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INTRODUCTION: REVIEW OF ELECTROACTIVE BIOMOLECULES AND ELECTROCHEMICAL SENSORS
1.1 INTRODUCTION

Dopamine (DA), ascorbic acid (AA) and uric acid (UA) are the physiologically important biomolecules which co-exist in the extracellular fluids of mammalian brain. DA is a very important catechol amine functioning as a neurotransmitter. It activates the metabolism helping the body to establish a healthy weight. Additionally, it helps the brain to generate sufficient energy to run the body. It is also useful for the treatment of drug addiction, parkinson and alzheimers diseases [1].

AA is also called as vitamin C and it is water soluble. Due to its antioxidant [2] and pH regulatory activities, it is added to a variety of food products and pharmaceuticals in their preservation. It facilitates the absorption of iron, maintains elasticity of the skin and improves resistance to infection. It is useful in controlling scurvy, common cold, mental illness, cancer, infertility and AIDS [3].

UA is a vital nitrogenous compound and it is a product of purine metabolism. Its abnormal quantities in blood fluids lead to many clinical disorders [4, 5]. Higher levels of UA in blood called hyperuricemia is linked with the disorders like gout, kidney problems and lesch-nyhan syndrome [6]. According to some epidemiological studies, elevated serum UA levels is the indicator of cardiovascular diseases [7, 8].

Owing to the importance of these biomolecules, it is quite essential to resolve and estimate each of the biomolecules separately to identify the disorders. However, the resolution of these compounds using cyclic voltammetry at bare carbon paste electrode (BCPE) in real biological samples is difficult because all these compounds have similar reduction potentials [9] giving rise to overlapping voltammetric peaks. Also, BCPEs often suffer from fouling of the surface by the oxidation products of the biomolecules resulting in rather poor reproducibility and sensitivity. Hence, there is an urgent need to develop the novel modified electrodes for their resolution.

In this chapter, an attempt has been made to understand in detail, the structures, properties, biological significance of these biomolecules. Also, development of novel sensors, literature related to the investigation of these biomolecules using various techniques are described. Finally, in a nut shell, the objectives and scope of the work is described.
1.2 DOPAMINE

1.2.1 History and development

Dopamine was first synthesized in 1910 by George Barger and James Ewens at Welcome Laboratories in London, England [10] and first identified in the human brain by Kathleen Montagu in 1957. It was named dopamine because it is a monoamine whose precursor in the Barger-Ewens synthesis is 3, 4-dihydroxyphenylalanine (levodopa or L-DOPA). Dopamine's function as a neurotransmitter was first recognized in 1958 by Arvid Carlsson and Nils-Åke Hillarp at the Laboratory for Chemical Pharmacology of the National Heart Institute of Sweden. Carlsson was awarded the 2000 Nobel Prize in Physiology or Medicine for showing that the dopamine is not only a precursor of norepinephrine (noradrenaline) and epinephrine (adrenaline), but is also itself a neurotransmitter [11].

1.2.2 Structure and properties

Dopamine (3, 4-Dihydroxyphenethylamine) is an organic chemical of the catecholamine and phenethylamine families. A dopamine molecule consists of a catechoi structure (a benzene ring with two hydroxyl side groups) with one amine group attached via an ethyl chain as shown in the figure 1.1. Its molecular formula is C_8H_{11}NO_2. It is freely soluble in water, methanol and hot 95% ethanol, but is practically insoluble in ether, petroleum ether, chloroform, benzene and toluene. Its water solubility is 600 g/L. As such, dopamine is the simplest possible catecholamine, a family that also includes the neurotransmitters norepinephrine and epinephrine.

![Figure 1.1 Structure of dopamine](image-url)
The presence of a benzene ring with this amine attachment makes it a substituted phenethylamine, a family that includes numerous psychoactive drugs. Like most amines, dopamine is an organic base [12]. As a base, it is generally protonated in acidic environments (in an acid-base reaction). The protonated form is highly water-soluble and relatively stable, but can become oxidized if exposed to oxygen or other oxidants [12]. In basic environments, dopamine is not protonated. In this free base form, it is less water-soluble and also more highly reactive. Because of the increased stability and water-solubility of the protonated form, dopamine is supplied for chemical or pharmaceutical use as dopamine hydrochloride, that is, the hydrochloride salt that is created when dopamine is combined with hydrochloric acid. In dry form, dopamine hydrochloride is a fine colorless powder. Dopamine, being electroactive undergoes oxidation to give dopaquinone as shown in the figure 1.2.

![Chemical structure of dopamine and dopaquinone](image)

**Figure 1.2 Oxidation of dopamine**

1.2.3 Occurrence

1.2.3.1 Microorganisms

Dopamine has been detected in some types of bacteria and in the protozoan called Tetrahymena [13]. Perhaps more importantly, there are types of bacteria that contain homologs of all the enzymes that animals use to synthesize dopamine [14]. It has been proposed that animals derived their dopamine-synthesizing machinery from bacteria, via horizontal gene transfer that may have occurred relatively late in evolutionary time, perhaps as a result of the symbiotic incorporation of bacteria into eukaryotic cells that gave rise to mitochondria.
1.2.3.2 Animals

Dopamine is used as a neurotransmitter in most multicellular animals [15]. In every type of animal that has been examined, dopamine has been seen to modify motor behavior. In the model organism, nematode Caenorhabditis elegans, it reduces locomotion and increases food-exploratory movements; in flatworms it produces "screw-like" movements; in leeches it inhibits swimming and promotes crawling. Across a wide range of vertebrates, dopamine has an "activating" effect on behavior-switching and response selection, comparable to its effect in mammals.

Dopamine has also consistently been shown to play a role in reward learning, in all animal groups. As in all vertebrates — invertebrates such as roundworms, flatworms, molluscs and common fruit flies can all be trained to repeat an action if it is consistently followed by an increase in dopamine levels [15]. It had long been believed that arthropods were an exception to this with dopamine being seen as having an adverse effect. Reward was seen to be mediated instead by octopamine, a neurotransmitter closely related to nor epinephrine [16]. More recent studies however have shown that the dopamine does play a part in reward learning in fruit flies. Also it has been found that the rewarding effect of octopamine is due to its activating a set of dopaminergic neurons not previously accessed in the research.

1.2.3.3 Plants

Dopamine can be found in the peel and fruit pulp of bananas. Many plants, including a variety of food plants, synthesize dopamine to varying degrees [17]. The highest concentrations have been observed in bananas—the fruit pulp of red and yellow bananas contains dopamine at levels of 40 to 50 parts per million by weight. Potatoes, avocados, broccoli and brussels sprouts may also contain dopamine at levels of 1 part per million or more. Oranges, tomatoes, spinach, beans and other plants contain measurable concentrations less than 1 part per million. The dopamine in plants is synthesized from the amino acid tyrosine, by biochemical mechanisms similar to those that animals use. It can be metabolized in a variety of ways, producing melanin and a variety of alkaloids as byproducts [17]. The functions of plant catecholamines have not been clearly established, but there is an evidence that they play a role in response to stressors such as bacterial infection, act as growth-
promoting factors in some situations and modify the way that sugars are metabolized. The receptors that mediate these actions have not yet been identified, nor have the intracellular mechanisms that they activate. Dopamine consumed in food cannot act on the brain, because it cannot cross the blood–brain barrier [12].

1.2.4 Biosynthesis

![Biosynthesis of dopamine](image)

Figure 1.3 Biosynthesis of dopamine

Dopamine is synthesized in a restricted set of cell types, mainly neurons and cells in the medulla of the adrenal glands [18]. The metabolic pathway is shown in the figure 1.3.
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The direct precursor of dopamine, L-DOPA, can be synthesized indirectly from the essential amino acid phenylalanine or directly from the non-essential amino acid tyrosine. These amino acids are found in nearly every protein and so are readily available in food, with tyrosine being the most common. Although dopamine is also found in many types of food, it is incapable of crossing the blood–brain barrier that surrounds and protects the brain. It must therefore be synthesized inside the brain to perform its neuronal activity. L-Phenylalanine is converted into L-tyrosine by the enzyme phenylalanine hydroxylase, with molecular oxygen (O₂) and tetrahydrobiopterin as cofactors. L-Tyrosine is converted into L-DOPA by the enzyme tyrosine hydroxylase, with tetrahydrobiopterin, O₂, and iron (Fe²⁺) as cofactors. L-DOPA is converted into dopamine by the enzyme aromatic L-amino acid decarboxylase (also known as DOPA decarboxylase), with pyridoxal phosphate as the cofactor. Dopamine itself is used as precursor in the synthesis of the neurotransmitters (and hormones) norepinephrine and epinephrine. Dopamine is converted into nor epinephrine by the enzyme dopamine β-hydroxylase, with O₂ and L-ascorbic acid as cofactors. Norepinephrine is converted into epinephrine by the enzyme phenyl ethanolamine N-methyltransferase with S-adenosyl-L-methionine as the cofactor. Some of the cofactors also require their own synthesis. Deficiency in any required amino acid or cofactor can impair the synthesis of dopamine, nor epinephrine, and epinephrine.

1.2.5 Metabolism

Dopamine is broken down into inactive metabolites by a set of enzymes—monoamine oxidase (MAO), catechol-O-methyl transferase (COMT) and aldehyde dehydrogenase, acting in sequence [19]. Both isoforms of monoamine oxidase, MAO-A and MAO-B, effectively metabolize dopamine. Different breakdown pathways exist but the main end-product is homovanillic acid as shown in the figure 1.4, which has no known biological activity. From the bloodstream, homovanillic acid is filtered out by the kidneys and then excreted in the urine. In clinical research on schizophrenia, measurements of homovanillic acid in plasma have been used to estimate the levels of dopamine activity in the brain. A difficulty in this approach however, is separating the high level of plasma homovanillic acid contributed by the metabolism of norepinephrine [20]. Although dopamine is normally broken down by
an oxidoreductase enzyme, it is also susceptible to oxidation by direct reaction with oxygen, yielding quinones plus various free radicals as products [21]. The rate of oxidation can be increased by the presence of ferric iron or other factors. Quinones and free radicals produced by autoxidation of dopamine can poison the cells and there is an evidence that this mechanism may contribute to the cell loss that occurs in Parkinson's disease and other conditions [22].

Figure 1.4 Metabolism of dopamine

1.2.6 Biological significance

1.2.6.1 Neurotransmission

Neurotransmission involves the conversion of an electrical impulse to a chemical event and then to another electrical event, is extremely rapid. Action potentials and neurotransmitters represents the bricks with which the internal representation of the external world is build. Neurotransmitter such as dopamine release is initiated by an electrical impulse called an action potential. Each neuron has a resting membrane. When an appropriate neurotransmitter binds to receptors on the dendrites or cell bodies, ion channels open, allowing an influx of Na\(^+\) that changes the membrane potential and initiates an action potential or firing. It then propagates down the axon to the terminal at a rate of 0.5 m/s [23]. This firing causes voltage-gated Ca\(^{2+}\)
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channels to open in the terminals. The resultant Ca\(^{2+}\) influx triggers the vesicles to fuse with the cell membrane and release their contents, a process termed as exocytosis. Because some vesicles are docked adjacent to the membrane, exocytosis occurs on a millisecond timescale [24].

1.2.6.2 Deficiency of dopamine

The brain neurotransmitter dopamine activates the metabolism helping the body to establish a healthy weight. Additionally, dopamine helps the brain to generate sufficient energy to run the body. DA affects the basal ganglia motor loop which in turn affects the brain functions. Shortage of DA, particularly the death of DA neurons in the nigrostriatal pathway, causes Parkinson's disease, in which a person loses the ability to execute smooth, controlled movements. Degeneration of DA leads to decline of cognitive functions, aging, breakdown and death. Since DA acts as a neurotransmitter which is vital for message transfer functions, the use of illegal drugs or substances such as heroin, cocaine, nicotine and alcohol blocks the DA transport that inhibits the re-uptake of DA and eventually increases the DA levels, causing an increased risk of depression and drug addiction [25].

1.2.6.3 Outside the nervous system

Dopamine not only exists in the brain, but it also exists in the peripheral areas in the body. It does not cross the blood–brain barrier, so its synthesis and functions in peripheral areas are to a large degree independent of its synthesis and functions in the brain [12]. Dopamine is found in blood plasma at levels comparable to those of epinephrine, but in humans, over 95% of the dopamine in the plasma is in the form of dopamine sulfate, a conjugate produced by the enzyme sulfotransferase 1A3/1A4 acting on free dopamine. The bulk of this dopamine sulfate is produced in the mesentery that surrounds parts of the digestive system. The production of it is thought to be a mechanism for detoxifying dopamine that is ingested as food or produced by the digestive process. Levels of dopamine in the plasma typically rise more than fifty-folds after a meal. Dopamine sulfate has no known biological functions and is excreted in urine. The relatively small quantity of unconjugated dopamine in the bloodstream may be produced by the sympathetic nervous system, the digestive system or possibly other organs. It may act on dopamine receptors in peripheral
tissues or be metabolized or be converted to norepinephrine by the enzyme dopamine beta hydroxylase, which is released into the bloodstream by the adrenal medulla. Some dopamine receptors are located in the walls of arteries, where they act as vasodilator and an inhibitor of norepinephrine release [26]. Beyond its role in modulating blood flow, there are several peripheral systems in which dopamine circulates within a limited area and performs an exocrine or paracrine function [19]. The peripheral systems in which dopamine plays an important role include the immune system, the kidneys and the pancreas.

1.2.7 Dopamine deficiency-related diseases and disorders

As described in section 1.2.6, deficiency of dopamine causes several diseases/disorders including parkinson's disease, schizophrenia, drug addiction, psychosis, attention deficit hyperactivity disorder, pain, nausea etc. At this juncture, it is necessary to briefly describe the role of dopamine in all these disorders.

1.2.7.1 Parkinson's disease

Parkinson's disease is an age-related disorder characterized by movement disorders such as stiffness of the body, slowing of movement and trembling of limbs when they are not in use [27]. In advanced stages it progresses to dementia and eventually death. The main symptoms are caused by the loss of dopamine-secreting cells in the substantia nigra. Dopamine cells are vulnerable to damage due to encephalitis, repeated sports-related concussions and some forms of chemical poisoning such as MPTP, resulting in parkinsonian syndrome that is similar in its main features to Parkinson's disease [28]. Most cases of Parkinson's disease, however, are idiopathic, meaning that the cause of cell death cannot be identified.

The most widely used treatment for parkinsonism is administration of L-DOPA, the metabolic precursor for dopamine. L-DOPA is converted to dopamine in the brain and various parts of the body by the enzyme DOPA decarboxylase. L-DOPA is used rather than dopamine itself because, unlike dopamine, it is capable of crossing the blood-brain barrier. It is often co-administered with an enzyme inhibitor of peripheral decarboxylation such as carbidopa or benserazide, to reduce the amount converted to dopamine in the periphery and thereby increase the amount of L-DOPA
that enters the brain. When L-DOPA is administered regularly over a long time period, a variety of unpleasant side effects such as dyskinesia often begin to appear, even so, it is considered as the best available long-term treatment option for most cases of Parkinson's disease. L-DOPA treatment cannot restore the dopamine cells that have been lost, but it causes the remaining cells to produce more dopamine, thereby compensating for the loss to at least some degree.

1.2.7.2 Drug addiction

Cocaine, substituted amphetamines (including methamphetamine), adderall, methylphenidate, MDMA (ecstasy) and other psycho stimulants exert their effects primarily or partly by increasing dopamine levels in the brain by a variety of mechanisms [29]. Cocaine and methylphenidate are dopamine transporter (DAT) blockers or reuptake inhibitors as shown in the figure 1.5. They non-competitively inhibit dopamine reuptake, resulting in increased dopamine concentrations in the synaptic cleft. Like cocaine, substituted amphetamines and amphetamine also increase the concentration of dopamine in the synaptic cleft, but through different mechanisms.

Figure 1.5 Dopamine transporter (DAT) blocking mechanism by cocaine

This increased levels of dopamine, appear to be the primary factor in causing addiction. When people addicted to stimulants, go through withdrawal, they do not
experience the physical suffering associated with alcohol withdrawal or withdrawal from opiates. Instead they experience craving, an intense desire for the drug characterized by irritability, restlessness and other arousal symptoms [30] brought about by psychological dependence.

1.2.7.3 Psychosis

Psychiatrists in the early 1950s discovered that a class of drugs known as typical antipsychotics (also known as major tranquilizers), were often effective at reducing the psychotic symptoms of schizophrenia [31]. The first widely used antipsychotic drug, chlorpromazine (Thorazine), in the 1950s, led to the release of many patients with schizophrenia from institutions in the years that followed, but in 1970s researchers understood that this antipsychotic worked as antagonists on the D2 receptors [31]. This realization led to the so-called dopamine hypothesis of schizophrenia, which postulates that schizophrenia is largely caused by hyperactivity of brain dopamine systems. The dopamine hypothesis drew additional support from the observation that the psychotic symptoms were often intensified by dopamine-enhancing stimulants such as methamphetamine and that these drugs could also produce psychosis in healthy people if taken in large enough doses. In the following decades other atypical antipsychotics that had fewer serious side effects were developed. Many of these newer drugs do not act directly on dopamine receptors, but instead produce alterations in dopamine activity indirectly [32].

However, the widespread use of antipsychotic drugs has long been controversial. There are several reasons for this. First, antipsychotic drugs are perceived as very aversive by people who have to take them, because they produce a general dullness of thought and suppress the ability to experience pleasure [33]. Second, it is difficult to show that they act specifically against psychotic behaviors rather than merely suppressing all types of active behavior. Third, they can produce a range of serious side effects, including weight gain, diabetes, fatigue, sexual dysfunction, hormonal changes and a type of serious movement disorder known as tardive dyskinesia. Some of these side effects may continue long after the cessation of drug use or even permanently.
1.2.7.4 Attention deficit hyperactivity disorder

Altered dopamine neurotransmission is implicated in attention deficit hyperactivity disorder (ADHD), a condition associated with impaired cognitive control, in turn leading to problems with regulating attention (attentional control), inhibiting behaviors (inhibitory control) and forgetting things or missing details (working memory), among other problems. There are genetic links between dopamine receptors, the dopamine transporter and ADHD, in addition to links to other neurotransmitter receptors and transporters [34]. The most important relationship between dopamine and ADHD involves the drugs that are used to treat ADHD. Some of the most effective therapeutic agents for ADHD are psycho stimulants such as methylphenidate (Ritalin, Concerta) and amphetamine (Adderall, Dexedrine), drugs that increase both dopamine and norepinephrine levels in the brain. The clinical effects of these psycho stimulants in treating ADHD are mediated through the indirect activation of dopamine and nor epinephrine receptors, specifically dopamine receptor D1 and adrenoceptor A2, in the prefrontal cortex [35].

1.2.8 Medical uses

Dopamine as a manufactured medication is sold under the trade names Intropin, Dopastat and Revimine among others. It is on the World Health Organization's list of Essential Medicines [36]. It is most commonly used as a stimulant drug in the treatment of severe low blood pressure, slow heart rate and cardiac arrest. It is especially important in treating the newborn infants. It is given intravenously. Its effects, depending on dosage, include an increase in sodium excretion by the kidneys, an increase in urine output, an increase in heart rate and an increase in blood pressure. At low doses it acts through the sympathetic nervous system to increase heart muscle contraction force and heart rate, thereby increasing cardiac output and blood pressure. Higher doses also cause vasoconstriction that further increases blood pressure [37].

Side effects of dopamine include negative effects on kidney function and irregular heartbeats. The LD₅₀ or lethal dose which is expected to prove fatal in 50% of the population, has been found to be 59 mg/kg (mouse-administered
intravenously), 950 mg/kg (mouse-administered intraperitoneally), 163 mg/kg (rat-administered intraperitoneally) and 79 mg/kg (dog-administered intravenously) [38].

1.3 URIC ACID

1.3.1 History

Carl Wilhelm Scheele, a Swedish-German scientist, worked at the University of Uppsala in Sweden. At the same university and at the same time, the famous gout sufferer Carl Linnaeus, a professor of Natural History was also working. In 1776 Scheele [39] examined what he called urinary concretions (solid matter) and discovered a new acid, which he named lithic acid. It later became known as uric acid, most of which in the body is in the form of urate. In the same year his fellow countryman, Tobern Bergman analysed a bladder stone and found the same acid in it. In 1797, William Hyde Wollaston, a British scientist, learnt that the same acid Scheele had found (lithic/urate acid) in urinary concretions was also found in tophi.

It had been known that the uric acid was found in the blood for some time - not just only in tophi or kidneys stones or urinary concretions In 1848 a British physician, Sir Alfred Garrod, who had made gout and rheumatism just about his life's work, declared in a lecture that an excess of uric acid in the blood was the cause of gout. Garrod had developed what he called his “Uric Acid Thread Experiment”, which others have since renamed his “Thread Test.” In 1882, the Ukrainian chemist Ivan Horbaczewski first synthesized uric acid by melting urea with glycine.

1.3.2 Structure and properties

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen and hydrogen with the molecular formula C₅H₄N₄O₃. Its structure is shown in the figure 1.6. It forms ions and salts known as urates and acid urates, such as ammonium acid urate. Uric acid is a product of the metabolic breakdown of purine nucleotides. It is a white crystalline substance with a melting point of 300°C. The water solubility of uric acid and its alkali metal and alkaline earth metal salts is rather low. All these salts exhibit greater solubility in hot water than cold allowing for easy recrystallization. This low solubility is significant for the etiology of gout. The solubility of the acid and its salts in ethanol is very low or negligible. In ethanol/water mixtures, the
solubilities are somewhere between the end values for pure ethanol and pure water. Its solubility in water is 0.6 mg/100 mL (at 20 °C). UA is a weak organic acid with pK$_a$ of 5.75 and is present principally as monosodium urate (MSU) at

![Figure 1.6 Structure of uric acid](image)

physiological pH. Uric acid undergoes electrochemical oxidation as per the reaction shown in the figure 1.7.

![Figure 1.7 Uric acid oxidation](image)

1.3.3 Dietary sources

In humans, purines are excreted as uric acid. Purines are found in high amounts in animal food products, such as liver and sardines. A moderate amount of purine is also contained in beef, pork, poultry, fish and seafood, asparagus, cauliflower, spinach, mushrooms, green peas, lentils, dried peas, beans, oatmeal, wheat bran and wheat germ. Examples of high purine and iron sources include: sweetbreads, anchovies, sardines, liver, beef kidneys, brains, meat.
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extracts (e.g., Oxo, Bovril), herring, mackerel, scallops, game meats, beer and gravy. Moderate and even high intake of purine-containing vegetables is not associated with an increased risk of gout. One serving of meat or seafood (85 g) mildly increases risk of gout, while two servings increase risk by at least 40%. Milk products reduce the risk of gout notably, whereas total protein intake has no effect [40].

High levels of uric acid lead to several diseases such as gout, Lesch-Nyhan syndrome, cardiovascular disease, Type 2 diabetes, Uric acid stone formation etc. So, to control the uric acid concentration, our diet should contain foods rich in vitamin C and also fibrous foods. Amla, guava, kiwi, oranges, sweet lime, capsicum, tomatoes, green leafy vegetables etc are the rich sources of vitamin C. These vitamin C rich foods disintegrate uric acid and forces it to be excreted through the urine. Fibrous foods include beans, whole grains, vegetables, fruits, nuts and seeds. Dietary fibre may help the blood to absorb uric acid well and then it would be excreted through the kidneys. Low levels of uric acid (hypouricemia) also leads to some diseases such as multiple sclerosis etc.

1.3.4 Biosynthesis and metabolism

Purines are absorbed from the diet through the intestine, synthesized in the body and/or derived from the degradation of endogenous DNA and RNA. The enzyme xanthine oxidase catalyzes the formation of uric acid from xanthine and hypoxanthine, which in turn are produced from other purines. Xanthine oxidase is a large enzyme whose active site consists of the metal molybdenum bound to sulfur and oxygen [41]. Within cells, xanthine oxidase can exist as xanthine dehydrogenase and xanthine oxidoreductase, which has also been purified from bovine milk and spleen extracts. Uric acid is released in hypoxic conditions [42]. In humans and higher primates, uric acid is the final oxidation (breakdown) product of purine metabolism and is excreted in urine as shown in the figure 1.8. In most other mammals, the enzyme uricase further oxidizes uric acid to allantoin (figure 1.9).
Figure 1.8 Metabolism of purines in human beings

The loss of uricase in higher primates parallels the similar loss of the ability to synthesize ascorbic acid, leading to the suggestion that urate may partially substitute for ascorbate in such species [43]. Both uric acid and ascorbic acid are strong reducing agents (electron donors) and potent antioxidants. In humans, over half the antioxidant capacity of blood plasma comes from uric acid. In humans, about 70% of daily uric acid disposal occurs via the kidneys, and in 5–25% of humans, impaired renal (kidney) excretion leads to hyperuricemia [44].

The Dalmatian dog has a genetic defect in uric acid uptake by the liver and kidneys, resulting in decreased conversion to allantoin, so this breed excretes uric acid, and not allantoin, in the urine [45].

In birds, reptiles and in some desert dwelling mammals such as the kangaroo, rat, uric acid also is the end-product of purine metabolism, but it is excreted
in feces as a dry mass. This involves a complex metabolic pathway that is energetically costly in comparison with the processing of other nitrogenous wastes.

Figure 1.9 Biosynthesis and metabolism of uric acid in humans and other mammals such as urea (from urea cycle) or ammonia, but has the advantages of reducing water loss and preventing dehydration. Platynereis dumerilii, a marine Polychaete worm, uses uric acid as a sexual pheromone released into the water by females during mating to induce males to release sperm [46].
1.3.5 Biological significance

In human blood plasma, the reference range of uric acid is typically 3.4–7.2 mg/dL (200–430 μmol/L) for men, and 2.4–6.1 mg/dL for women (140–360 μmol/L) [47]. Uric acid concentrations in blood plasma above and below the normal range are known as, respectively, the hyperuricemia and hypouricemia. Likewise, uric acid concentrations in urine above and below normal are known as hyperuricosuria and hypouricosuria.

Diseases pathway of uric acid levels

Figure 1.10 Diseases caused by excess uric acid

Such abnormal concentrations of uric acid are not medical conditions, but are associated with a variety of medical conditions. Hyperuricemia (high levels of uric acid) can lead to diseases such as gout, Lesch-Nyhan syndrome, cardiovascular
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disease, Type 2 diabetes, uric acid stone formation etc. Similarly, lower levels of uric acid (hypouricemia) also leads to diseases such as multiple sclerosis etc. Diseases caused by the excess of uric acid in the body is pictorially shown in the figure 1.10.

1.3.6 Causes of abnormal uric acid levels in the body

1.3.6.1 High uric acid levels

The following causes are responsible for higher uric acid levels in the body.

1. Diet may be a factor. High intake of dietary purine, high-fructose corn syrup and table sugar can cause increased levels of uric acid [48]

2. Serum uric acid can be elevated due to reduced excretion by the kidneys

3. Fasting or rapid weight loss can temporarily elevate uric acid levels

4. Certain drugs such as thiazide diuretics can increase uric acid levels in the blood.

1.3.6.2 Low uric acid levels

Low uric acid (hypouricemia) can have numerous causes.

1. Low dietary zinc intakes cause lower uric acid levels. This effect can be even more pronounced in women taking oral contraceptive medication [49].

2. Xanthine oxidase (XO) is an iron–molybdenum enzyme, so people with iron deficiency (the most common cause of anemia in young women) or molybdenum deficiency can experience hypouricemia.

3. Xanthine oxidase loses its function and gains ascorbate function when some of the iron atoms in XO are replaced with copper atoms. As such, people with high copper/iron can experience hypouricemia and vitamin C deficiency, resulting in oxidative damage. Since estrogen increases the half-life of copper, people with very high estrogen levels and intense blood loss during menstruation are likely to have high copper/iron and present with hypouricemia.
4. Sevelamer, a drug indicated for prevention of hyperphosphataemia in patients with chronic renal failure, can significantly reduce serum uric acid [50].

5. But the main cause of congenitally low uric acid, sometimes as low as zero, remains the molybdenum cofactor deficiency.

1.3.7 Diseases and disorders

Abnormal levels of uric acid in blood and urine cause several diseases as discussed in section 1.3.5. It is necessary to discuss in detail about each of the diseases.

1.3.7.1 Diseases due to higher levels of uric acid

Gout, Lesch-Nyhan syndrome, cardiovascular disease, type 2 diabetes, metabolic syndrome, stone formation in kidneys (Urolithiasis) are the main diseases resulting from higher levels of uric acid.

1.3.7.1.1 Gout

Excess serum accumulation of uric acid (hyperuricemia) in the blood can lead to gout, which is a type of arthritis. This painful condition is the result of needle-like crystals of uric acid (sodium urate) precipitating in joints (figure 1.11), capillaries, skin and other tissues [51].

![Figure 1.11 Figure showing normal joint and joint with gout](image-url)
Kidney stones can also form through the process of formation and deposition of sodium urate microcrystals. Its prevalence is 3/1000 and males are affected more than females (8-10:1). It usually occurs in males over 30 years of age and females after the menopause. A study found that men who drink two or more sugar-sweetened beverages a day have 85% higher chance of developing gout than those who drank such beverages infrequently [52]. Gout can occur where serum uric acid levels are as low as 6 mg/dL (~357 µmol/L), but an individual can have serum values as high as 9.6 mg/dL (~565 µmol/L) and not have gout.

There are four stages in the development of this disorder:

1. **Asymptomatic hyperuricaemia:**

   Hyperuricaemia is usually present for many years before the onset of symptoms.

   Only 1 in 20 subjects with hyperuricaemia will eventually develop clinical gout.

2. **Acute gouty arthritis:**

   Classical presentation is an acute inflammation of the metatarsophalangeal joint of the big toe (70%) and the first attack is usually monoarticular (affects only 1 joint). Other joints that may be involved are the ankle, knee, wrist, elbow and small joints of hands and feet.

3. **Intercritical gout:**

   Some patients may have only 1 attack, whilst others have recurrent attacks at shorter intervals. Between attacks the patient is usually asymptomatic except for hyperuricaemia.

4. **Chronic tophaceous gout:**

   This follows recurrent attacks and is characterised by the development of tophi (swellings containing uric acid crystals) in the periarticular tissue. Other sites include the helix of the ear, bursae and tendons.
One treatment for gout, in the 19th century, had been the administration of lithium salts. The resulting lithium urate is more soluble in water. Today, inflammation during attacks is more commonly treated with NSAIDs, colchicine or corticosteroids and urate levels are managed with allopurinol. Allopurinol, developed over 30 years ago by Elion et al. It weakly inhibits xanthine oxidase. It is an analog of hypoxanthine that is hydroxylated by xanthine oxidoreductase at the 2-position to give oxipurinol. Oxipurinol has been supposed to bind tightly to the reduced molybdenum ion in the enzyme and thus, inhibits uric acid synthesis [53].

1.3.7.1.2 Lesch-Nyhan syndrome

The Lesch-Nyhan syndrome is an X-linked recessive disorder due to severe deficiency of Hypoxanthine-guanine phosphoribosyl transferase (HGPRT). It is characterised by hyperuricaemia, mental deficiency, spasticity, choreoathetosis and self-mutilation. Hyperuricaemia is due to decreased activity of the salvage pathway causing decreased purine reutilization and increased uric acid synthesis. Relatively low levels of nucleotides result in the decreased inhibition of de novo synthesis, resulting in further overload of the non-functioning salvage pathway and increased uric acid production. It is an extremely rare inherited disorder, is also associated with very high serum uric acid levels [54]. Spasticity, involuntary movement and cognitive retardation as well as manifestations of gout are seen in cases of this syndrome.

1.3.7.1.3 Cardiovascular disease

Hyperuricemia may be associated with an increase in risk factors for cardiovascular disease [55]. An epidemiological link between elevated serum uric acid and an increased cardiovascular risk has been recognized for many years. Observational studies show that serum uric acid concentrations are higher in patients with established coronary heart disease compared with healthy controls. Elevated serum uric acid concentrations are also found in healthy offspring of parents with coronary artery disease, indicating a possible causal relationship. However, hyperuricaemia is also associated with the elevated serum triglyceride and cholesterol concentrations, blood glucose, fasting and post-carbohydrate plasma insulin concentrations, waist-hip ratio and body mass index [56]. About one quarter
of hypertensive patients have co-existent hyperuricaemia and interestingly, asymptomatic hyperuricaemia predicts future development of hypertension, irrespective of renal function.

Some studies have suggested that the importance of uric acid may be independent of certain risk factors. Multivariate analysis of data from the MONICA cohort of 1044 males showed a significant association between raised serum uric acid and cardiovascular mortality, independent of body mass index, serum cholesterol concentration, hypertension, diuretic use, alcohol intake and smoking habits [57]. In contrast to these findings, several studies have suggested that the relationship between elevated serum uric acid and cardiovascular risk does not persist after correcting for other risk factors. The British Regional Heart Study of 7688 men aged 40 to 59 years showed a significant association between elevated serum uric acid and fatal and non-fatal coronary disease over a mean of 16.8 years. However, this relationship disappeared after correcting for other risk factors, particularly serum cholesterol concentration.

In summary, although there is overwhelming evidence that elevated serum uric acid concentrations are strongly associated with increased cardiovascular risk and poor outcome, prospective population studies are often confounded by co-existent risk factors. It remains unclear whether the uric acid is an independent predictor of poor cardiovascular outcome.

1.3.7.1.4 Stone formation in kidneys (Urolithiasis)

Saturation levels of uric acid in blood may result in one form of kidney stones when the urate crystallizes in the kidney. These uric acid stones are radiolucent and so do not appear on an abdominal plain X-ray and thus their presence must be diagnosed by ultrasound or stone protocol CT. Very large stones may be detected on X-ray by their displacement of the surrounding kidney tissues.

Uric acid stones, which form in the absence of secondary causes such as chronic diarrhea, vigorous exercise, dehydration and animal protein loading are felt to be secondary to obesity and insulin resistance seen in metabolic syndrome. Increased dietary acid leads to the increased endogenous acid production in the liver and
muscles which in turn leads to an increased acid load to the kidneys. This load is handled more poorly because of renal fat infiltration and insulin resistance, which are felt to impair ammonia excretion (a buffer). The urine is, therefore, quite acidic and uric acid becomes insoluble, crystallizes and stones are formed (figure 1.12). In addition, naturally present promoter and inhibitor factors may be affected. This explains the high prevalence of uric stones and unusually acidic urine seen in patients with type 2 diabetes. Uric acid crystals (sodium urate) can also promote the formation of calcium oxalate stones, acting as "seed crystals" (heterogeneous nucleation) [58].

1.3.7.1.5 Type 2 diabetes

The association of high serum uric acid with insulin resistance has been known since the early part of the 20th century, but the hypothesis that the high serum uric acid is a risk factor for diabetes has long been a matter of debate. In fact, hyperuricemia was presumed to be a consequence of insulin resistance rather than its precursor. However, a prospective follow-up study showed high serum uric acid is associated with higher risk of type 2 diabetes, independent of obesity, dyslipidemia, and hypertension [59].
13.7.2 Disease due to lower levels of uric acid - Multiple sclerosis

Lower serum values of uric acid have been associated with multiple sclerosis (MS). MS patients have been found to have serum levels of 194 \( \mu \text{mol/L} \), with patients in relapse averaging 160 \( \mu \text{mol/L} \) and patients in remission averaging 230 \( \mu \text{mol/L} \). Serum uric acid in healthy controls was 290 \( \mu \text{mol/L} \) [60]. Conversion factor: 1 mg/dL = 59.48 \( \mu \text{mol/L} \).

A 1998 study completed a statistical analysis of 20 million patient records, comparing serum uric acid values in patients with gout and patients with multiple sclerosis. Almost no overlap between the groups was found [61].

Uric acid has been successfully used in the treatment and prevention of the animal (murine) model of MS. A 2006 study found elevation of serum uric acid values in multiple sclerosis patients, by oral supplementation with inosine, resulted in lower relapse rates and no adverse effects [62].

1.3.8 Normalizing low uric acid

Correcting low or deficient zinc levels can help to elevate serum uric acid [63]. Inosine can be used to elevate uric acid levels. Zinc inhibits copper absorption, helping to reduce the high copper/iron in some people with hypouricemia. Iron supplements can ensure adequate iron reserves (ferritin above 25 ng/dl), also correcting the high copper/iron.

1.4 ASCORBIC ACID

1.4.1 History

It was noted from the middle of the 18th century that lemon and lime juice could help to prevent sailors from getting scurvy. At first, it was supposed that the acid properties were responsible for this benefit. However, it soon became clear that other dietary acids such as vinegar had no such benefits. In 1907, two Norwegian physicians reported an essential disease-preventing compound in foods that was distinct from the one that prevented beriberi. These physicians were investigating dietary-deficiency diseases using the new animal model of guinea pigs, which are
susceptible to scurvy. The newly discovered food-factor was eventually called vitamin C.

From 1928 to 1932, the Hungarian research team led by Albert Szent-Györgyi, as well as that of the American researcher Charles Glen King, identified the antiscorbutic factor as a particular single chemical substance. At the Mayo clinic, Szent-Györgyi had isolated the chemical hexuronic acid from animal adrenal glands. He suspected it to be the antiscorbutic factor but could not prove it without a biological assay. This assay was finally conducted at the University of Pittsburgh in the laboratory of King, which had been working on the problem for years, using guinea pigs. In late 1931, King's lab obtained adrenal hexuronic acid indirectly from Szent-Györgyi and using their animal model, proved that it is vitamin C by early 1932.

This was the last of the compound from animal sources, but, later that year, Szent-Györgyi's group discovered that paprika pepper, a common spice in the Hungarian diet, is a rich source of hexuronic acid. He sent some of this more available chemical to Walter Norman Haworth, a British sugar chemist. In 1933, working with the then-Assistant Director of Research (later Sir) Edmund Hirst and their research teams, Haworth deduced the correct structure and optical-isomeric nature of vitamin C and in 1934 reported the first synthesis of the vitamin. In honor of the compound's antiscorbutic properties, Haworth and Szent-Györgyi now proposed the new name of "a-scorbic acid" for the compound. It was named L-ascorbic acid by Haworth and Szent-Györgyi when its structure was finally proven by synthesis.

In 1937, the Nobel Prize for chemistry was awarded to Haworth for his work in determining the structure of ascorbic acid, shared with Paul Karrer. The Noble prize for Physiology that year went to Albert Szent-Györgyi for his studies of the biological functions of L-ascorbic acid. The American physician Fred R. Klenner, promoted vitamin C as a cure for many diseases in the 1950s by elevating the dosages greatly to as much as tens of grams vitamin C daily orally and by injection. From 1967 on, Nobel prize winner Linus Pauling recommended high doses of ascorbic acid as a prevention against cold and cancer. However, modern evidence does not support
a role for high-dose vitamin C in the treatment of cancer or the prevention of the common cold in the general population [64].

1.4.2 Structure and properties

Ascorbic acid is also called vitamin C. Its IUPAC name is (5R)-[(1S)-1, 2-Dihydroxyethyl]-3, 4-dihydroxyfuran-2(5H)-one. Its molecular formula is C₆H₈O₆. Its structure is shown in the figure 1.13. It is a white solid, but impure samples can appear yellowish with molecular weight of 176.12 g.mol⁻¹ and density 1.65 g/cm³. It is soluble in water, ethanol, glycerol, propylene glycol etc. It is insoluble in solvents such as ether, chloroform, benzene, petroleum ether, oils and fats. Its solubility in water is 330g/L and its melting temperature is between 190 to 192°C. It is an acid with first pKa value of 4.10 and second pKa value of 11.6. Lethal dose or concentration (LD, LC): LD₅₀ (median dose) is 11.9 g/kg (oral, rat). It is derived from glucose, many non-human animals are able to produce it, but humans require it as part of their nutrition. Other vertebrates which lack the ability to produce ascorbic acid include some primates, guinea pigs, teleost fishes, bats and some birds, all of which require it as a dietary micronutrient (that is in vitamin form) [65]. It readily undergoes reversible oxidation to dehydroascorbic acid as shown in figure 1.14.
1.4.3 Dietary sources

Ascorbate is found in many fruits and vegetables [66]. Citrus fruits and juices are particularly rich sources of vitamin C but other fruits including cantaloupe, honeydew melon, cherries, kiwi fruits, mangoes, papaya, strawberries, tangelo, watermelon and tomatoes also contain variable amounts of vitamin C. Vegetables such as cabbage, broccoli, brussels sprouts, bean sprouts, cauliflower, kale, mustard greens, red and green peppers, peas, tomatoes and potatoes may be more important sources of vitamin C than fruits. This is particularly true because the vegetable supply often extends for longer periods during the year than the fruit supply.

1.4.3.1 Limitations in the supply of vitamin C

In many developing countries, limitations in the supply of vitamin C are often determined by seasonal factors (i.e., the availability of water, time and labour for the management of household gardens and the short harvesting season of many fruits). For example, mean monthly ascorbate intakes ranged from 0 to 115 mg/day in one Gambian community in which the peak intakes coincided with the seasonal duration of the mango crop and to a lesser extent with orange and grapefruit harvests. These fluctuations in dietary ascorbate intake were closely reflected by the corresponding variations in plasma ascorbate (11.4-68.4 mmol/L) and human milk ascorbate (143-342 mmol/L) [67].
1.4.3.2 Effect of various factors on vitamin C content

Vitamin C is also very labile and the loss of vitamin C on boiling milk provides one dramatic example of a cause of infantile scurvy. The vitamin C content of food is strongly influenced by season, transport to market, shelf life, time of storage, cooking practices and chlorination of water. Blanching techniques, low pH inactivate the oxidase enzyme and help to preserve ascorbate, as in the preparation of sauerkraut (pickled cabbage). In contrast, heating and exposure to copper or iron or to mildly alkaline conditions destroys the vitamin and too much water can leach it from the tissues during cooking.

However, it is important to realise that the amount of vitamin C in a food is usually not the major determinant of a food’s importance for supply, but rather regularity of intake. For example, in countries where the potato is an important staple food and refrigeration facilities are limited, seasonal variations in plasma ascorbate are due to the considerable deterioration in the potato’s vitamin C content during storage. The content can decrease from 30 to 8 mg/100 g over 8-9 months. Such data can indicate the important contribution that the potato can make to human vitamin C requirements even though the potato vitamin C concentration is low. An extensive study has been made of losses of vitamin C during the packaging, storage and cooking of blended foods (maize and soya-based relief foods). Data from a US Agency for International Development Programme show that the vitamin C losses from packaging and storage in polythene bags of such relief foods are much less significant than the 52-82 percent losses attributable to conventional cooking procedures [68].

1.4.4 Biosynthesis and metabolism

Ascorbic acid is produced from glucose in plants and animals [69]. Animals must either produce it or digest it, otherwise a lack of vitamin C may cause scurvy, which may eventually lead to death. Reptiles and older orders of the birds make ascorbic acid in their kidneys. Recent orders of birds and most mammals make ascorbic acid in their liver where the enzyme L-gulonolactone oxidase is required to convert glucose to ascorbic acid [69]. Humans, other higher primates, guinea pigs and most bats require dietary L-gulonolactone oxidase because the enzyme catalysing the last step in the biosynthesis is highly mutated and non-functional, therefore, unable to
make ascorbic acid. Synthesis and signalling properties are still under investigation [70]. Animal and plant ascorbic acid biosynthesis pathways are shown in the figure 1.15.

1.4.5 Biological significance, diseases and disorders

Vitamin C is an electron donor (reducing agent or antioxidant) and probably all of its biochemical and molecular functions can be accounted for by this property. It plays an important role in forming collagen, a protein that gives structure to bones, cartilage, muscle and blood vessels. It also helps blood capillaries, bones, teeth and aids in the absorption of iron. AA is a reducing agent, necessary to maintain the enzyme prolyl hydroxylase in an active form, most likely by keeping its iron atom in a reduced state. The precursor molecule to the protein collagen, procollagen contains an unusual amino acid sequence, in that every third amino acid is a glycine, which consists of amino acids with a high frequency that was not found in any other proteins like hydroxyproline and hydroxylysine. These two amino acids are converted from
proline and lysine respectively, after the procollagen molecule has been synthesized. The hydroxylation of proline and lysine into procollagen is carried out by the enzyme prolyl hydroxylase using ascorbic acid as a cofactor. The natural form of the vitamin C is the L-isomer. AA plays a vital role in the synthesis of carnitine also, however, its most vital role is as a water-soluble vitamin in the human body [71]. AA is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. As a scavenger of reactive oxygen and nitrogen oxide species, ascorbic acid has been shown to be effective against the superoxide radical ion, hydrogen peroxide, the hydroxyl radical and singlet oxygen [72]. AA protects folic acid reductase, which converts folic acid to folinic acid and may help to release free folic acid from its conjugates in food. It facilitates the absorption of iron, maintains elasticity of the skin, improves resistance to infection, used in the treatment of scurvy and may prevent the occurrence and development of cancer.

1.4.5.1 Role in human metabolic processes

1. Enzymatic functions: Vitamin C acts as an electron donor for 11 enzymes [73]. Three of those enzymes are found in fungi but not in humans or other mammals. They are involved in reutilisation pathways for pyrimidines and the deoxyribose moiety of deoxynucleosides. Of the 8 remaining human enzymes, three participate in collagen hydroxylation [74] and two in carnitine biosynthesis. Of the three enzymes which participate in collagen hydroxylation, one is necessary for the biosynthesis of the catecholamine nor epinephrine, one is necessary for amidation of peptide hormones and one is involved in tyrosine metabolism [75]. Ascorbate interacts with enzymes having either monooxygenase or dioxygenase activity. The monooxygenases, dopamine b-monooxygenase and peptidyl-glycine a-monooxygenase incorporate a single oxygen atom into a substrate, either a dopamine or a glycine-terminating peptide. The remaining enzymes are dioxygenases which incorporate two oxygen atoms in two different ways. The enzyme 4-hydroxyphenylpyruvate dioxygenase incorporates two oxygen atoms into one product. The other dioxygenase incorporates one oxygen atom into succinate and one into the enzyme-specific substrate.
2. **Miscellaneous functions:** The concentrations of vitamin C in gastric juice were several fold higher (median, 249 mmol/l and range 43-909 mmol/l) than those found in the plasma of the same normal subjects (39 mmol/l, 14-101 mmol/l) [76]. Gastric juice vitamin C may prevent the formation of N-nitroso compounds, which are potentially mutagenic. High intakes of vitamin C correlate with reduced gastric cancer risk, but a cause-and-effect relationship has not been established. Vitamin C protects low-density lipoproteins ex vivo against oxidation and may function similarly in the blood [77].

### 1.4.5.2 Diseases and disorders

Deficiency of vitamin C can cause anemia, scurvy, infections, bleeding gums, muscle degeneration, poor wound healing, atherosclerotic plaques and capillary hemorrhaging. Neurotic disturbances consisting of hypochondriasis, hysteria and depression followed by the decreased psychomotor performances have been reported in ascorbic acid deficiency. Vitamin C deficiency is often associated with gingivitis.

1. **Anaemia:** A common feature of vitamin C deficiency is anaemia. The antioxidant properties of vitamin C may stabilise folate in food and in plasma and increased excretion of oxidized folate derivatives in human scurvy was reported [78]. Vitamin C promotes absorption of soluble non-haem iron possibly by chelation or simply by maintaining the iron in the reduced (ferrous, Fe²⁺) form. The effect can be achieved with the amounts of vitamin C obtained in foods. However, the amount of dietary vitamin C required to increase the iron absorption ranges from 25 mg upwards and depends largely on the amount of inhibitors such as phytates and polyphenols present in the meal.

2. **Scurvy:** Three important manifestations of scurvy - gingival changes, pain in the extremities and haemorrhagic manifestations - preceded oedema, ulcerations and ultimately death. Skeletal and vascular lesions in scurvy probably arise from a failure of osteoid formation. In infantile scurvy, the changes are mainly at the sites of most active bone growth. Characteristic signs are a pseudo paralysis of the limbs caused by extreme pain on movement and caused by haemorrhages under the periosteum, as well as swelling and
haemorrhages in areas of the gums surrounding erupting teeth. In adults one of
the early, principle adverse effects of the collagen-related pathology may be
impaired wound healing. Vitamin C deficiency can be detected from the early
signs of clinical deficiency, such as the follicular hyperkeratosis, petechial
haemorrhages, swollen or bleeding gums and joint pain or from the very low
concentrations of ascorbate in plasma, blood or leukocytes. The Sheffield
studies and later studies in Lowa [79] were the first major attempts made to
quantify vitamin C requirements. The studies indicated that the amount of
vitamin C required to prevent or cure early signs of deficiency was between
6.5 and 10 mg/day. This range represents the lowest physiological
requirement. The Lowa studies [79] and Kallner et al. established that at tissue
saturation, whole body vitamin C content is approximately 20 mg/kg or 1500
mg and that during depletion vitamin C is lost at 3 percent of whole body
content per day.

Clinical signs of scurvy appear in men at intakes lower than 10 mg/day
or when the whole body content falls below 300 mg. Such intakes are
associated with plasma ascorbate concentrations below 11 mmol/l or
leukocyte levels less than 2 nmol/10^8 cells. However, the plasma
concentrations fall to around 11 mmol/l when dietary vitamin C is between 10
and 20 mg/day. At intakes greater than 25-35 mg/day, plasma concentrations
start to rise steeply, indicating a greater availability of vitamin C for metabolic
needs. In general, plasma ascorbate closely reflects the dietary intake and
ranges between 20 and 80 mmol/l. Note that during infection or physical
trauma, an increase in the number of circulating leukocytes occurs and these
take up vitamin C from the plasma [80]. Therefore, both plasma and leukocyte
levels may not be very precise indicators of body content or status at such
times.

Intestinal absorption of vitamin C is by an active, sodium-dependent,
energy-requiring, carrier-mediated transport mechanism and as intakes
increase, the tissues progressively become more saturated. The physiologically
efficient, renal-tubular reabsorption mechanism retains vitamin C in the tissues
up to a whole body content of ascorbate of about 20 mg/kg of body weight.
Chapter 1

However, under steady state conditions, as intakes rise from around 100 mg/day there is an increase in urinary output and at 1000 mg/day almost all the absorbed vitamin C is excreted [81].

1.4.6 Vitamin C- Immunity and infections

Infection means the entrance, growth and multiplication of a microorganism (pathogen) in the body of a host resulting in the establishment of a disease process. An infectious disease represents a combat between two living forces- the organism invading and the organism invaded. The invader may be bacterium, fungus, virus or rickettsia and in human pathology, the human body is invaded. Infections initiate bi-directional interactions with the defense mechanisms of the host, both immunological and nonspecific and also interact with the nutritional status of the host.

Vitamin C can enhance the body’s resistance to an assortment of diseases, including infectious disorders. It strengthens and protects the immune system by stimulating the activity of antibodies and immune system cells such as phagocytes and neutrophils [82]. Vitamin C works by stimulating the immune system and protecting against damage by the free radicals released by the body in its fight against the infection. Vitamin C helps the immune system to fight against viruses. It acts as an antiviral agent, elevating body’s interferon level. Even taken in small amounts, it appears to reduce the duration and severity of illness [83].

In laboratories, Vitamin C has been found to inhibit HIV replication. With its antioxidant and immunity-enhancing abilities, it is an excellent supplement for HIV patients, as it may help with disease resistance and overall well being. Vitamin C, taken at levels of 2 g/day, may help the body to fight against infection via hepatitis-contaminated blood. Vitamin E deficiencies are often found in hepatitis patients. Intake of Vitamin C (1200 IU) daily reduces the liver damage in adult patients. It is a confident preventive measure; however, more promising is the use of Vitamin C as a treatment for infected patients [84].

1.4.7 Applications of ascorbic acid

Ascorbic acid has applications in diverse fields such as- food preservation, plastic manufacturing, fluorescence microscopy, photographic developing etc.
Chapter - 1

1. Ascorbic acid and its sodium, potassium and calcium salts are commonly used as antioxidant food additives. These compounds are water-soluble and thus cannot protect fats from oxidation. For this purpose, the fat-soluble esters of ascorbic acid with long-chain fatty acids (ascorbyl palmitate or ascorbyl stearate) can be used as food antioxidants. Eighty percent of the world's supply of ascorbic acid is produced in China.

2. It is a cofactor in tyrosine oxidation [85].

3. In fluorescence microscopy and related fluorescence-based techniques, ascorbic acid can be used as an antioxidant to increase fluorescent signal and chemically retard dyephotobleaching [86].

4. In plastic manufacturing, ascorbic acid is used to assemble molecular chains more quickly and with less waste.

5. Heroin users are known to use ascorbic acid as a means to convert heroin base to a water-soluble salt so that it can be injected [87].

6. As justified by its reaction with iodine, it is used to negate the effects of iodine tablets in water purification. It reacts with the sterilized water, removing the taste, color and smell of the iodine. This is why it is often sold as a second set of tablets in most sporting goods stores as Portable Aqua-Neutralizing Tablets, along with the potassium iodide tablets.

7. Ascorbic acid is easily oxidized and so is used as a reductant in photographic developer solutions (among others) and as a preservative.

8. It is also commonly used to remove dissolved metal stains such as iron from fiberglass swimming pool surfaces.

9. Intravenous high-dose ascorbate is being used as a chemotherapeutic and biological response modifying agent. Currently it is still under clinical trials.
1.4.8 Vitamin C toxicity

1. **Kidney stone formation:** Oxalate is an end product of ascorbate catabolism and plays an important role in kidney stone formation. Excessive daily intake of vitamin C produces hyperoxaluria. In four volunteers who received vitamin C in the range of 5-10 g/day, this amounted to approximately a doubling of urinary oxalate excretion, from 50 to 87 mg/day (range 60-126 mg/day) [88]. However, the risk of oxalate stones formation may become significant at high intakes of vitamin C (>1 g) [89], particularly in subjects with high amounts of urinary calcium.

2. **Haemolysis:** Vitamin C may precipitate haemolysis in some people, including those with glucose-6-phosphate dehydrogenase deficiency [90], paroxysmal nocturnal haemoglobinuria or other conditions where increased risk of red cell haemolysis may occur or where protection against the removal of the products of iron metabolism may be impaired, as in people with the haptoglobin Hp2-2 phenotype [91]. Of these conditions, only the haptoglobin Hp 2-2 condition was associated with abnormal vitamin C metabolism (lower plasma ascorbate than expected) under conditions where intake of vitamin C was provided mainly from dietary sources. Therefore, 1g vitamin C appears to be the advisable upper limit of dietary intake.

3. **Gastrointestinal disturbances:** The potential toxicity of excessive doses of supplemental vitamin C relates to intra-intestinal events and to the effects of metabolites in the urinary system. Intakes of 2-3 g/day of vitamin C produce unpleasant diarrhoea from the osmotic effects of the unabsorbed vitamin in the intestinal lumen in a majority of the people [92]. Gastrointestinal disturbances can occur after ingestion of as little as 1 g because approximately half of the amount would not be absorbed at this dose.

1.5 LITERATURE REVIEW

George Barger and James Ewens at Welcome Laboratories in London, England, in 1910, first synthesized dopamine [10]. It was first identified in the human brain by Kathleen Montagu in 1957. Neurotransmission properties of dopamine was
first recognized in 1958 by Arvid Carlsson and Nils-Åke Hillarp at the Laboratory for Chemical Pharmacology of the National Heart Institute of Sweden. Carlsson was awarded the 2000 Nobel Prize in Physiology or Medicine for showing that dopamine is not only a precursor of norepinephrine (noradrenaline) and epinephrine (adrenaline), but is also itself a neurotransmitter [11].

Carl Wilhelm Scheele, a Swedish-German scientist, in 1776, examined what he called urinary concretions (solid matter) and discovered a new acid, which he named lithic acid [53]. It later became known as uric acid, most of which in the body is in the form of urate. In the same year his fellow countryman, Tobern Bergman analysed a bladder stone and found the same acid in it. William Hyde Wollaston in 1797 learnt that the same acid Scheele had found (lithic/uric acid) in urinary concretions was also found in tophi. Sir Alfred Garrod, a British physician, in 1848 declared in a lecture that an excess of uric acid in the blood was the cause for gout and rheumatism. The Ukrainian chemist Ivan Horbaczewski first synthesized uric acid by melting urea with glycine in 1882.

Two Norwegian physicians, in 1907, reported an essential disease-preventing compound in foods that was distinct from the one that prevented beriberi, was eventually called vitamin C. In late 1931, King's lab in the University of Pittsburgh obtained adrenal hexuronic acid and proved that it is vitamin C by early 1932. Haworth deduced the correct structure and optical-isomeric nature of vitamin C and in 1934 reported the first synthesis of vitamin C. In honor of the compound's antiscorbutic properties, Haworth and Szent-Györgyi proposed the new name of "a-scorbic acid" for the compound. It was named L-ascorbic acid by Haworth and Szent-Györgyi when its structure was finally proven by synthesis.

Ever since, these biomolecules were discovered, investigations on them, owing to their biological significance, were carried out using various techniques. The traditional analytical methods used for the detection of biomolecules include chemiluminescence [93], fluorimetry [94, 95], ultraviolet-visible spectrometry [96], spectrophotometry [97], HPLC [98, 99], flow injection methods [100, 101] and capillary electrophoresis [102]. The other methods like ion exchange membranes (anionic and cationic) have been developed to electrostatically accumulate oppositely
charged analyte molecules. Few examples for this are- Nafion [103], polyester sulphonic acid [104], poly(4-vinylpyridine) [105], stearate [106], w-mercapto carboxylic acid [107], poly(monomericeugenol) [108], overoxidised poly(1-(2-carboxyethyl) pyrrole [109], 4-aminophenylacetic acid [110], ionic liquid [111], overoxidised polypyrrole [112,113] etc.

In recent years, the development of voltammetric sensors for the determination of biomolecules has received a great interest. This is because, there are certain advantages of cyclic voltammetric technique over the other traditional analytical techniques, which made the investigators to select this for investigation of biomolecules. The advantages are given below.

1. It requires simple and inexpensive instrumentation,
2. Specially trained people are not required for its operation,
3. Investigation process is quite fast compared to other methods,
4. The instrumentation is compact and does not require more work space,
5. It is the most widely used technique for acquiring qualitative information about electrochemical species,
6. The power of cyclic voltammetry is revealed from its ability to provide considerable information on the thermodynamics of redox processes,
7. Rapid location of redox potentials of the electroactive species,
8. It helps to determine the mechanism, rates of oxidation / reduction of electrochemical reactions.

A major problem in the detection of the biomolecules DA, UA and AA, which co-exist in biological fluids, using cyclic voltammetry, however, is the interference of their voltammetric peaks, due to similar reduction potentials at bare carbon paste electrodes [114, 115]. Also, it is known that the direct electro-oxidation of DA, UA and AA at bare electrodes is irreversible and requires high potentials. Oxidation often suffers from a pronounced fouling effect, which results in rather poor selectivity,
sensitivity and reproducibility. Both sensitivity and selectivity are, therefore of equal importance in biomolecules determination. One of the most common routes is to use modified carbon paste electrodes in the place of bare carbon paste electrodes, which have the ability to eliminate the interfering substances from DA determination. Different types of modifications quoted in the literature are- bulk modification, surface modification by electropolymerisation, surface modification by immobilization and both bulk and surface modifications.

In bulk modification, a variety of modifiers are being used, such as, metal oxides, organic redox mediators, nano particles of metals, metal oxides, carbon nano tubes, carbon ceramic electrodes etc. J. H. Chen et al., used an organic redox mediator poly (4-(2-pyridylazo)-resorcinol) on modified glassy carbon electrode [116], F. H. Li et al., used a nano composite made up of Pt/ionic liquid/graphene [117], J. S. Huang et al., used palladium nanoparticle-loaded carbon nanofibers modified electrode [118], N.F. Atta made use of nanoclusters-coated polyfuran [119], Y. Z. Zhang et al., used functionalized single-wall carbon nanotube modified electrode [120], Sathish Reddy et al., used CdO Nanoparticles [121], R.P. Silva et al., used pyrolytic graphite modified electrode [122], A. Safavi et al., used carbon ionic liquid electrode [123], K. S. Prasad et al., used screen-printed carbon electrode [124], for the determination of DA, UA and AA.

In surface modification, electrode surface is coated with a polymer by electropolymerisation. Such electrodes are widely used now-a-days to determine the biomolecules DA, UA and AA. For instance, Patton and Reeder’s reagent [125], xylenol orange [126], maleic acid [127], L-arginine [128], methyl orange [129], eriochrome black-T [130] etc., were used for the surface modification of carbon paste electrodes by electropolymerisation to determine the biomolecules.

Surface modification of the carbon paste electrodes by immobilization is also a commonly used method. Immobilisation by acetone/water [131], 1-butyl-4-methyl pyridinium tetrafluoroborate ionic liquid [132] were used in the past for the determination of biomolecules.
Bulk modification by ZnO and surface modification by glycine is reported by Satish Reddy et al., [133]. Similarly, carbon nano tubes for bulk modification and room temperature ionic liquid (RTIL) of 1- Octyl-3-methylimidazolium hexafluorophosphate for surface immobilization was used by Yifang Zhao et al., [134] for the determination of the biomolecules.

1.6 SENSORS

1.6.1 Sensor

A sensor, in its broadest sense, is an object whose purpose is to detect events or changes in its environment and then provide a corresponding output. A sensor is a type of transducer. Sensors may provide various types of output, but typically use electrical or optical signals. For example, a thermocouple generates a known voltage (the output) in response to its temperature (the environment). A mercury-in-glass thermometer, similarly, converts measured temperature into expansion and contraction of a liquid, which can be read on a calibrated glass tube.

1.6.2 Sensitivity

A sensor's sensitivity indicates how much the sensor's output changes when the input quantity being measured changes. A good sensor obeys the following rules.

1. It is sensitive to the measured property,
2. It is insensitive to any other property likely to be encountered in its application, and
3. It does not influence the measured property.

The sensitivity is, therefore, defined as the ratio between the output signal and measured property. For example, if a sensor measures temperature and has a voltage output, the sensitivity is a constant with the unit [V/K], this sensor is linear because the ratio is constant at all points of measurement.

1.6.3 Resolution

The resolution of a sensor is the smallest change it can detect in the quantity that it is measuring. Often in a digital display, the least significant digit will fluctuate,
indicating that the changes of that magnitude are only just resolved. The resolution is related to the precision with which the measurement is made. For example, a scanning tunneling probe (a fine tip near a surface collects an electron tunneling current) can resolve atoms and molecules.

### 1.6.4 Sensors in living organisms

All living organisms contain biological sensors with functions similar to those of the mechanical devices described. Most of these are specialized cells that are sensitive to:

1. Light, motion, temperature, magnetic fields, gravity, humidity, moisture, vibration, pressure, electrical fields, sound, and other physical aspects of the external environment
2. Physical aspects of the internal environment, such as stretch, motion of the organism, and position of appendages (proprioception)
3. Environmental molecules, including toxins, nutrients, and pheromones
4. Estimation of biomolecules interaction and some kinetics parameters
5. Internal metabolic indicators, such as glucose level, oxygen level or osmolality
6. Internal signal molecules, such as hormones, neurotransmitters, and cytokines
7. Differences between proteins of the organism itself and of the environment or alien creatures.

### 1.6.5 Classification

Sensors can be broadly classified into two categories such as biosensors and chemical sensors.

#### 1.6.5.1 Biosensors

Biosensors are defined as the sensors that use biomolecules and/or structures to measure something with biological significance or bioactivity such as proteins.
nucleotides and even tissues [135]. Within these sensors, the active sensing material on the electrode should act as a catalyst and catalyze the reaction of the biochemical compounds to obtain the output signals [136, 137]. This terminology can be applied for both in vitro and in vivo applications. The encapsulation of the biological component in biosensors, presents a slightly different problem when compared to the ordinary sensors, this can either be done by means of a semi-permeable barrier such as a dialysis membrane or by a hydrogel, which either physically constrains the sensing macromolecule or chemically constrains the macromolecule by bounding it to the scaffold.

1.6.5.2 Chemical Sensors

Chemical sensors (CS) are defined as "Devices which give the result of a chemical interaction or process between the analyte and the sensor device, transforms chemical or biochemical information of a quantitative or qualitative type into an analytically useful signal". It provides information about chemical composition, phase i.e. whether liquid or a gas phase of the analyte [138].

![Image of a chemical sensor]

**Figure 1.16** Schematic picture of a chemical sensor

One can say that the chemical sensor is the "eye" of the process control system. CS contains a physical transducer and a chemically sensitive layer or
recognition layer. The functioning of a chemical sensor involves two main steps, they are

A) Recognition- Analyte molecules interact selectively with receptor molecules or sites included in the structure of the element of the sensor.

B) Transduction- It is a physical parameter that provides output signal. The block diagram of the typical chemical sensor is shown in figure 1.16, where the output may be in the form of light, potential etc.

1.6.6 Types of Chemical Sensors

Chemical sensors are categorized into the following groups depending on the transducer type: (a) Electrochemical Sensor, (b) Mass Sensitive sensor, (c) Heat Sensitive sensor, (d) Optical sensor. Various sensing processes are shown in the figure 1.17.

![Figure 1.17 Various sensing processes](Image)
1.6.6.1 Electrochemical Sensors (ES)

Electrochemical Sensors (ES) are the oldest group of chemical sensors. According to IUPAC (International Union of Pure and Applied Chemistry), an electrochemical sensor is defined as a self contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition which is retained in the direct spatial contact with an electrochemical transduction element [139].

The principle of electrochemistry is the transfer of electron(s) to/from an electrode from/to a solution redox species. During this process, some chemical changes take place at the electrodes and the change is conducted through the bulk of the sample phases. Both the electrode reactions and/or the charge transport can be modulated chemically and serve as the basis to the sensing process.

Electrochemical measurements require a closed circuit. This means at least two electrodes are essential to constitute an electrochemical cell. Electrochemical cells are divided into two groups. Galvanic cells transform spontaneously chemical energy into electrical energy where as electrolytic cells need an applied potential higher than their own reversible potential to force the desired chemical reaction. The charge transport in the sample can be electronic, ionic or mixed but within the transducer part of the sensor and/or inside the supporting instrumentation which is a part of the whole circuit, it is always electronic.

1.6.6.2 Mass Sensitive Sensors (MSS)

These make use of the piezoelectric effect, include devices such as the surface acoustic wave sensor and are particularly useful as gas sensors. They rely on a change in mass on the surface of an oscillating crystal which shifts the frequency of oscillation. The extent of the frequency shift is a function of the amount of material absorbed on the surface.

1.6.6.3 Heat Sensitive Sensors (HSS)

The heat of a chemical reaction involving the analyte is monitored with transducers such as thermistor or a Platinum thermometer. They are often called calorimetric sensors. Compared to optical, mass and thermal sensors, electrochemical
sensors are especially attractive because of their remarkable detectability, experimental simplicity and low cost. They have a leading position among the presently available sensors that have reached the commercial stage and which have found a vast range of important applications in the fields of clinical, industrial, environmental and agricultural analyses.

1.6.6.4 Optical Sensors (OS)

Optical Sensor is a device that consists basically of a light source, wave-guide arrangement and an appropriate optical detector change in optical parameters like alteration in light absorption as well as refraction or the effects of chemiluminescence, fluorescence and phosphorescence etc., caused by the analyte are used as the signals on optical sensing. Some optical sensors are based on non-linear optical effects like the evanescent wave or the surface plasmons or light scattering. For use in a biosensor, photodiodes have been proposed in combination with light emitting diodes to detect absorption changes promoted by a biocatalyst. Optical fibers can also be used as sensors e.g. as catheters. There are two concepts in applying fiber-optics sensors. In chemically mediated fiber optic biosensors, both fibers lead to a reaction chamber containing some dye or indicator solution, which is separated from the sample solution by a gas-permeable membrane as shown in figure1.18. Fiber optic sensors are built very small. Thus a small triple sensor of 1 mm diameter measuring pH, oxygen and CO₂ in blood has been commercialized. A needle type fiber-optic sensor of 0.75 mm for in vivo measurements of pH, O₂ and the O₂ has been employed to measure O₂ levels in dog eyes as an animal model for diabetic retinopathy. The general knowledge of optic devices over other systems is the ability to monitor multiple wavelengths with a single optical fiber. In addition, a separate reference elements are not required and the intensity ratio measurements minimize the need for sensor recalibration.
1.6.6.5 Amperometric sensors (AMS)

In the field of electrochemical sensors amperometric measurements play a vital role. The AMS produces current signal, which is related to the concentration of the analyte by Faraday’s law and the laws of mass transport. The schematic picture of AMS is shown in figure 1.19. It is operated in a region where mass transport is limiting and therefore it has a linear response with the concentration of the analyte. This type of sensors was developed with different geometries for a broad range of analytes, such as CO, nitrogen oxides, H₂S, O₂, glucose, unique gas like hydrazine and many other vapours [140]. By using micro fabrication techniques, the entire sensor can be assembled on a chip or be part of a m-TAS (micro fabricated total analytical system).
1.6.6.6 Potentiometric Sensors (PS)

These sensors measure the potential difference between the transuding electrode and a reference electrode under zero current flow. Working principle of potentiometric sensors are electric potential develops at the surface of a solid material immersed in solution containing ions that exchange at the surface.

1. The potential is proportional to the number or density of ions in the solution. When potential difference between the surface of the solid and the solution occurs because of charge separation at the surface.

2. To ensure that the potential is measured accurately, and therefore that the ion concentration is properly represented by the potential, it is critical that the current drawn by the measuring instrument is as small as possible (any current is a load on the cell and therefore reduces the measured potential).

3. For a sensor of this type to be useful, the potential generated must be ion specific that is, the electrodes must be able to distinguish between solutions.

4. These are called ion-specific electrodes or membranes.

5. The four types of membranes are:

6. Glass membranes selective for $H^+$, $Na^+$ and $NH_4^+$ and similar ions.

7. The electrochemical interface senses the concentration of some products of the reactions by monitoring the consumption or formation of ions ($H^+$, $NH_4^+$).

1.6.6.7 Solid electrolyte sensors (SES)

These sensors are designed to operate at high temperature and can operate in either a potentiometric or amperometric mode as shown in figure 1.20. The sensor response is described by using the Nernst equation (equilibrium). The surface of a solid electrolyte is coated with an auxiliary phase which will react electrochemically and reversibly with the analyte and generate with an interfacial potential. Sensitivity and selectivity of the analytes are provided by the auxiliary phase. An important advantage of this approach is the development of detection methods that survive, harsh conditions where typical liquid electrochemical sensors would be inappropriate.
The catalytic activity of the electrode material is particularly important, e.g., the Pt/Au sensor can measure CO and hydrocarbons due to the difference in catalytic activities between the Pt and Au electrodes. Solid electrolytes are used to replace the liquid electrolyte in an electrochemical sensor.

**Figure 1.20 Solid Electrolyte Sensor**

1.6.6.8 Piezoelectric sensors (PZS)

Piezoelectric is a greek word derived from the piezen, it was discovered in 1880 by French physicists Jacques and Pierre Curie. The word Piezoelectricity means electricity released due to pressure. It is defined as the electric charge that accumulated on certain solid materials like ceramics, crystals and biological matters like bone, DNA etc. The principle of piezoelectric sensor is a physical dimension, transformed into a force that acts on two opposing faces of the sensing element and it is used for the measurement in gas phase. These types of sensors are especially used with high frequency sound in ultrasonic transducers for medical imaging and also industrial non destructive testing (NDT). It effects linear electrochemical interaction between the electrical and mechanical state in crystalline materials with no inversion symmetry.

1.6.6.9 Conductometric Sensors (CS)

The conductance of the cell is measured by alternating current bridge method. Many chemical reactions produce or consume ions. These result in a change of the
conductivity of the electrolyte. By applying an alternating current (with a frequency of approximately 1 KHz), migration rates of all the species of ions in the electrolyte can be measured. The resistance of the electrolyte is determined by all ions present in the solution. The advantages of these sensors are that they can be easily manufactured by common microelectronic process.

1.7 OBJECTIVES AND SCOPE

The main objective of this study is to develop new electrochemical sensors for the effective resolution of some physiologically important biomolecules such as dopamine, ascorbic acid and uric acid, which co-exist in the extracellular body fluids of mammalian brain. The specific research objectives are as follows:

- Fabrication of cost effective, easy to prepare modified carbon paste electrodes for the simultaneous determination of DA, AA and UA.
- Finding the effect of double modification i.e. both bulk modification and surface modification, on the sensitivity and selectivity of the electrodes for the detection of the biomolecules.
- Using cheap and simple organic, inorganic compounds for both bulk modification and surface modification to achieve good detection and quantification limits.
- Investigation on the optimum conditions of pH, number of cycles of polymerization and electrode mechanism.
- Testing of fabricated electrodes in real sample analysis for their efficacy.
- Proposing possible and appropriate mechanisms for the interaction between the analytes and the modified electrodes.
- In the present research work four types of modified electrodes were prepared as given below.

1. Zirconia/poly(oxalic acid) modified carbon paste electrode for the detection of UA in the presence of AA and DA.

2. Poly(benzoic acid) modified carbon paste electrode for the detection of UA in the presence of AA and DA.
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3. Poly(cinnamic acid) modified carbon paste electrode for the determination of DA in the presence of AA and UA.

4. Montmorillonite K_{10}-clay /poly(glycine) modified carbon paste electrode for the determination of AA in the presence of DA and UA.

If the modified electrodes prepared have better resolution ability, detection and quantification limits, stability, cost effectiveness, easy to fabricate, then they can be fabricated commercially and have the scope to be used in clinical, biochemical and pharmaceutical industries for the detection of all biologically significant biomolecules. The detailed description of the present study i.e. experimental work, results and analysis, conclusions drawn are presented in the forthcoming chapters of the thesis.
1.8 REFERENCES


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