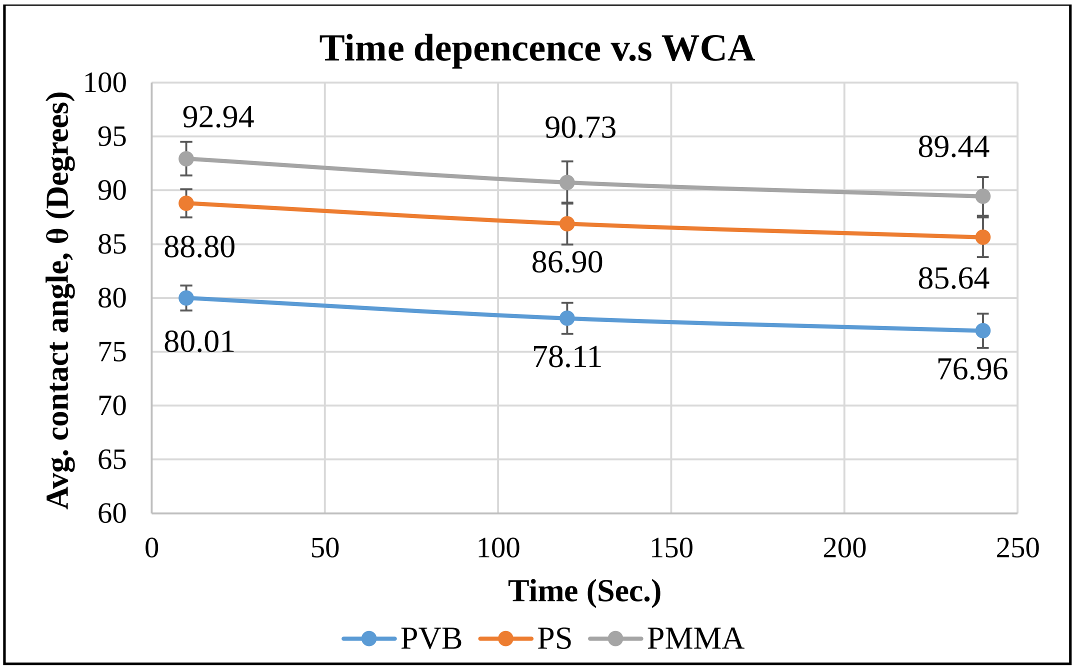


Figure 42. Time dependence for WCA

To observe the change in WCA images were captured for each drop sample on various polymer composite surface at a different time interval. Images were captured after 10 secs, 120 secs, and 240 secs for each and every drop sample. Then it was analyzed several time to obtain an accurate result of a change in the WCA. Initially, after 10 sec measured WCA of PZT +PVB is 80.01° then after 120 secs 1.9° change observed and it was gradually decreased to 76.96° after 240 secs. So it could be possible to state WCA of PVB has gradually decrement of 2° after every 120 secs. Moreover, a similar effect has been observed with PS binding polymer initially 88.80° observed then it decreased to 86.90° and finally, after 240 secs, it decreased to 85.64°. For PMMA binding polymer it was 92.94° at 10 secs it decreases to 90.73° after 120 secs and at last, after 240 secs it was observed at 89.44°.

So for all three polymers binding material, no significant change observed during first 120 secs but there is an observable difference in contact angle between initial and final measurement. The values of measurement are presented in Table 22.

Table 22 Measured values of WCA dependence on time

Time (Sec)	PVB, WCA (θ°)	PS, WCA (θ°)	PMMA, WCA (θ°)
10	80.01 ± 1.16	88.80 ± 1.31	92.94 ± 1.56
120	78.11 ± 1.44	86.90 ± 1.94	90.73 ± 1.96
240	76.96 ± 1.59	85.64 ± 1.84	89.44 ± 1.79

Also, Comparison of WCA (water contact angle) between ceramics and PZT polymer composite materials (PVB, PS, and PMMA) done using same experimental methods to identify the hydrophobicity property of PZT ceramic and PZT composite polymer solid surface. Obtained average values of the contact angle of water drop on PZT ceramic is 29.82 ± 0.43° and for PZT composite polymer are PVB – 80.01 ± 1.16°, PS – 88.80 ± 1.31°, and PMMA – 92.94 ± 1.56°. comparison of WCA for different solid surfaces are presented in Figure 43.

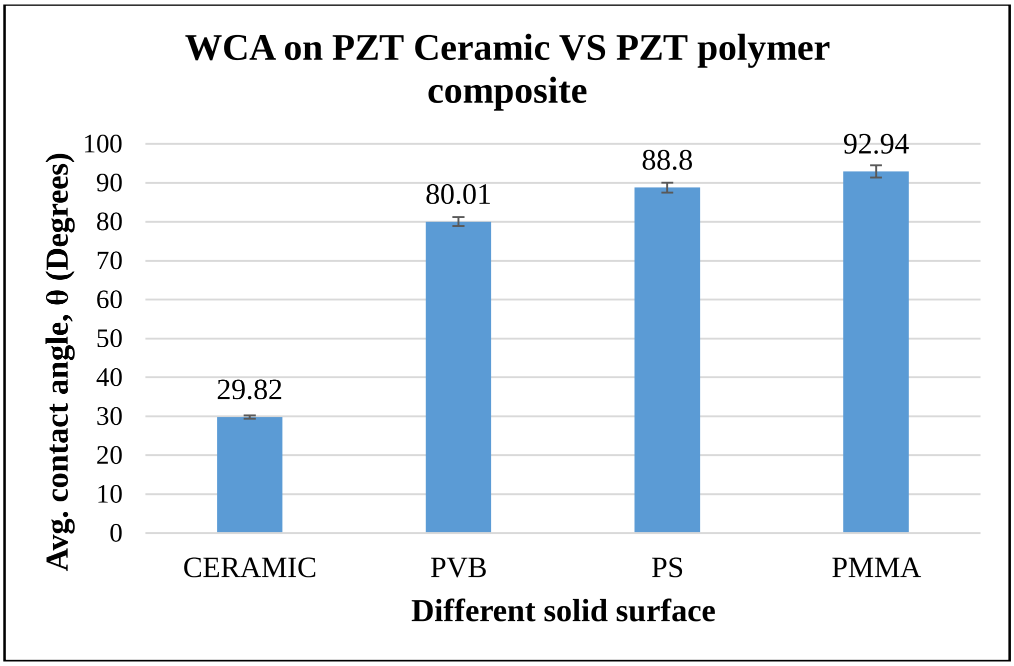
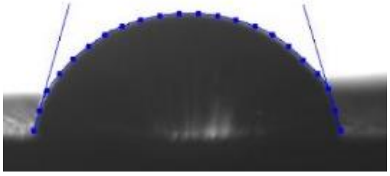
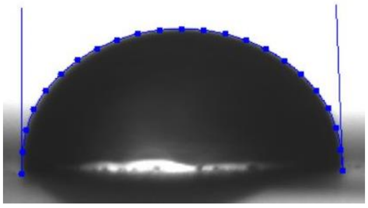
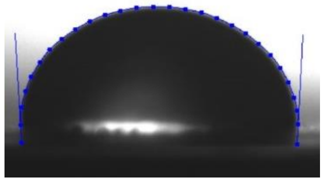
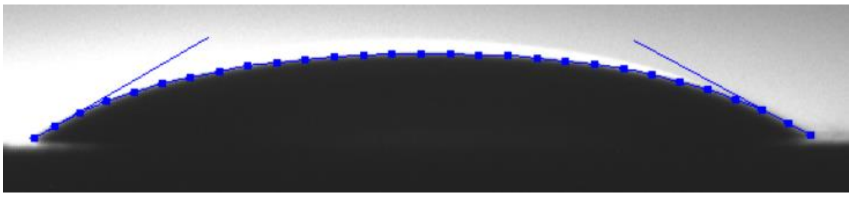


Figure 43. WCA on PZT ceramic VS PZT polymer composite

From the results, it is found that contact angle of water drop on PZT ceramic is (28.82 ± 0.43°) less than 90°. On the other hand, PZT composite materials show much higher contact angle for water drop almost between 80° to 93°. Moreover, amongst of three PZT composite PMMA shows highest contact angle value (92.94 ± 1.56°). So, from the results, it could be possible to state that PZT polymer composite material surfaces indicate more hydrophobic properties than PZT ceramic material. Captured images of WCA on the different solid surface are presented in Table 23.

Table 23. Water contact angle for PZT ceramic and PZT composite material

Liquid	PZT composite binding materials		
	PVB	PS	PMMA
Water	CA: L = 80.02 R= 80.06 	CA: L = 89.50 R= 88.38 	CA: L = 93.6 R= 92.3 
	PZT Ceramic		
	CA: L = 29.95 R= 28.45 		

3.3. Application of PZT composite material

To check the possible application of proposed PZT composite materials decided to prepare microchannel with controllable parameters using thermal reapplication process. Model of the microchannel is presented in Figure 44.

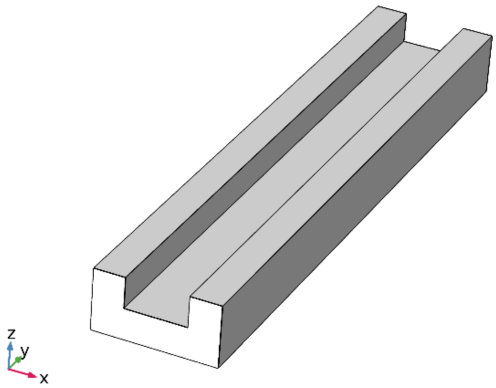


Figure 44. Microchannel model

Dimension of the microchannel are length (L) = 20 µm, period (P) = 4 µm, total thickness (T) = 2 µm, depth of the inner slot (d) = 1 µm, width of the slot (w) = 2 µm, and thickness of the two ridge (2r) = 2 µm as shown in the Figure 45. Investigation of the microchannel is done by using COMSOL Multiphysics 5.2a simulation software.

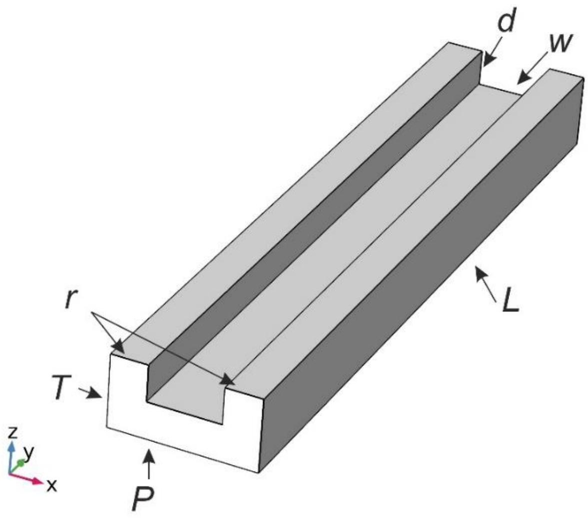


Figure 45. The dimension of the finite element model of the microchannel

Just one microchannel was analyzed with the symmetrical boundary conditions because this is a periodic system. As shown in Figure 46 boundary conditions were applied to finite element model in COMSOL Multiphysics 5.0 simulation software.

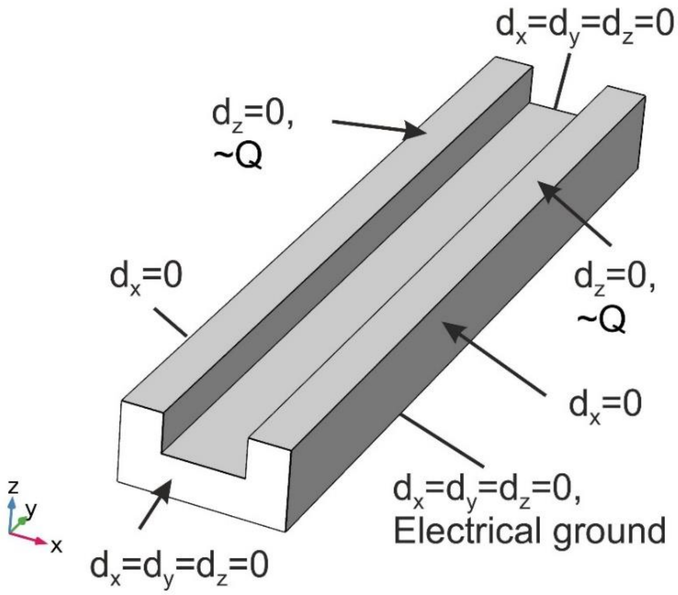


Figure 46. The boundary condition of the finite element model of the microchannel

As shown in Figure 46 left and the right surface of the microchannel was kept fixed by using rollers. The front and back surfaces of the microchannel were totally in fixed condition because these surfaces are connected to the fluid container. The bottom surface of the model will be connected to the other element of the sensor, therefore it is also in unmovable fixed condition and its serves as an electrical ground. The top portion of the microchannel is enclosed by electrodes and high strength transparent layer. It allows for the visual inspection of the fluid flow and confirms that pressure inside the channel is high.

Then the system of the microchannel is excited by applying the sinusoidal alternating electrical potential of 20 V. there is three sample test has been done for the dynamic response of the suggested microchannel at a different frequency.

The total deformation and X component of the displacement field of the electrically excited microchannel system at 110 MHz frequency shows that shape of the channel is deformed and it looks like a mechanical valve it means the cross-section area of the channel is eventually increased or decreased through all the length of the microchannel as shown in Figure 47.

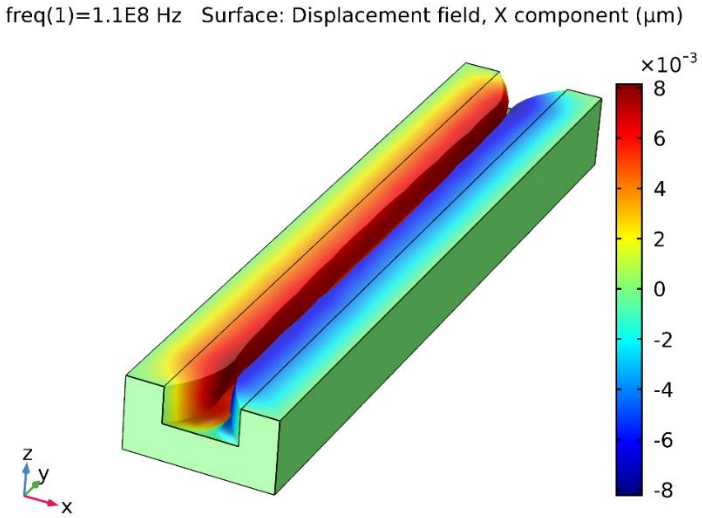


Figure 47. Total deformation of periodically at 110 MHz frequency excited microchannel with visualized X component of the displacement field

The frequency is increased to 117 MHz then the cross-section of the microchannel is start to divide into two segments with different concentrations of bioparticles as shown in Figure 48.

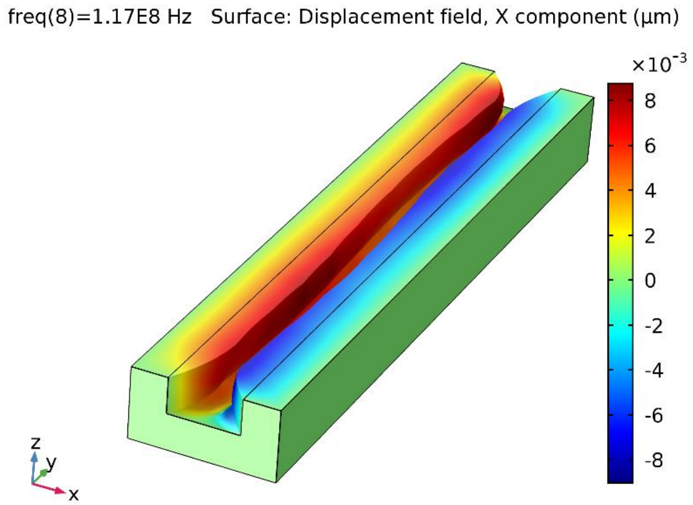


Figure 48. Total deformation of periodically at 117 MHz frequency excited microchannel with visualized X component of the displacement field

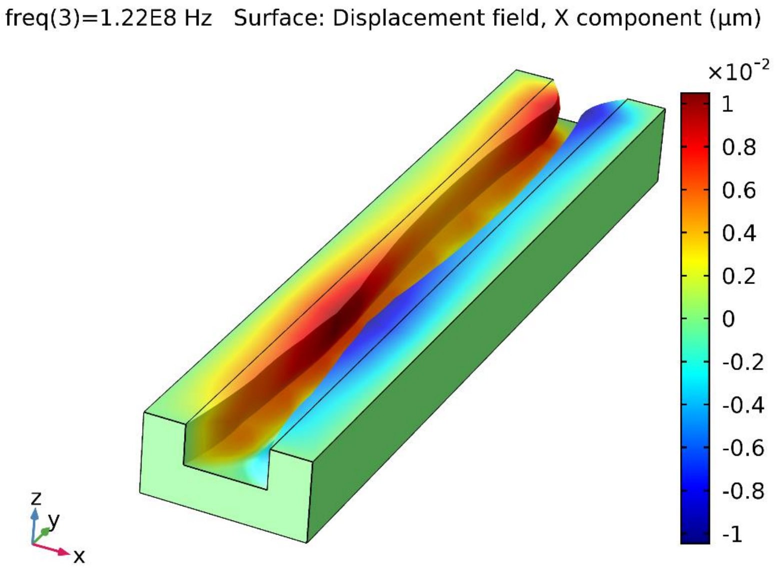


Figure 49. Total deformation of periodically at 122 MHz frequency excited microchannel with visualized X component of the displacement field

While frequency increased to 122 MHz then the cross-section of the microchannel actually shows that it is divided into two segments with different concentration of bioparticles as shown in Figure 49.

Conclusion

1. New type of PZT composite material was proposed for biosensor applications. Experimental results have demonstrated the natural frequency of PVB, PS, and PMMA composite material are 32.1 HZ, 43.02 HZ, and 46.17 HZ respectively. From the simulation result obtained natural frequency are 31.5 HZ, 43.15 Hz, and 46.99 Hz for PVB, PS, and PMMA respectively. It shows the very low difference between experimental and theoretical values (maximum deviation of 1.8%). So obtained values of Young's modulus from the results can be clearly stated for these PZT composite materials. Young's modulus of PZT Nanopowder mixed with PVB polymer value is 3.9 GPa, PS – 5.3 GPa, and PMMA – 6.3 GPa. A high Q factor of the proposed composite materials would permit to effectively control the microdevice as microchannels.

2. Designed experimental setup for contact angle measurement allows us to measure CA with the accuracy level 1.5°. It is possible to capture excellent quality image due to the high-speed camera (60 fps) used. Also, setup could be useful to measure dynamic contact angle, time dependence, and advancing and receding contact angle. Used image analysis software is a freely available platform (ImageJ) DropSnake plugins retuned the precise and stable values of contact angle in both the relative high ($\theta \geq 90°$) and low ($\theta \leq 40°$) degree region. Also, it is possible to measure contact angle for different solid substrate and liquids.

3. Experimental results have shown the average contact angle for PZT composite material PVB, PS, and PMMA with water are 80.71 ± 1.56°, 88.08 ± 1.31°, and 92.94 ± 1.56° respectively. Also, contact angle measured with different liquids and results are with glycerin PVB – 73.49 ± 1.92°, PS – 86.92 ± 0.7°, and 72.52 ± 1.44°, with spirit PVB – 33.43 ± 1.76°, PS – 38.01 ± 1.89°, and PMMA – 18.48 ± 0.5°, and with olive oil PVB – 23.18 ± 0.4°, PS – 25.86 ± 1.14°, and PMMA – 23.57 ± 0.7°. From the obtained values of result only contact angle of the water drop is noted highest (92.94 ± 1.56°) on the PZT + PMMA composite surface. Moreover, other values of results are falling in the range of ≤ 90°. So from the results, it is clearly stated that only PMMA with PZT composite material surface is falling in the hydrophobic segment and rest of all the surfaces are hydrophilic. Moreover, it is found that there is no influence of the different thickness of coating and different base materials on the contact angle.

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