In this thesis, the biophysical properties of human renal excretory fluid i.e. urine has been studied in both *in vitro* and *in vivo*. The urine is a bio fluid; the metabolic wastes in the human body are excreted in the form of urine. The urine has a significant place in medicine and pathology to social customs and agriculture. The one - fifth of world population is suffering from urine related problems, such as kidney malfunction called nephrological problems and urological problems such as kidney stone formation. In mythology, it is considered as untouchable in some religions. Some argue that there is a strong possibility of there being several beneficial substances not yet known to science. The auto-urine therapy is an ancient therapy which believes that the intake of early morning urine supplements the essential nutrients and strengthens the body. In agriculture, the stored urine is used as a fertilizer. The characteristics and compositions of urine such as ammonia, electrical conductivity, and pH change with storage time. In medicine and pathology the important parameters are specific gravity, refractive index, surface tension, viscosity, pH and electrical conductivity. These parameters are also very important for water reclamation from urine, used in space vehicles.

The urine is studied extensively by physiologist, bio-chemists, and biomedical engineers. But it has not drawn much attention of physicists. The application of concepts, principles and techniques of physics for the solution of problems in biology at different levels of complexity to get internal picture is drawing the attention of many researchers. The aim of the thesis is to develop these ideas at molecular level by studying the biophysical properties of human urine at different physiological conditions.
In this thesis the physical properties of urine such as specific gravity, refractive index, viscosity, surface tension, pH, electrical conductivity and Fourier transform infrared spectroscopy has been studied for both normal subjects and physiological disorders.

The thesis consists of five chapters:

1. Introduction
2. Theoretical Aspects
3. Material and methods
4. Results
5. Discussion and Conclusions

Chapter – I

INTRODUCTION

The human body has a complex system for balancing the volume and composition of body fluid. The system circulates the blood, which transports nutrients, oxygen, hormones, carbon-dioxide and waste materials. There are four tracts which eliminates the body waste i) urine excretory tract which is the main system of elimination of body fluid ii) the lungs eliminates the carbon dioxide iii) the digestive tract eliminates indigestible solids and bacteria iv) sweat glands eliminates excess heat and salt. The excretion is controlled by Anti-Diuretic Hormone (ADH) produced in the hypothalamus of brain and released from pituitary gland.

The urine excretory system consists of two kidneys, urinary bladder and urethra. The kidney is the main organ in the purification of blood. It removes the metabolic waste and performs the homeostatic function. The kidneys are located retroperitoneally in the upper dorsal region of abdominal cavity, each weighs 150 gm has length 10 cm, and wide 5cm has a thickness of about 2.5 cm. The important parts of kidney are cortex, medulla and pelvis.
The nephrons are the important and basic functional unit of the kidney. There are about 1 to 1.3 millions of nephrons present in each kidney, which drains in to renal pelvis, they start from cortex and ends in medulla, the total length ranges from 45 to 66 mm. The important parts of nephrons are i) Bowman’s capsule ii) Glomerulus iii) Proximal convoluted tubule (PCT) iv) Loop of henle v) Distal convoluted tubule(DST) vi) Collecting tubule. The Bowman’s capsule is a cup around the glomerulus, a space present between them is called bowman’s space. The blood from renal artery enters in to the glomerulus which contains capillary loops, and get filtered at pressure of 45 mm of Hg, this filtration is called glomerulur filtrate contain all constituents of blood plasma except protein and is collected in bowman’s space. The normal rate of filtration is 125 ml/min, it is called glomerulur filtration rate (GFR). The glomerulur filtrate enters in to the proximal convoluted tubule (PCT) which is surrounded by millions of microvilli which absorbs glucose, amino acids, proteins of low molecular weights, water and some ions and at the same time unwanted nitrogenous molecules are added to the filtrate. There occurs secretion of toxin ions $K^+$, $H^+$, $NH_4$ and then the filtration goes down to the loop of henle.

The PCT consists of ascending and descending limbs. The descending limb has a length of 2 to 4 mm. In this segment more water is reabsorbed by counter current mechanism. The ascending limb reabsorbs ions of Na and Cl. It is connected to the distal convoluted tubule (DCT). The cells lining the tubule have large mitochondria, which enable the active transport of fluid. The DCT reabsorbs more calcium and excrete more phosphate, hydrogen and ammonia to regulate pH. Hence urine is formed in three steps filtration, filtration, reabsorption and secretion.

The roles and function of kidney are i) Homeostatic function ii) Endocrine function iii) Gluconeogenesis function. The homeostatic function is regulation of fluid in the body by blood plasma regulation,
regulation of pH, regulating plasma osmolarity, and by removing metabolic waste and foreign substances. The endocrine function is secretion of renins, renal erythroprotiens which stimulates red blood cells production. The gluconeogenesis function is one in which the kidney synthesis and secretes glucose produced from non-carbohydrate sources, example gulatine in case of long fasting and respiratory acidosis.

Some of the important constituents of human urine are Urea, Uric acid, Sodium, Potassium, Calcium, Inorganic phosphorus, Sulfur, Iodine, Arsenic, Hippuric acid, Creatinine, Phenol, Oxalic acid, Ketone bodies, Lead, Creatine, Magnesium, Ammonia, Hormones, Vitamins and some Enzymes. The composition and concentration of urine change continuously during the course of 24 hours. The urine concentration changes according to water intake & activities before the test. It is important and necessary to regulate some aspects of urine collection, like time of collection, length of collection period (2hrs, 12hrs, 24hrs and mid day clean catch), dietary intake and medicinal intake and method of collection to represent patient’s truly metabolic state. The time of the day when specimen is collected influence the test result and findings. The random specimen, clean-catch (mid stream) and second morning specimen (double voided) have most common characteristics and minimum bacterial count. These types of specimens are used for physico-chemical screening, routine screening, bacterial culture and microscopic examination.

The physical examination of urine includes Urine volume, colour and appearance, Specific gravity, urine odour, pH and reaction and urine blood. The chemical examinations includes human chronic gonadotropin test, urine estrogen, urine drug investigation and for electrolytes such as sodium, potassium, chloride, calcium etc.

Different types of kidney diseases are caused due to different reasons and they show different symptoms. The kidney diseases can be broadly
classified into two (i) acute kidney disease (AKD), (ii) chronic kidney disease (CKD). The acute kidney may develop suddenly due to various reasons while the chronic kidney disease develops over a long period of time. The polycystic kidney disease (PKD) is one of most common AKD, due to many cyst or cavities formed in the kidney. Pyelonephritis also called urinary tract infection is kidney disorder that refers to infection of kidney usually due to bacteria but could be caused due to viruses.

**Chapter – II**

**THEORTICAL ASPECTS**

**Theory of surface tension**

The surface tension is a property of liquid that has no counter parts in solids or gases. It is a property of surface of liquid, causes the surface portion of liquid to be attracted to another surface. The surface tension is caused by cohesion. The cohesive forces among the liquid molecules are responsible for surface tension. A molecule in the bulk of a liquid is equally attracted and pulled by the surrounding molecules in all directions results a net zero force. A molecule of the liquid at its surface does not have other like molecules on all sides of it. There by it cohere more strongly to those directly associated with them on the surface. As a result the molecules at the surface are subjected a force acting inwards at right angles to the surface. So the surface of the liquid is always in a tension and behaves like a stretched membrane. This property of a liquid is known as surface tension. The surface of a liquid is defined as the tangential force acting on unit length of a line drawn on the surface of a liquid, tending to pull it apart.

Different methods of finding surface tension of liquids are Spinning drop method, Capillary rise method, Wilhelmy plate method, Pendent drop method, Stalagmometer method and Du Noüy ring detachment method.
Theory of viscosity

The viscosity is an inherent property of a liquid, which determines its resistance to shear stress. It measures the internal fluid friction which causes the resistance to flow of the liquid. The viscosity occurs due to cohesion and molecular movement between fluid layers.

A perfect or ideal fluid has no viscosity. But in practice there is no perfect fluid. All the fluids are compressible, and when they flow, they are capable of shearing stress on account of friction between the adjacent layers of the fluid. The temporary resistance offered by fluid to the shearing stress is called viscosity. A liquid may be considered as a pile of thin sheet or layers placed one on other. The velocity gradient between the two the adjacent layers is \( \frac{dv}{dx} \), caused by a force acting parallel to upper layer in the direction of motion. In the absence of this force i.e. if there is no viscosity, this velocity gradient is zero. This tangential force ‘F’ is proportional to velocity gradient and area.

\[
F = A \frac{dv}{dx}
\]

\( F = \eta A \frac{dv}{dx} \) is called Newton’s law of viscous flow.

Where ‘\( \eta \)’ is called coefficient of viscosity

The viscosity is temperature dependent. In gases the viscosity increases with increasing temperature. In liquids the viscosity decreases with increasing temperature and decreasing pressure. The viscosity of a solution changes with concentration of the solution, it depends on the amount of cohesive and adhesive forces between the same kind of molecule and different kind of molecules respectively. The cgs unit of viscosity is gm/cm/Sec it is called ‘poise’. Centipoise and mill poise are also used. 1 poise=0.1kg m\(^{-1}\) s\(^{-1}\)

The viscosity of a liquid of a liquid can be determined with the Pioseulle’s equation. It is given by \( \eta = \pi P r^4 t / 8 l V \). The viscosity of a liquid can be determined with respect to that of water, it is called relative viscosity. Let \( t_1 \) and \( t_2 \) are the time of flow for a fixed
volume of two liquids of density $\rho_1$ and $\rho_2$ respectively, then the ratio of their viscosities are $\eta_1 / \eta_2 = \pi \frac{P_1 r^4 t_1}{8 \pi V^* 8 \pi V/ \pi P_2 r^4 t_2}$

Therefore, $\eta_1 / \eta_2 = \rho_1 t_1 / \rho_2 t_2$, where $\rho_1$ and $\rho_2$ are densities of the two liquids.

The different types and methods of finding viscosity are Dead-load viscometers, Gas viscometers or constant pressure viscometers, Counter pressure viscometers, Constant Rate capillary viscometers, Dead-Load viscometers with variable loads, Glass viscometers, Rotational viscometers, Falling cylinder viscometer, sotwald viscometer. The viscometer is simplest type of viscometer, with which the viscosity of a fluid can be compared with that of a reference liquid.

**Theory of electrical conductivity of liquids**

Like metals the solutions conduct electrical current through them by the migration of ions under the influence of an electric field, obeying Ohm’s law. For an applied electromotive force $E$, maintained constant but at a value that exceeds the decomposition voltage of the electrolyte, the current $I$ flowing between the electrodes immersed in the electrolyte will vary inversely with the resistance of the electrolyte $R$. The electrical conductance of a solution is a summation of contributions from all the ions present. It depends upon the number of ions per unit volume of the solution and upon the velocities with which these ions move under the influence of the applied electromotive force. As a solution of an electrolyte is diluted, the specific conductance ‘$\kappa$’ will decrease and fewer ions to carry the electric current are present in each cubic centimeter of solution. As the conductivity is due to large number of ions, in order to express the ability of individual ions to conduct, a function called Equivalent conductance ‘$\Lambda$’ can be used. The equivalent conductance of a volume of an electrolyte is defined as the conductance of a volume of a solution containing one gram equivalent of electrolyte placed between two parallel electrodes one centimeter apart. It is also defined as the
conductance of an electrolyte obtained by dissolving one gram–equivalent of it in Vcc volume of water.
The equivalent conductance is the product of specific conductance $\kappa$ and $V$ in cubic centimeters containing one gram–equivalent of electrolyte.

$$\Lambda = \kappa V$$

If an electrolytic solution containing N grams-equivalent in 1000cc of the solution, then the volume of solution containing 1 gram equivalent will be $1000/N$

Therefore $\Lambda = \kappa*1000/N$

The unit for equivalent conductivity is ohm$^{-1}$ cm$^2$ eqvt$^{-1}$ or semens Cm$^2$.

**Effect of dilution**

When the electrolytic solution is diluted, the number of ions per cubic centimeter will be decreased and hence the specific conductance decreases, but the equivalent conductance goes on increases with dilution till it reaches a maximum value called equivalent conductance at infinite dilution ‘$\Lambda\omega$’. This is because, on diluting the degree of disassociation of electrolyte increases, thereby large number of ions becomes available for conduction.

The vibration spectra occur in infra red region. When infrared radiations of some frequency falls on molecules; the molecules absorbs energy and get excited to higher vibrational levels. The molecules absorb a quantum of energy give rise to characteristic based of the molecules from 50 to 12000 cm$^{-1}$. It is generally subdivided into three regions. Far IR 400 – 50 cm$^{-1}$, mid IR 4000 to 400 cm$^{-1}$, and near IR 12,500 to 4000 cm$^{-1}$. The mid IR region is the most commonly used for standard research investigations. For solid samples the Far IR is also equally important requires special instruments & techniques.
MATERIALS AND METHODS

Identification and selections of sample: healthy donors are identified in the age group of 22-26, 34-38 and 55-64. The donors have taken normal protein diet a day before urine sample collection. The pathological diabetic urines are collected from Hyderabad Diagnostic centre, malakpet, Hyderabad. The chronic kidney disease (CKD) and that with diabetic are collected from Hyderabad kidney and laproscopic centre, Hyderabad from patients undergoing dialysis.

Sample collection, Maintenance and preservation: The normal urine samples are collected for both first morning urine and second void of the day. The diabetic urines are of first morning urine and post lunch. The CKD urines are collected from patients who can afford it before proceeding for dialysis. All the samples are collected in plastic bottles of 150ml volume, and are kept at constant room temperature in water bath.

Sample preparation: The different concentrations of treated urines with glucose, urea, albumin and bilirubin are prepared by diluting a standard solution by un-treated urine.

The specific gravity was determined by a specific gravity bottle of 5 ml and weighing it by an electronic balance of accuracy of 0.1 mg. The electrical conductivity and temperature of urine has been determined by Elico conductivity meter and pH by Elico pH meter.

The research grade urea (H$_2$NCONH$_2$), glucose anhydride (C$_6$H$_{12}$O$_6$) (D-glucose), creatinine (C$_4$N$_7$N$_3$O) and albumin are Merck made and bilirubin(C$_{33}$H$_{36}$N$_4$O$_6$) is Lobal Chem made is purchased from the market. The viscosity has been found by comparing with viscosity of water by Ostwald Viscometer and surface tension by capillary rise method. The refractive index has been measured by Abbes’ refractometer under sodium vapor lamp.
The FT-IR spectra of urinary samples are recorded at IICT Hyderabad on Thermo Nicolet Nexus 670 FT-IR Spectrometer in absorption mode, Mid-IR region and also between 2000 to 700 cm$^{-1}$.

Chapter IV

RESULTS

The parameters specific gravity, refractive index, viscosity, surface tension, pH and electrical conductivity were measured for both first morning urine and random samples and the results are tabulated. All the parameters are found for different concentrations of glucose, urea, albumin and bilirubin for different samples of various subjects, and the results are tabulated. The concentrations of urea and glucose are 10 gm/dl, 5 gm/dl, 2.5 gm/dl, 1.25 gm/dl and 0.625 gm/dl. The concentrations for albumin are 1000 mg/dl, 500 mg/dl, 250 mg/dl, 125 mg/dl and 0.625 gm/dl, and for bilirubin the concentrations are 40, 20, 10, 5, 2.5 and 1.25 gm/dl. All the above parameters are found for chronic kidney disease (CKD) urine and also CKD with diabetic were found. All these parameters are found for diabetic mellitus patients of different glucose levels, and the results are tabulated.

FT-IR spectra for normal urine and urine treated with glucose, urea, creatinine and albumin are recorded and analysed.

Chapter V

DISCUSSION AND CONCLUSIONS

The values of specific gravity, refractive index, viscosity and electrical conductivity of first morning urine are high compared to that of randomly collected. The refractive index increases with increasing specific gravity.
The refractive index is an alternate to the specific gravity. There by the urine specific gravity can also be approximated by refractometry. The parameter specific gravity, refractive index, surface tension, viscosity, pH and electrical conductivity have been found at various concentrations of urea, albumin, bilirubin and glucose.

The two factors urea and albumin are very much useful in determining the kidney disease, in which the blood urea nitrogen (BUN) and albumin to creatinine ratio (ACR) increases. It has been observed that as the concentration of urea increases in urine in vitro the parameter specific gravity and refractive index have been increased. The electrical conductivity decreases with the increasing concentration, the decreased electrical conductivity can be attributed to the decreased mean free path of electrolytes in the urine. The pH and surface tension have not been affected significantly. The presence of urea in urine has increased its viscosity.

In normal urine the value of urea is up to 23,300 mg/liter. But in case of chronic kidney disease (CKD), the excretion of urea ranges from 900 to 1290 mg/dl, very less compared to normal urine. So the specific gravity is less and viscosity is low compared to that of normal urine. In case of CKD diabetic cases it is close to normal value due to glucose present in it.

As the concentration of albumin is increased in urine in vitro, the increase in the specific gravity and refractive index are not significant. An increase of 0.001 in refractive index and 0.0035 of specific gravity are observed for 1000 mg/dl of albumin added. The surface tension and viscosity decrease with the concentration of albumin. The parameters pH and electrical conductivity are not affected by the concentration of urine. In all the urine specimen analysed the urine pH value ranges from 5.5 to 6.5

The value of albumin in normal urine is up to 7 mg/dl. In case of CKD the excretion of albumin is very high up to 500 mg/dl. The excretion of
urine creatinine in CKD is about 30mg/dL, which is very low compared to 1.5gm/dL in normal urine. The urine albumin to creatinine ratio (UACR) in kidney has been found high. According to National Kidney Foundation (NKF), if the UACR is more than 30mg/gm or 0.03 which is abnormally high, then it is a case of CKD, where the GFR is less than 60ml/min.

In case of chronic kidney disease the value of UACR has been found more than 1.60 which is abnormally high compared to normal value around 0.0046. It has also been found that, the specific gravity, refractive index, viscosity and surface tension of urine in Chronic Kidney Disease are low compared that of to normal urine.

The decreased value of surface tension can be attributed to the shape of albumin protein molecule. The albumin protein molecules are ellipsoidal in shape, they need time to develop an orientation at air-water interface, a process that results in surface tension. At higher concentrations of albumin in urine, the molecules have more difficulty in re-orienting to the surface of urine due to greater electrostatic repulsion. The decreased surface tension and specific gravity of urine is an accurate method of finding the kidney disease. This method of detecting kidney disease or proteinuria is a quick and cheaper method, and it suggests that the glomerulus filtration rate is low.

As the concentration of glucose is increased in vitro, it has been observed that the specific gravity, refractive index, viscosity increases with increasing concentrations. The electrical conductivity and surface tension have been decreased with increasing concentration of glucose. It has been observed that the urines of patients with diabetic mellitus is much concentrated having high specific gravity, refractive index and viscosity. The surface tension has been found decreased in diabetic mellitus urine. In diabetic insipidus, there is a decrease or absence of anti-diuretic hormone, which makes large intake of water. There by the
kidney produces an excessive amount of urine even up to 20 liters/day. As this urine is diluted, the specific gravity decreases.

The presence and addition of bilirubin in urine does not increase the specific gravity, refractive index, viscosity and electrical conductivity. The surface tension is decreased with increasing concentration of bilirubin.

It is observed in the present investigation by FT-IR spectral studies that 1033 cm\(^{-1}\) is the most specific peak for glucose and 1640 cm\(^{-1}\) is the most specific peak for urea in urine. The specific peaks for albumin a protein is 1450 cm\(^{-1}\) and creatinine 1493 cm\(^{-1}\). It is also observed as the concentration of these urine constituents increases the absorption at these peaks also increase and the absorption band becomes strong.

It can be concluded that the diseased or pathological urine has less surface tension compared to normal urine. The urine constituents can be quantified by FT-IR spectra.