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This work and its defense approved by:

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SiC-C Composite Microelectrode for Biomedical Applications

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ABSTRACT

Microelectrodes implanted in the brain as transducers for neuronal impulses should ideally constitute dual functionality, i.e. stimulating and recording to avoid use of separate electrodes, smaller size for highly localized response, impedance stability for lesser power consumption, and non-metallic behavior to avoid MRI interaction. In this work a composite fiber microelectrode (140 µm), was developed, such that it has two conducting elements (Carbon) well insulated and separated with Silicon Carbide (SiC), concentrically. In vitro studies were done to use the outer carbon layer as concentric ring stimulating electrode while recording from inner carbon core. An electrolytic etching technique was developed for fabricating electrode tips from SiC-C composite fiber. SEM was used to study carbon surface morphology, different tip geometries and shapes. Cyclic Voltammetry and Electrochemical Impedance Spectroscopy were used for the electrochemical characterization and modeling. The mechanics of redox and transport properties of the system were studied using ferrocene oxidation experiment. Effects of various stimulus waveforms and frequencies on charge transfer capability were studied. Different multielectrode configurations and designs were fabricated and tested for inductive charge development, electrical cross-talk and impedance change. Reversible charge injection limits were established using current and potential transient measurements, PH and temperature change, and direct observation of faradic processes. Biochemical sensing of neurotransmitters including Dopamine Hydrochloride and Vitamin C was done with the SiC-C composite electrodes and oxidation currents were found to vary linearly with concentration. In-Vivo action potential recordings from anesthetized rat’s brain with very high signal to noise ratio and firing response to
external pressure stimulus were successfully obtained using novel SiC-C microelectrodes. The effectiveness of SiC-C composite microelectrodes, to electrically stimulate rat’s brain, was established by inserting these electrodes into motor cortex of rat’s brain and studying evoked motor functions.
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Chapter-1
Background and Literature Review

**Introduction**

Neurotechnology attempts to connect the electronic world to the neural world, via electrode systems, with the goal of restoring sensory and neuromuscular deficits in affected individuals [1]. Each type of cell exhibits a characteristic electrical activity and measurement of this activity yields important information relating to the cellular function. From this fact has developed the clinical study of bioelectric signals, which deals with the measurement of the electrical activity of large number of cells. Because dysfunction frequently reveals itself in the bioelectric signal, much diagnostic information can be obtained from such recordings [2]. The electrical excitability phenomenon of the nerve, through which propagated action potentials can be initiated, can help to restore function in people with neurological handicaps [3]. One of such phenomenon called DBS, Deep Brain Stimulation, has helped in ameliorating the various symptoms of Parkinson Disease (PD). DBS uses voltage pulses (~2V amplitude) at frequencies of (100-150Hz) through implanted electrodes for stimulation of the thalamus or basal ganglia to treat movement disorders [4, 5]. The efficacy of DBS therapy can be improved by localizing the current density into specific population of neurons and by increasing the power efficiency through proper selection of electrode geometry. During MRI procedure, wave interaction with the magnetic field, particularly for metal (Pt/Ir) electrodes, tend to obscure proper imaging [6, 7]. In order to precisely identify the area of the brain, microelectrode recording is performed prior to the implantation of the stimulating electrode. Recording electrodes form repeated passages during implantation and withdrawal, followed by stimulation electrode insertion. But still the stimulation site could easily be shifted, due to
loss of cerebral spinal fluid during recording procedure. Thus, it is very important to address the above mentioned issues in the design and fabrication phases of an electrode for recording and stimulation of physiological activities. A new electrode design should assure dual functionality, i.e. stimulating and recording to avoid use of separate electrodes, smaller size for highly localized response, impedance stability for lesser power consumption, non-metallic behavior to avoid MRI interaction. Stimulation therapy has also been extended to the treatment of epilepsy, control of pain, dystonia, as well as certain psychological affliction. While significant effort has been put into finding optimal anatomical targets for electrode implantation, relatively little effort has been devoted to design of electrodes [8, 9]. These procedures can be made more accurate, less invasive and streamlined further by using advanced and improved microelectrodes.


### Biological Background

#### 1.1 Concept of Action Potential

The human body is made up of trillions of cells. The cells of the nervous system, called nerve cells or neurons, are specialized to carry messages through an electrochemical process [10]. Neurons are non-spherical cells consisting of a cell body (soma), many short dendritic processes, and one large protrusion called an axon (Fig.1.1). Axonal diameter may vary between <1 um and > 20 um. Muscles are controlled by up to a few hundred motor neurons per muscle. Neurons communicate information among themselves, muscles and organs via electrical potentials, called Action Potentials (Spike).

Action potentials are short excursion, transient waveforms with a typical duration of 1 ms. The transmembrane resting potential, is approximately -70 mV inside versus outside of the cell and is due to the ionic gradient across the enveloping membrane [11]. The concentration of Na$^+$ outside the cell is 10 times greater than that inside the cell and concentration of K$^+$ inside the cell is 20 times greater than that outside the cell. The interior of the cell is negative with respect to the exterior. The semipermeable cell membrane has Na$^+$ and K$^+$ ion channels that plays a critical role in the generation and

---

**Figure 1.1:** Picture Showing a neuron cell and its different parts. [12]
propagation of action potential, as they carry ionic currents through the membrane, thereby perturbing the resting potential.

Certain external stimuli of adequate intensity reduce the charge across the plasma membrane to cause local depolarization. This external stimuli can be mechanical stimuli (e.g., stretching, sound waves) or through neurotransmitters, both of which help to open sodium channels in the membrane. Diffusion of sodium into the cell reduces the resting potential to threshold voltage. The sudden complete depolarization of the membrane opens up more of the voltage-gated sodium channels in adjacent portions of the membrane (Fig 1.2). In this way, a wave of depolarization sweeps along the cell, and an action potential is generated in the cell. As shown in the figure 1.3, after the depolarizing phase, repolarization is established by the facilitated diffusion of potassium ions out of the cell. The neuron is finally rested only when the sodium ions that came in at each impulse are actively transported back out of the cell. An undershoot phase is due to the hyper-polarization caused by the opening of chloride and potassium channels by neuro-transmitters which inhibit the transmission of nerve impulses. In each case, opening of the channels increases the membrane potential by letting negatively-charged chloride ions (Cl\(^-\)) IN and positively-charged potassium ions (K\(^+\)) OUT. A single neuron,
especially one in the central nervous system has thousands of other neurons synapsing on it. Some of these release activating (depolarizing) neurotransmitters; others release inhibitory (hyperpolarizing) neurotransmitters.

![Diagram of action potential phases](image)

**Figure 1.3:** Figure showing different phases involved in action potential [12]

### 1.2 Neural and Muscular Stimulation

Neural and muscular tissue can be activated electrically, either in the natural way or by artificial stimulation, to perform two basic functions: information transport and muscular contraction, respectively [2]. Local extracellular application of a depolarizing (cathodic), monophasic, short current pulse may excite an action potential. Negative going current pulse close to the cell membrane which already has a potential gradient such that inside of the cell is more negative, produces a depolarizing effect and an action potential is generated. An effective stimulus is an environmental change that produces a characteristic and reversible response in a tissue. The response occurs after initiation of the stimulus and usually outlasts it [3]. The chemically reversible charge injection is very important as it should not leave any reaction byproducts near the stimulation site [13]. The charge densities required for some types of neural stimulation (Intracortical; 400-3200 \( \mu \text{C/cm}^2 \)) clearly exceed the reversible charge injection limits for most of the conventional electrode materials including platinum (250 \( \mu \text{C/cm}^2 \)) and its alloys [14].
Stimulation requires flow of ionic charge in the biological tissue, which can be induced by two mechanisms: Capacitive and Faradic.

### 1.2.1 Capacitive mechanism:

The capacitive mechanism involves charge delivery without any electron transfer. A layer of adsorbed oriented water molecules on the metal electrode surface acts as a dielectric double layer. Charging and discharging of this double layer is an ideal process as no chemical changes can occur in the tissue, but this tissue limits the amount of charge delivered to the tissue. The structure is equivalent to a parallel plate capacitor. The electrical characteristics of the double layer can be expressed in terms of the differential capacity or double layer capacitance, \( C_{\text{dl}} \):

\[
C_{\text{dl}} = \frac{dQ}{dE} \quad (1.1)
\]

Where \( E \) is the electrode potential with respect to reference electrode and \( Q \) is the surface charge density [15]. The value of \( C_{\text{dl}} \) is about 10-20 µF/cm\(^2\) of real electrode area. This value is large as compared to normal capacitance standards because of the very thin one water molecule dielectric double layer.

The amount of charge that can be injected with a metal electrode by double layer charging is only about 20 µC/cm\(^2\). Excess charge densities than this can lead to dielectric breakdown and the onset of faradic reactions. Highly dielectric materials can provide an increase in the quantity of charge delivered involving purely capacitive processes.

### 1.2.2 Faradic Mechanism:

The faradic mechanism involves electron transfer and thus results in oxidation and reduction of some chemical specie. This mechanism is widely accepted because the charge required to elicit a physiological response is much higher than can be obtained from the charging and discharging of a double layer capacitor [3]. This mechanism is
further divided into reversible and irreversible processes, depending on whether the oxidation-reduction reactions introduce new chemical species into the solution.

1.2.2.1 Reversible Faradic Processes:

Reversible reaction involves species that remain bound to the electrode surface and can be reversed by passing a current in the opposite direction, so that no new chemical specie is produced in the bulk solution. Oxide formation and H-atom plating on platinum and other noble metals are examples of reversible electrochemical interaction.

**Oxide formation and reduction:**  
\[ \text{Pt} + \text{H}_2\text{O} \rightleftharpoons \text{PtO} + 2\text{H}^+ + 2\text{e}^- \]  
(1.2)

**H-atom plating [16]:**  
\[ \text{Pt} + \text{H}^+ + \text{e}^- \rightleftharpoons \text{Pt-H} \]  
(1.3)

\[ \text{Pt} + \text{H}_2\text{O} + \text{e}^- \rightleftharpoons \text{Pt-H} + \text{OH}^- \]  
(1.4)

Valence transition and proton or hydroxyl ion transfer reactions also come under reversible process category. Multilayer oxide films of Iridium, Ruthenium or Rhodium typically show these reactions [17].

1.2.2.2 Irreversible Faradic Processes:

Irreversible reactions, on the other hand leave some products which have escaped from the electrode surface in the bulk. Electrode corrosion and oxidation of organic molecules contained in the tissue are outcomes of irreversible faradic process. e.g.

(i)  
\[ \text{Pt} + 4\text{Cl}^- \rightleftharpoons [\text{PtCl}_4]^{2-} + 2\text{e}^- \]  
(1.5)

(ii) The electrolysis of water to form H\(_2\) and O\(_2\) accompanied by shifts in pH.  
\[ 2\text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons \text{H}_2 + 2\text{OH}^- \]  
(1.6)

\[ 2\text{H}_2\text{O} \rightleftharpoons \text{O}_2 + 4\text{H}^+ + 4\text{e}^- \]  
(1.7)

The above reactions may alter the chemical composition of the tissue fluid, producing biologically toxic products, or generating extremes of alkanity or acidity, which are highly undesirable.
### 1.2.3 Stimulation Charge and Charge Densities:

Table 1.1 shows typical charge densities and charge/pulse for different sized and material electrodes implanted at various locations in the body.

<table>
<thead>
<tr>
<th>Application</th>
<th>Electrode material</th>
<th>Electrode surface area</th>
<th>Charge/Pulse (nC)</th>
<th>Charge Density µC/cm² (geom.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intramuscular</td>
<td>Stainless 316LVM</td>
<td>0.1 cm²</td>
<td>5000</td>
<td>50</td>
</tr>
<tr>
<td>Peripheral Nerve</td>
<td>Platinum</td>
<td>0.1 cm²</td>
<td>600-2500</td>
<td>6-25</td>
</tr>
<tr>
<td>Cortical Surface</td>
<td>Platinum</td>
<td>0.01 cm²</td>
<td>200-1000</td>
<td>20-100</td>
</tr>
<tr>
<td>Intracochlear</td>
<td>Platinum</td>
<td>$5.6 \times 10^{-3}$ cm²</td>
<td>100-180</td>
<td>18-32</td>
</tr>
<tr>
<td>Intracochlear</td>
<td>Platinum</td>
<td>$10^{-3}$ cm²</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Intracortical</td>
<td>Pt - Ir</td>
<td>$5 \times 10^{-4}$ cm²</td>
<td>2-86</td>
<td>4-172</td>
</tr>
<tr>
<td>Intracortical</td>
<td>Pt – Ir Activated Ir</td>
<td>$20 \times 10^{-6}$ cm²</td>
<td>8-64</td>
<td>400-3200</td>
</tr>
</tbody>
</table>

Table 1.1: Charge Densities Employed in Neural Stimulation [2].

Stimulation sites requiring high stimulus charge should employ electrodes with large surface area in order to maintain the charge densities within their reversible charge injection limits. Auditory prostheses typically require lower stimulus charges, but the electrodes must be small enough to fit into the cochlea.

### 1.2.4 A Surgical technique: Deep Brain Stimulation

This surgical approach consists of implanting an electrode in the brain, which is then connected to an implantable pulse generator (battery) by an extension wire placed underneath the skin (fig 1.4). DBS involves the delivery of voltage pulses (about 2V in amplitude) at high frequencies (100-150Hz) through an implanted electrode in which the tip is located in either the subthalmus nucleus (STN) or the globus pallidus interna (GPi) of the brain. The DBS procedure is less invasive, allows for bilateral stimulation, creates fewer complications, and is reversible [18]. Various mechanisms have been proposed in an attempt to explain how Deep Brain Stimulation (DBS) works. Effects produced by
DBS are similar to those produced by making a lesion, so it has been proposed that the overall effect of DBS is to inhibit the neural activity in the region stimulated [19].

There has been an observed increase in the incidence of Parkinson’s disease (PD) amongst the population aged 60 and above. PD results from degeneration of the dopamine-synthesizing neurons in the substantia nigra of the brain. Various surgical procedures have been performed to treat patients with PD. The outcome has been somewhat variable, with some patients getting relief while others showed no improvement. Following the implementation of drug treatment in the 1960’s, the number of patients undergoing surgery diminished greatly [20].

Unfortunately for these patients, prolonged usage of levodopa and other dopamine-receptor agonists led to the development of motor complications. Animals by the inhalation route developed the manifestation of Parkinsonian symptoms as seen in

Figure 1.4 Picture showing Deep Brain Stimulation surgical procedure [18]
humans. Monkeys treated with MPTP display all the cardinal symptoms of PD. Physiological recordings from the animals treated with the synthetic chemical 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) showed that the neuronal firing rate of cells in both the GPi and STN is higher, whereas the firing rate of cells in the globus pallidus externa (GPe) is lower when compared to control animals [21]. Similar changes in neuronal activities are also found in PD patients undergoing surgery [22]. Evaluation of the effectiveness of high frequency stimulation in PD patients indicated that their tremor was controlled adequately [23]. The chronic implantation of stimulating electrodes deep in the thalamus gave rise to the procedure known as Deep Brain Stimulation (DBS). Reduction in the effectiveness of stimulation with time has been observed.

1.3 Neuronal Recording

Nerve fibers attached to sensors such as muscle spindle, tendon organs, and cutaneous sensors, gives selective feedback information which help in attaining control of muscle via artificial stimulation. Recording of the action potential is done by inserting electrodes in the tissue which measures potential difference across them. Depending on the position of microelectrode in the vicinity of neuron, neuronal recording is divided into following categories:

1.3.1 Extracellular Recording:

The coordinated activity of neurons organized into networks is believed to act as the major computational units in the brain. In order to understand how the spatio-temporal organization of activity in networks of neurons transform information it is necessary to use high resolution monitoring techniques. Extracellular electrophysiology is currently the best tool for performing high-resolution recording from neural tissue. It offers
information about the spiking (output) and synaptic activity (input) of neurons in the recorded area. These neurons are understood to be organized into networks, layers, maps, and systems in which their coordinated activity mediates the emergence of a large diversity of behaviors [24].

During extracellular recording a conductive probe inserted into the space between living neurons. There, it measures the combined electrical potential of the local population of cells, relative to some reference. When cells in the neighborhood fire synchronously, which we can often persuade them to do, the combined potentials can be large enough to be measured effectively. Since the fluctuations in the local field potential that occur in the brain are commonly less than a millivolt, the signal must be amplified so that it may be detected and recorded by a common personal computer. In the process of amplifying the signal, it is useful to filter the signal to remove very low (<1Hz) and very high (>3kHz) frequencies. Multiple-electrode arrays can be used to help localize the foci of these electrical events. Recording local field potentials from extracellular electrodes can provide 3D information about the cooperating synaptic inputs into a recorded area. When neurons in a network simultaneously receive many concerted inputs, this population event can be recorded as local voltage fluctuations in the extracellular field. If these population events occur regularly, the resulting voltage fluctuations can be seen to rhythmically oscillate. Recording field potentials has revealed that network activity in the brain regularly shifts between two functional states and shows different types of population synchrony within these states [25].

1.3.2: Intracellular-Recording:

Intracellular recording involves measuring voltage and/or current across the
membrane of a cell. To make an intracellular recording, the tip of a fine (sharp) microelectrode must be inserted inside the cell, so that the membrane potential can be measured. Typically, the resting membrane potential of a healthy cell will be -60 to -80 mV, and during an action potential the membrane potential might reach +40 mV. Most microelectrodes used for intracellular recording are glass micropipettes, with a tip diameter of < 1 micrometre, and a resistance of several megaohms [26]. The micropipettes are filled with a solution that has a similar ionic composition to the intracellular fluid of the cell. A chlorided silver wire inserted into the pipet connects the electrolyte electrically to the amplifier and signal processing circuit. The voltage measured by the electrode is compared to the voltage of a reference electrode, usually a silver-silver chloride wire in contact with the extracellular fluid around the cell (figure 1.5). In general, the smaller the electrode tip, the higher its electrical resistance, so an electrode is a compromise between being small enough to penetrate a single cell with minimum damage to the cell, while having a low-enough resistance that small neuronal signals can be discerned from thermal noise in the electrode tip.

Figure 1.5 Voltage clamp measurement circuit[27].
Intracellular technique can be further divided into voltage clamp and current clamp measurements. The “voltage clamp” technique allows an experimenter to "clamp" the cell potential at a chosen value. This makes it possible to measure how much ionic current crosses a cell's membrane at any given voltage. This is important because many of the ion channels in the membrane of a neuron are voltage-gated channels -i.e. they are open only in a certain voltage range, and understanding how they work is important to understanding how neurons process information.

"Current Clamp" describes recording the trans-membrane voltage with the ability to inject current into a cell through the recording electrode. Unlike in the voltage clamp mode, where the membrane potential is held at a level determined by the experimenter, in "current clamp" mode the membrane potential is free to vary, and the amplifier records whatever voltage the cell generates on its own or as a result of stimulation. This technique is used to study how a cell responds when electrical current enters a cell; this is important for instance for understanding how neurons respond to neurotransmitters that act by opening membrane ion channels.

1.3.3 Patch Clamp Recording [27]: Patch clamp recording uses a similar electrode as intracellular, but rather than piercing the cell membrane, the electrode tip is sealed to the membrane without breaking it (figure 1.6). With this technique, current flow through individual ion channels can be measured directly (if the patch is sufficiently small, it may contain a solitary ion channel). The patch may also be pulled right off the cell, allowing control of the environment on both sides of the membrane. Or, the patch may be ruptured, leaving the cell attached directly to
the electrode. A disadvantage (compared to conventional intracellular recording with sharp electrodes) is that the intracellular fluid of the cell mixes with the solution inside the recording electrode, and so some important components of the intracellular fluid can be diluted.

1.4 General Considerations for Electrode Design:

The design of the electrodes is important for selective and controlled activation of populations of neurons in three ways. First, the electrode geometry can affect the spatial distribution of current density over the electrode surface, a cofactor with charge in stimulation induced neural damage [28]. Second, the electrode geometry can affect the pattern of neural excitation by determining the electric field generated in the tissue medium [29]. Third, the electrode design can affect electrode impedance, a concern in designing electrodes as it impacts power consumption. These elements are linked, in that current density \( J \) and electric field \( E \) are related by Ohm’s law,

\[
J = \sigma E
\]  

(1.8)

and the impedance \( Z \) depends on the current density distribution over the electrode surface,

\[
Z = \frac{V}{\int_S J \cdot dS},
\]

(1.9)

where \( V \) is the potential drop across the electrode–electrolyte interface and \( S \) is the electrode surface area. Also, Implantable electrodes must be able to survive within the rather hostile environment of the body without causing significant tissue reaction. Implantation of an object into the body initiates a “foreign body response” involving the body’s natural defensive systems that can range from simple encapsulation of the object.
to hemorrhage, inflammation, and necrosis [30]. There are three basic biocompatibility issues which need to be considered while designing an electrode system:

1.4.1 Chemical Biocompatibility:

Chemical reactions occur at the interface between body tissue and implanted electrode which can result in severe pH shifts in the tissue around the electrodes. Besides being concerned with local pH shifts, which can cause irreversible changes in the tissue protein, one also has to be wary of metallic dissolution products, gross hydrogen and oxygen gas bubbles, and oxidized organic and inorganic species [31]. This occurs as a result of faradic (oxidation-reduction) reactions. Electrode materials thus used should be inert and if any chemical reaction is expected, it should be minimal with inert resulting products. The use of biphasic stimulus waveform with zero net charge flow, or balanced waveforms, help in minimizing faradic products. This promotes reversible reactions as discussed in section 1.2.2.1. Also capacitive charge injection mechanism with charging and discharging of double layer also gives a safer alternative to faradic stimulation process.

1.4.2 Mechanical and Geometrical Biocompatibility:

Flexibility, Strength, and durability of the implanted electrode constitute the mechanical compatibility of device with the surrounding tissue. Implanted device should have similar mechanical properties to the surrounding tissue. It is also important to consider the likelihood of motion within the implant site. Nerves and tissues which are subjected to a wide range of motion must be as soft and flexible as possible to prevent mechanically induced neural damage [32]. Surface displacements in anesthetized rats were found to be in the order of 2–25 μm due to respiration and 1–3 μm due to vascular
pulsations. In chronic microelectrodes, probe ‘micromotion’, relative displacements of the probe with respect to the brain tissue, could be attributed to forces resulting from rotational acceleration [33]. ‘Micromotion’ is believed to be the causative factor for tethering forces acting on the probe. Researchers suggest that stiffness of the substrate and the interconnect could translate the tethering forces acting on them into interfacial strains at the probe–tissue interface, hence causing damage to the surrounding brain tissue [34]. It is also believed that a relatively inflexible probe in the brain microenvironment could cause a shear-induced inflammatory response [35]. This could be due to the large mismatch in the stiffness of the probe substrate and the brain tissue (Young’s moduli of bulk silicon and brain are ~200 GPa [2] and 6 kPa [1] respectively). Hence, there is a good basis for positing a direct correlation between the strains induced around the implant and the chronic reactive tissue response.

Subbaroyn et. al. developed a finite element model of the mechanical effects of implantable microelectrodes in the cerebral cortex [37]. They observed that the strain along the sidewall of implants is due to frictional shear stresses induced by the longitudinal displacement or ‘poking’ action of the probe. The shear stress increases from the surface of the brain to the tip of the probe track (figure 1.7(a)).

The radial deformation of the brain tissue could also be compared to crack propagation in brittle materials. The ‘poking’ action of the probe is analogous to mode I or opening mode of a crack. From basic fracture mechanics, the primary tensile stress in front of the crack tip varies with distance [36], as

$$\sigma = \frac{K}{(2\pi r)^{0.5}} = \frac{k}{r^{0.5}}. \tag{1.10}$$
where $K$ is the stress intensity factor, $\sigma$ is the tensile stress in front of the crack tip and $r$ is the distance from the tip,

$$k = \frac{K}{(2\pi)^{0.5}}. \quad (1.11)$$

The von Mises stress data for a radial force when fitted with an inverse square root relation to the longitudinal distance from the tip resulted in an $R^2 = 0.9872$ (figure 1.7(b)). This result suggests that a ‘poking’ deformation could lead to extensive compression, or in the worst case, tearing of the tissue underneath the tip.

![Figure 1.7](image)

Figure 1.7. (a) Frictional shear stress increased with the distance from the surface of the brain tissue and was maximum at the tip. (b) von Mises stress has an inverse square root dependence on the longitudinal distance from the tip, analogous to mode I of a crack [37].

Howard et. al. calculated the peak force associated with inserting a microelectrode wire in live brain and compared to 0.5 % agar gel and 1 % agar gel (figure 1.8). It is seen that around 1 mN force is needed to insert the microelectrode into the human brain [38].

To maximize their flexibility and compliance, electrode assemblies should be made as small as is practical and lead wires are often wound into helical coils. A primary goal is also to minimize the size of the electrode (µm range), except when constriction of charge density may occur. Sharp edges and blunt corners of the implant should be avoided in
favor of a tapered configuration. Surfaces can be smooth or rough depending on the tissue ingrowth requirements. If tissue ingrowth is to be avoided, so as to preserve mobility, the implant should be smooth [39]. If it is desirable that the implant be anchored by connective tissue, a rough, porous, or open structure is recommended.

Figure 1.8: Peak force associated with inserting microelectrode wire 2 cm at approximately 0.33 mm/s into 0.5% and 1% agar gel for comparison to peak force extrapolated from Howard (1999) data [38]
1.5 Microelectrode Background

1.5 Microelectrodes

A microelectrode is an electrode with at least one dimension small enough that its properties, e.g. mass transport regime, are a function of size. Microelectrodes also are defined as electrodes whose critical dimension is in the micrometer range. If the geometric dimensions of a voltammetric working electrode become progressively smaller then the behavior of the electrode begins to depart from that of a large electrode which can be approximated by an electrode of infinite dimension. These differences are caused by changing conditions of the mass transport from the bulk of solution toward the electrode and have several important practical implications, such as a decreased ohmic drop of potential, $IR$, fast establishment of a steady-state signal, a current increase due to enhanced mass transport at the electrode boundary, and increased signal-to-noise ratio. These effects make sufficiently small electrodes advantageous in many areas of electroanalytical chemistry. The development in microelectronics technology made it possible to measure reliably very low currents and to construct reproducibly very small electrodes. The application of small-size electrodes ranges from analytical chemistry (e.g., the need for electrodes in miniature cells in detection for high-performance separations or in electrochemical sensors) to biochemistry (in situ electroanalytical measurements on living organisms) [40].

1.5.1 Theoretical Aspect

The most important factor determining the behavior of an electrode is the mass transport in solution in the vicinity of the electrode. When considering an uncomplicated charge-
transfer reaction, then the voltammetric current signal is proportional to the flux of the electroactive substance toward the solution–electrode interface described by Fick’s first law. Fick’s second law then describes the time-dependent changes in the concentration of the electroactive substance amount caused by the flux—the partial derivative of the concentration with respect to time equals the divergence of the flux vector [41]. When the electrode potential is switched from a value at which no electrode reaction occurs to that corresponding to the limiting current of the electrode reaction, then the concentration of the electroactive substance at the electrode surface decreases to zero and a concentration gradient develops over a certain distance from the electrode surface into the bulk of the solution; this distance increases with increasing electrolysis time. The solution volume within which the diffusional flux of the substance occurs is termed the diffusion layer. The flux of the substance toward the electrode is then described by the product of the diffusion coefficient of the substance, \( D \), and its bulk concentration divided by the diffusion layer thickness, \( \delta \). This quantity is defined, for planar semi-infinite diffusion, by the relationship:

\[
\delta = \pi Dt
\]  

(1.12)

The diffusion layer thickness can be rigorously calculated for a spherical electrode and for a planar disk electrode placed in a plane of an insulator; the formulae for electrodes with other geometries are approximations that differ in dependence on the initial assumptions [42]. If the electrode geometry is approximated by the model of an infinitely large planar electrode with the substance flux perpendicular to the electrode plane, then the flux is uniform over the entire electrode surface and the substance concentration attains the bulk value at a distance of a few diffusion layer thickness values. However,
the flux is not uniform over the electrode surface (edge effect) for other geometries. For example, if a convex electrode is embedded in a planar insulator, then the size of the electrode can be described by twice the linear dimension of the electrode (e.g., 2r, where r is the radius of a disk or hemispherical electrode). Two limiting cases can then be distinguished, namely, $\delta$ is much smaller than r and $\delta$ is much larger than r.

For $\delta \ll r$, the perturbation of the linear diffusion flux caused by the hemispherical diffusion at the electrode edges extends to a short distance of several $\delta$ from the edge. Thus, only a small part of the electrode surface is affected, and the behavior of the electrode can be approximated by the infinite electrode model.

For $\delta \gg r$, the edge effect plays a predominant role, and the diffusional flux toward the electrode is constant with time but inhomogeneous over the electrode surface; it increases with decreasing distance from the electrode edge. Concentration of the electroactive substance attains the limiting value described by the equation for steady-state transport, which corresponds to the time derivative being zero in Fick’s second law.

An extremely important conclusion follows from the effects described above, namely, that the diffusion layer thickness depends on time. Therefore, the differentiation between the two cases considered above also depends on time. At a sufficiently short time, any planar electrode, no matter how small (with the obvious limitations of the diffusion model), behaves as an infinitely large planar electrode [43]. With increasing time, the electrode behavior gradually changes toward the second case. In fact, a single dimensionless parameter, $\delta/r$, is sufficient for describing the electrode behavior.

After sufficiently long time, (e.g. ~ 400 sec. for spherical 50 µm dia.), a steady state is established for electrodes of certain geometries (e.g., planar and spherical). However, a
steady state cannot be attained for an infinitely long cylindrical electrode. Spherical electrodes exhibit an especially simple flux pattern—the concentration gradient equals the bulk substance concentration, $C_0$, multiplied by the sum of the reciprocals of $\delta$ and $r$, the first term corresponding to the transient state and the second to the steady state;

$$\left( \frac{\partial c(x,t)}{\partial x} \right)_{x=r} = c_0 \left[ \frac{1}{\sqrt{\pi Dt}} + \frac{1}{r} \right] = c_0 \left[ \frac{1}{\frac{\delta}{\delta}} + \frac{1}{r} \right]$$

(1.13)

The first term predominates at short times ($\delta << r$), while the second at a sufficiently long time ($\delta >> r$). It can be seen that the time required to attain a steady state strongly depends on the electrode dimensions. That is, the smaller the electrode, the shorter the time. More precisely, this time is proportional to $r$ squared, that is, to the electrode surface area.

### 1.5.2 Multiple Electrode Theory:

Three principal cases can be distinguished when considering the diffusional flux toward a microelectrode array [44, 45]:

The gaps between the electrodes are wide compared to the diffusion layer thickness. Thus, the diffusion layers of the individual electrodes do not overlap. This separation of diffusion layers occurs for $\delta < W/2$, where the total width is $W = We + Wg$, $We$ being the electrode width equivalent to $2r$ and $Wg$ being the gap width. The electrodes then do not affect one another and the overall current measured is the sum of the currents passing through the individual electrodes. If $\delta > W/2$, then the diffusion layers of the individual electrodes, partially overlap. The angle $\psi$ represents the degree of overlap. The overall current is then smaller than the sum of the currents passing through the electrodes when they operate independently. For the value $\psi = \pi/2$ (i.e., when $\delta >> W/2$), the diffusion layers totally overlap so that the array behaves as a continuous electrode having the
surface area equal to that of the whole array. Under these conditions, the signal is proportional to the surface area of the whole array, while noise is proportional only to the area of the electrodes. Thus, the signal-to-noise ratio is greatly improved.

1.5.3 Microelectrode Properties:

The principal consequences of the properties of microelectrodes are as follows: [46]

• A steady state for a faradaic process is attained very rapidly.

• The faradaic-to-charging current ratio, $I^F/I^C$, is improved, as the charging current decreases in proportion to decreasing area of the electrode, while the steady-state faradaic current is proportional to its characteristic dimension. Therefore, the $I^F/I^C$ ratio increases with the reciprocal of the characteristic dimension.

• The ohmic drop of potential, $IR$, is decreased as the measured currents are very low.

• The applied potential can be scanned very rapidly because the charging current is suppressed.

• The signal-to-noise ratio is greatly improved when an individual microelectrode performs under steady-state conditions or when the diffusion layers totally overlap in an array of microelectrodes.

• With microelectrode arrays used for measurements in flowing liquids, the amperometric signal (i.e., the current intensity) is enhanced not only through the above effects, but also by replenishment of the electroactive substance in the diffusion layer during the solution passage over the gaps between the electrodes.

• Interactions between the individual electrodes in the microelectrode arrays permit regeneration of the electroactive substance through redox cycling and derivatization of substrates to obtain products of desired properties.
• Small size of the electrodes permits measurements on very limited solution volumes.

1.6 Electrode Materials and Limitations

1.6.1 Metallic electrodes: The so called noble metals, platinum, iridium, rhodium, gold, and palladium are preferred for electrical stimulation because of their resistance to corrosion. The relative importance of a given electrode varies according to application. Stainless steel electrodes are often used where mechanical stability is highly desirable. Cortical stimulation and cortical surface electrodes demands little mechanical strength and pure platinum electrodes are commonly used [47]. When higher mechanical strength is needed with high charge densities, pure platinum is alloyed with iridium, since the pure platinum is soft and not suitable for all neural prosthesis. Tungsten microelectrodes are highly favorable in the most demanding application of all that involves microelectrode stimulation with electrodes the size of single neuron. For acute multiple-unit neural recording, microelectrode arrays are used that consist of multiple metal shanks projecting from a silicon supporting bulk [48]. However tungsten is unacceptable with biphasic current pulses due to surface reactions and rapid changes in impedance. Dissolution of platinum electrodes is an unavoidable consequence of electrical stimulation. The reversible charge injection limit (i.e. the amount of charge that can be injected without causing irreversible reaction at the interface) for a platinum electrode is relatively low, reflecting its low surface area. It is known also that in the presence of chloride platinum can dissolve during an anodic pulse [49]. As shown in figure 1.9a, pitting corrosion occurred after the charge was injected beyond the safe limits of the Pt alloy and Pt dissolution into the tissue environment increased with time (figure 1.9b).
1.6.2 Ceramics and Oxides: It is clear that alternatives to Pt are needed since both its electrochemical and mechanical properties are not adequate, especially for very small electrodes.

1.6.2.1 Iridium Oxide: When appropriately activated, multilayered oxide coatings can be produced on the iridium surface which can store a large amount of charge [50]. Iridium and rhodium (1400 µC/cm$^2$) have higher charge injection limits than platinum (700 µC/cm$^2$). Ir subjected to repetitive potential sweep, forms a thick oxide film that is a valence change oxide, and therefore has high values of charge injection density upon voltage cycling in electrolytes. The charge is injected via valence change reactions between two stable oxides Ir$_2$O$_3$ ↔ IrO$_2$, without complete
reduction of the oxide (figure 1.10). This reaction enhances the limits of reversible charge injection [51]. Ir also has high resistance to dissolution and excellent biocompatibility. The iridium oxide layers can be formed by electrochemical activation of iridium metal, by thermal decomposition of an iridium salt on a metal substrate or by reactive sputtering from an iridium target.

1.6.2.2 Ti and TiN Coated electrodes: TiN coating as a surface layer on an electrode promotes safe stimulation through double layer charging and discharging. TiN has found increasing interest because of its metal-like conductivity, excellent mechanical and chemical properties and the fact that it can be deposited with a high surface area, which increases the safe charge injection limits [52]. A larger effective surface area gives a higher capacitance and also a lower polarization. These electrodes are very useful for high resolution stimulation and to enhance spatial resolution for neural recording. Despite the above properties, in vivo experiments done by Ducheyne et. al. showed accumulation of titanium ions adjacent to implants, which, therefore, limits the use of these electrodes for long term implantation [53].

1.6.2.3 Capacitor Electrodes: Capacitor electrodes are ideal stimulation electrodes, since a thin dielectric layer at the surface prevents electrochemical reactions and, therefore, charge is injected without any faradic reactions. Tantalum pentoxide has very low dc leakage currents and its charge storage capacity can be increased through surface area enhancement using sintered tantalum powder [54]. Anodized titanium or

Figure. 1.11: DC leakage current in dilute PBS electrolyte for BaTiO3 films deposited on Pt substrates [55]
thin films of barium titanate do not provide higher charge storage capacity, despite their higher dielectric constants, as their dc leakage currents are prohibitively high [55]. As seen in figure 1.11, DC leakage currents in barium titanate are significantly high in presence of PBS electrolyte solution.

1.6.3 Polymeric Electrodes:

Conducting polymers have recently been developed for various applications as an alternative to conventional conducting materials. They have been explored in applications such as biological analysis and microelectronic devices. Following is a brief description on some of the recent work done in the area of neuronal microelectrode development in the polymer field.

**Metallized Polymer fibers [56]:** Metallization of high performance polymer fibers (Kevlar) can yield electrically conductive fibers with greatly improved flexibility and mechanical stability which is a desirable interfacial property. They are used as intrafascicular microelectrodes

**Polyaniline Coated microelectrodes [57]:** Polyaniline, being a conducting polymer is very useful for microelectronic devices. Depositing polyaniline on metallic substrates, significantly stabilizes the ohmic impedance in a physiological media.

**PDMS Planar Electrodes [58]:** Poly(dimethylsiloxane) has low dielectric loss and can be micromolded into the shape of the tip of a micropipette, which can be used for patch clamp recordings. Surface chemistry can be altered to make it hydrophilic so that a high resistance seal with the membrane can be achieved.

**Polypyrrole based electrodes [59]:** An implantable Microelectrode array made of conducting polymer can be used for stimulating nervous system. Vapor phase
polymerization is carried and micro Patterns of PPy have been developed using the photochemical reaction of oxidative polymerization agents.

*Polymer Hydrogel Microelectrodes [60]:* Due to the interesting mechanical and chemical adaptability of the polymer hydrogel material and its large charge delivery capacity and low impedance, it can be used as a microelectrode for neural communication. A conducting polymer hydrogel electrode can be electrochemically deposited in micromachined via holes. The porous structure can enhance integration to nerve cells.

### 1.6.4 Carbon Fiber Microelectrodes

Carbon fiber electrodes are microelectrodes for recording from nerve cells using a single carbon fiber (or small group of fibers) as the conducting element. For neuroscience applications a single fiber is placed into a glass tube drawn out into a fine capillary (fig 1.12). The end result is somewhat like a protruding pencil: a short length of carbon protrudes from the end of the glass insulation [61]. The diameter of the carbon fiber is only a few microns (often 8 microns), these electrodes can be placed near single cells in dissociated cultures or placed in brain slices for electrical recording of single nerve cell activity.

The carbon fiber tip is formed by etching it to desired size by following methods [62]:

**Electrolytic etching:** A drop of electrolyte is placed in a circular shape platinum electrode, and the tip is dipped inside of it. Chromic acid is used as the electrolyte, and a variable transformer is used to supply 100-300 uA current. Different currents will result in different shaped tips.

Another electrolyte is 0.9% saline solution. 1-2 mA current, with .1-3 ms pulse duration, at 1-10 Hz is supplied. The strong current in this case, heats up the tip and burns it off.
**Spark Etching:** Tungsten electrode is used to produce a spark, which appear at a voltage of 400-600. AC etch is followed by DC etch to shape up the tip (fig 1.13).

![Figure 1.12 Carbon fiber electrode protruding from glass assembly [61]](image1)

![Figure 1.13 Spark Etching arrangement for carbon fiber electrodes [62]](image2)

Contact between wire and fiber is very critical as it can contribute a lot to the total noise of the signal. Following materials are used for this purpose: Saline solution in
micropipettes (Ag/AgCl wire), Mercury Silver containing resin, Wood’s metal (In, Sn alloy, low temp melting material), Silver bearing paste (Solder weld).

Carbon fibers are basically fine filaments of carbon which have very high tensile strength and are used for tennis racquets, golf clubs and fishing rods, as well as in some aerospace applications where light weight and high strength are at a premium. Combined with small size, excellent mechanical properties, high stiffness, good electrical conductivity and being non-toxic to neural tissue makes them uniquely useful for neuronal microelectrode applications [63]. Moreover, as carbon is a versatile electrode material that can undergo various chemical and electrochemical modifications it is possible to produce flexible surfaces ranging from purely inert to highly active. Carbon fibers have various electroanalytical applications and cover such diverse areas as coulometry, potentiometry, anodic stripping voltammetry, spectroelectrochemistry and kinetic measurements [64].

Carbon fiber electrodes already in use have the following features which favor their use neural electrodes:

- Carbon fiber is commercially available in tows of about 1000 fibers with 7-8 um diameter fibers in it. This abundant availability and lesser cost make it the material of choice.
- Carbon fibers are a low resistance form of graphite and they have resistivities in the range of 250 Ω/mm [65].
- Carbon fibers form a low-noise interface with electrolyte solutions.
- They are easier to make than metallic (W, Pt-Ir) electrodes.
- Carbon fibers are highly biocompatible.
Despite the above advantages carbon fibers electrode assembly have various limitation that needs to be addressed. The fibers are not as stiff as metallic electrodes and thus makes it harder to penetrate into the tissue environment, and they can even break with little extra stress. They have uniform diameter (8 µm) instead of a taper back from the tip. Taper back provides extra strength and it is easier to etch them to smaller sizes. The single fiber is more easily damaged while pulling fiber out of the tow or even while pulling the glass micropipette over it. Fibers being very small develop static charges which make them stick together. Special tweezers and microscopic heads are needed for handling.

A frequently used method for fabricating carbon fiber microelectrodes is to encase the conducting fiber in a glass insulating support like capillary tube and to seal the tip with heat pulling or by using epoxy resin. Unfortunately this technique leads to problems, associated with the conductor glass and conductor epoxy interfaces, that result in poor quality electrodes, non-reproducible electrode geometries, and electrochemical responses [66,67]. The glass insulation at the tip has a tendency to break or leak, as there is a large gradient of size between the tip and glass [68]. Glass pipette fabrication is a multi-step, time consuming process which requires a special glass puller with good glass handling dexterity. The fragile glass electrodes are difficult to mount in the standard holders of stereotaxic instruments, and chances of breakage are high, with attendant risk of contamination. Moreover it is difficult to make an electrode that reaches deeper than 10 mm below the skull surface, without making use of stainless steel guidance tubes. The thin glass, in the proximity of the tip can also store charge, which can lead to capacitive coupling and increase in the signal noise level. Sreeniwas et al. fabricated a novel sputter
deposited, thin film carbon microelectrode array using standard integrated technologies and silicon micromachining (figure 1.14) [69].

Such photolithographically produced arrays of microelectrodes exhibit reduced capacitive coupling and can be fabricated reproducibly. However, studies have shown that the silicon based multisite microelectrodes have decreased selectivity for biochemicals, higher background currents, and poorer detection limits as compared to typical single carbon fiber electrodes [69]. Considering all the above limitations in the glass pipette design, microelectrode array and single carbon fiber, there is clearly a need to develop novel electrode material and design that feature enhanced properties to overcome performance degradation of the carbon fiber design.

1.7 Available Electrode Designs

Depending on the end use of implant, different electrode designs are available. They differ in material, shape, and size. Their manufacturing processes and complexity vary accordingly, depending upon the implant purpose and location as described below.

1.7.1 Microelectrodes:

When it is desired to examine the electrical activity of a single cell, it is necessary to construct an electrode with dimensions that are small with respect to the cell so that insertion into the cell can be accomplished without its destruction. A detailed discussion on microelectrodes is presented in section 1.4.
The electrode shown in figure 1.15 is a Tungsten microelectrode with a micron sized tip and gold plated pin used as a connector. Because of the small tip size desired, it is difficult to mechanically sharpen metal rods or wire. These tips are fabricated in many different ways such as electrolytic etching, heat pulling, and electric arc etching.

![Tungsten Microelectrode](image)

**Figure 1.15 Tungsten Microelectrode [A-M systems*]**

There are several ways of making microelectrodes depending on the procedure of encapsulation. Platinum or Gold wire placed in a quartz or glass capillary could be heat pulled in a microburner to produce a glass insulated platinum electrode having an exposed tip of 1 um [70,71]. This procedure involves inserting metal wires into an open glass capillary and then pulling the metal glass assembly together using a pipette puller. Due to the fast and reproducible local heating of a glass capillary together with an inserted metal wire, the metal wire is pulled simultaneously with the glass leading to a drastic decrease in its diameter and a simultaneous tight seal of the metal within the glass capillary.

A similar process is used for glass tubes to form glass insulated micropipette electrodes. Different material electrodes such as carbon fiber are heat pulled through a glass micropipette and the fiber is connected to other conducting leads via some electrolyte or conducting epoxy inside the pipette [72]. Using a similar procedure, a 7 μm diameter carbon fiber was inserted into a commercial Teflon capillary, followed by pulling the capillary to produce a self-sealing thin Teflon coating. The carbon fiber was exposed by cutting with a microsurgical scalpel blade [73]. Figure 1.16a shows a carbon
fiber protruding out of a glass capillary. There are very fine capillaries attached around the circumference of the central cavity for drug delivery purposes.

Another type of design as shown in figure 1.16b consists of fused silica tubing instead of glass pipette [68]. This design provides simplicity in the manufacturing process and gives rigidity and stability to the probe system.

![Figure 1.16: a) Glass pipette electrode design with multiple pipettes attached around the carbon fiber, b) a carbon fiber inserted into fused silica tube [68]](image)

1.7.2 Microfabricated linear 2-D and 3-D Multielectrodes:

Microfabrication technology has been used in Electrode fabrication so as to enable reproducible construction of very small tips having an exact geometry. They have been microfabricated through the anisotropic etching of silicon, followed by thin-film deposition of silicon dioxide, platinum and silicon nitride. Photoresist is applied so that the top of the tips are left just emerging from the photoresist layer. The silicon nitride layer is then removed from the tip region by plasma etching, leaving a Pt finite cone having a radius of 1.25 μm and a height of 2 μm [74].

MEMS and CMOS production technologies have also been used to develop polycrystalline silicon micro-electrode arrays. Linear multielectrodes refer to a one-dimensional array of electrode sites mounted in/on a needle or incorporated in a glass or
silicon tip-shaped carrier. The needle is a hollow metal shaft in which a side-window perforation houses the tips of a number of leads threaded through the shaft. Lithographic patterning and deposition of thin-film metal leads and electrode sites onto glass, silicon, or polyimide carriers helps in more complex fabrications [75].

1.7.3 Industrial Design from Medtronics:

The electrode is 1.27 mm in diameter with quadripolar Pt/Ir leads, with contacts 1.5 mm long and separated by either 0.5 or 1.5 mm of insulation (figure 1.18). It is connected to an extension lead tunneled subcutaneously to an implanted pulse generator. This electrode is specifically designed for Deep Brain Stimulation. This design allows for effective control of stimulation over a distance along the axis of the device. Effective positions within the brain nucleus that are anterior, posterior, or lateral to the axis of the electrode may not be adequately stimulated or properly controlled.

Figure 1.18: Figure showing commercially available cylindrical quadripolar electrode [http://medtronic.com]
1.8 Electrode device packaging Issues:

1.8.1 Magnetic Resonance Imaging, Introduction:

Magnetic resonance imaging (MRI) has many advantages compared with x-ray–based diagnostic techniques, including its nonionizing nature and unparalleled ability to discriminate different soft tissues without contrast media. MRI has now become the image modality of choice for imaging the brain, spine, musculoskeletal system, head and neck, and other tissue structures. The number of patients with implantable devices is increasing steadily. The presence of these devices, however, is currently considered a contraindication to MRI [76]. Nevertheless, there is an increasing need to image patients with implantable devices because of the advantages offered by MRI.

1.8.2 MRI – Artifact in imaging: Locating the tip of the stimulating device or establishing the location of the stimulating electrodes is important in determining whether movement of the electrode occurred. In contrast, imaging of the presently available electrode with MRI technique produces an image that does not allow for an accurate determination of its location within a distance of a millimeter [76]. Presumably, the artifact is due to the metallic nature (platinum/iridium) of the electrode, which produces a diffraction pattern in the magnetic field and obscures the proper image. In order to overcome surgical problems, the electrode tip, at the minimum, needs to be MRI friendly, allowing the surgeon to observe with a high degree of accuracy the electrode placement. Therefore, a MRI useable electrode or a non-metallic electrode system would be of significant importance.

1.8.3 MRI - Tip Heating: As with all electronically activated devices in the MR environment, it is generally recommended that patients with a neurostimulation system
should not undergo MR imaging because of the potential for serious consequences, including movement of the leads or implantable pulse generator, excessive MR imaging–related heating, induced electric currents, and functional disruption of the operational aspects of the device [77–79]. From an MR safety point of view, the greatest concern for electronically activated or electrically conductive implants in the brain is excessive MR imaging–related heating, which can cause irreversible tissue damage [80,81]. Results from studies conducted to date [80,81] and a recent report [82] revealed that there is a realistic potential for injury due to excessive MR imaging–related heating of neurostimulation systems used for DBS. Recently, investigators have evaluated MR-related heating for the only neurostimulation system (Activa Tremor Control System; Medtronic) approved by the FDA for use in chronic DBS. This neurostimulation system is a fully implantable multiprogrammable device designed to deliver electric stimulation to the thalamus or other brain structures. The basic implantable system is composed of the neurostimulator (or implantable pulse generator), the DBS lead, and an extension that connects the lead to the implantable pulse generator. In their studies on neurostimulation systems, Rezai et al and Finelli et al indicated that MR safety for neurostimulation systems is highly dependent on a number of critical factors. To simulate a worst-case clinical application of DBS, these investigations evaluated bilateral DBS applications, such that two neurostimulators, two extensions, and two leads were assessed during in vitro experiments. The findings from these studies indicated that substantial heating occurs under certain conditions, while other conditions produced relatively minor physiologically inconsequential temperature increases. Furthermore, factors that strongly influenced local temperature increases at the electrode tip included the positioning of the
neurostimulation system (especially the electrode), the type of RF coil used. According to the study by Rezai et al [80], as long as highly specific guidelines pertaining to the positioning of these neurostimulation devices and to the parameters used for MR imaging are carefully adhered to, MR-related heating does not appear to present a major safety concern for patients with the bilateral neurostimulation systems.

1.8.4 Recording with simultaneous Stimulation:

Positioning of the stimulating electrode is critical to the outcome of the electrode implantation surgery. Placement of the electrode is complex. Operation problems can affect the accuracy in the positioning of the electrode. Because every brain is anatomically different, even with microelectrode recording, the surgeon must repeat electrode positioning, resulting in a trial and error procedure [6]. There is risk to the patient with every brain penetration. In order to overcome these problems, it is very important to develop a dual functional microelectrode. Simultaneous recording and stimulation can reduce the number of tracks necessary to locate the appropriate area of implantation. This can reduce cost and other drawbacks such as use of MRI. If one of the electrodes fails, other can be used to perform same function temporarily. Two electrodes with different impedance can be implanted together such that they have a proper insulation between them to prevent cross talk.

1.8.5 Guidance Delivery System:

To further improve the effectiveness of the stimulation a guidance delivery system needs to be developed. This will allow implantation of multiple electrodes, such that recording and stimulating electrodes are in the vicinity of each other. Improvement in guidance system design would also include simultaneous drug delivery. The ability to stimulate
multiple areas within the nucleus should permit better control of the symptoms manifested by the patient and enable modification of other parameters, e.g., lower voltage, used in the stimulation. It may even prevent the development of adaptation of the brain circuitry because of the ability to switch stimulation sites. Delivering drugs simultaneously with stimulation and recording can reduce both cost and time. Moreover the drugs can be delivered to the very proximate vicinity of the affected area. Suitable guidant electrode systems are desired such that they incorporate a number of different electrodes with proper insulation between them as well as extra tubing for drug delivery.
1.9 Biochemical Sensing Phenomenon

Neurons generally exchange messages when the sending neuron releases a chemical (neurotransmitter) which activates receptors in the receiving neuron. In most cases, neurons do not actually touch each other; instead, there is a tiny gap, called a synapse (fig 1.19). Neurotransmitter molecules may cross the synapse to activate a receptor on the receiving neuron, or may remain in the synapse unused until it is broken down or reabsorbed into the sending neuron to be recycled for later use.

![Image 1.19](www.bio.ic.ac.uk) Picture showing neurotransmitter action as communication between two neurons

In both food and medical diagnostics, it is often necessary to detect and quantify chemical and biochemical species rapidly with a high degree of specificity. Electrochemical techniques detect materials by oxidizing them in solution at a positively
polarized "working" electrode. The oxidized material gives up electrons which are collected by the working electrode and generate a current flow through the medium [83]. The detection of this current is the basis of the measurement method. Several compounds of biological interest are oxidizable under these conditions, and are therefore said to be "electroactive". These include the amine neurotransmitters dopamine, noradrenaline (norepinephrine) adrenaline and serotonin.

![Figure 1.20 Figure showing Dopamine reversible oxidation reaction [63]](image)

Other transmitters such as acetylcholine, GABA, glycine and glutamate are NOT electroactive and are therefore not detectable using this technique. The neurotransmitter gas nitric oxide is also electroactive and can be detected electrochemically, as can several other compounds of interest in the brain, including ascorbic acid, uric acid, and several metabolites of the amine transmitters.

![Figure 1.21 Oxidation of ascorbic Acid [84]](image)
Imbalanced dopamine activity can cause brain dysfunction and disease (schizophrenia, parkinson’s disease) or reveal abuse of drugs ranging from stimulants to depressants. Dopamine affects brain processes that control movement, emotional response, and ability to experience pleasure and pain.

A voltage waveform is applied to an inert microelectrode tip implanted in a given brain region. At a given critical voltage any monoamine present at the electrode surface oxidizes and surrenders electrons to the electrode [84]. These form a current that is proportional to the concentration of monoamine at the electrode surface. By appropriate calibration of the current, it is possible to calculate the concentration of monoamine present in unknown solutions or brain areas.

Carbon fiber and carbon disk microelectrodes have been used in the detection of oxidizable compounds [85,86]. Carbon is the most preferred material for biochemical sensing because of its high degree of biocompatibility, outstanding signal to noise ratio and due to the high conductivity of amorphous carbon. Carbon fibers are highly flow independent and they have high H₂ and O₂ overpotentials. They have excellent lifetime and can be passivated with SiC or SiN layers, ensuring excellent chemical and electrical protection.
Introduction to Electrode’s Electrochemical Concepts

1.10 Impedance Behavior of electrodes and Geometrical Effects

The performance of a microelectrode for stimulating and recording neuronal potentials depends on precise control of their geometrical, electrical and mechanical properties. Their narrow tapering profiles permit them to be positioned very close to the individual neurons and a very fine insulation within a few micrometer of the tip aids in recording highly localized extracellular potentials and in injecting localized currents to influence the excitability of the nearest neuron [87]. Charge carrying capabilities of these microelectrodes is limited by the size of the electrode. Thus, the electrode-electrolyte interface is designed to maximize the amount of charge that can be injected into a given site before voltages must be applied that would exceed the threshold for irreversible electrochemical reactions.

1.10.1 The Electrochemical Interface:

The neuron electrode interface is electrically characterized by three components: the neuron, the microelectrode, and the medium in between [1]. The minimum current needed to trigger an action potential will depend both on the electric field vector and on the nature and position of the cell. The field vector at a given point will be a function of electrode geometry and polarity of the electrolyte resistivity [14]. The versatility of microelectrodes arises due to their unique behavior, which is largely controlled by the interplay of Current ( I \( \propto r \) ), uncompensated resistance ( R \( \propto 1/r \) ), and double layer capacitance ( C \( \propto r \) ), where r is the radius of the electrode [88]. Various researchers have studied the spatial distribution of current density over the surface of electrode, as well as electrode geometry effects on the neural excitation. Analysis of the models showed that
current densities are concentrated at the tip of the microelectrode and the electrode insulator interface. Changing the surface area of the electrode, radius of curvature of the electrode tip, or applying a resistive coating to the electrode surface alters the current-density distribution on the surface of the electrode [89].

1.10.2 Impedance Behavior:

The electrode electrolyte interface can be expressed as a serial capacitance and a series resistance, together with a faradic resistance [90]. In a RC circuit representation both R and C depend on frequency in the range up to 10-20 KHz. The Impedance of such interface arrangement can be best described by the expression in figure 1.23. Various researchers have developed models for electrochemical interaction of carbon microelectrode. Impedance-frequency plots for a typical microelectrode system comes out to be as a S-shaped curve [52] (figure 1.22). This kind of curve can be better fitted by an equivalent circuit consisting of a leak resistor in parallel with the capacitor plus a series resistor, as shown in figure 1.19.

![Figure 1.22](image-url)  
**Figure 1.22** Bode plot of Pt electrode in PBS solution [52]

![Figure 1.23](image-url)  
**Figure 1.23** Equivalent circuit and impedance expression for microelectrode assembly [52]

\[ Z = \frac{1}{2} \left( R_1^2 + 2R_1R_2 + R_2^2 \right) \frac{1}{1 + (2R_1Cf)^2} \]
Impedance of the system depends on both resistor values as well as on capacitance value as shown in the expression above. The C term is due to formation of an electrolytic double layer at the electrode-electrolyte interface. \( R_1 \), the electrode tip resistance, depends on the cross sectional area and tip length. \( R_2 \) is a lumped resistance term, including the resistance of the electrolyte in the bath, and the resistance of the electrode - wire contact. Another model for stainless steel microelectrodes verified the Warburg’s postulate [90]. In the frequency range studied it was found that the reactance was very nearly equal to the series resistance and both varies almost inversely as the square root of the frequency. The impedance of an electrode-electrolyte interface decreases with increasing current density [90].

1.10.3 Surface Roughness effects:

To avoid irreversible reactions on the electrode surface, which may lead to degradation of the electrode or to undesired reaction products, it is desirable that the stimulation pulses are transferred through interfacial charging-discharging processes. For stimulation, the electrode needs to exhibit capacitive characteristics with enough charging magnitude to overcome the so called pacing threshold. For sensing, the electrode has to have low polarization, otherwise the signals might not be detected correctly. These parameters are to a large extent dependent on the material, surface structure of the electrode and electrochemical processes taking place at the electrode/electrolyte interface.

![Figure 1.24 Impedance Change before and after deposition of Pt and Ir onto smooth Pt [91]](image)
In modern pacemakers, the electrodes are small so that the output impedance is high for the battery, and therefore the current drain is low and the battery life is long. In neuronal studies microelectrodes need to be very small, which increases the impedance of the electrode. However, high impedance limits the sensitivity of the electrode and produce very high noise in the signal. Hence a balance must be created between the small size and high impedance. Enlarging the surface in the form of a porous coating or columnar structure can compensate for this phenomenon [1]. Rough-surfaced electrodes are also used to increase the effective surface area. A larger effective surface area gives a higher capacitance, and also a lower polarization [52]. Moreover it is the real surface area of an electrode, not the geometric area, which defines the charge injection limits of chemically reversible surface processes. Efforts have been made to calculate the real surface areas of platinum electrodes by hydrogen atom plating charge [92] and by hydrogen adsorption using cyclic voltammetry [93]. There is also a correlation between charge storage capacity and the morphology of the electrodes. Charge storage capacity increases linearly with the 2-D and 3-D roughness factor [94]. This shows that rough surfaces increase the real surface area of the electrodes and is highly desirable in order to obtain low impedance and high capacitance values. For sensing applications, it can lead to high signal to noise ratio and for stimulation it can deliver higher charge densities while staying within the reversible charge injection limits. Figure 1.24 shows the effect of deposition of a rough noble metal layer and Iridium separately. Impedance of the electrode can be decreased by almost two orders of magnitude and it can also be made to satisfy the requirement of constant low electrode impedance by roughening Pt surface and by depositing Iridium on it respectively [91]
1.11 Stimulation Parameters:

A primary consideration for the stimulation is to produce the desired physiologic or clinical effect. The gamut of possible stimulation protocol is constrained by the need to protect the integrity of the electrode and the surrounding tissue. Controlled current stimuli are preferable to controlled voltage stimuli in any application requiring maximum protection of the neural substrate and of the electrode itself. It is recognized that this increases the complexity of stimulator hardware. However, it is only by the use of controlled current regimes that the stimulus charge density and charge per phase can be controlled and only thus can we hope to simultaneously control electrode dissolution, generation of electrochemical toxic products at the electrode interface, the capacity of the stimulus to excite neurons, and its propensity to induce neural injury.

To minimize potentially hazardous electrodic processes, tissue should be pulsed with charge-balanced stimuli in which no net charge is injected, and the electrode should be fabricated from a noble material. This ensures that the net production of soluble toxic products is minimized. If the stimulus frequency is low it is in most cases acceptable to achieve charge balance by means of capacitor in series with a monophasic current pulse [96] rather than through the use of an actively charge- balanced or “Lilly” waveform [97]. The importance of stimulus charge density, independent of charge per phase, in the induction neural injury implies that the stimulating electrode’s active surface should be as large as possible, consistent with the required spatial localization of the stimulus and with mechanical biocompatibility. Thus, while the size of penetrating electrodes is restricted by the need to minimize mechanical injury (particularly if many electrodes are to be implanted), a larger electrode permits injection of the charge per phase necessary to
excite neurons within a larger required radius while also reducing the charge density near the electrode surface.

In selecting optimum pulse duration for neural stimulation, the charge per phase (of a pulse pair stimulus) required to activate a particular neuron or axon can be reduced by shortening the pulse length to approximately that of a chronaxie of the particular neuron or process. (Chronaxie is measurement of excitability of nervous or muscular tissue; the shortest duration of an effective electrical stimulus having a strength equal to twice the minimum strength required for excitation). Further shortening of the pulse reduces the required charge per phase only marginally. In fact, varying the pulse durations (with a fixed charge per phase) can vary the efficiency of the stimulus only to a limited degree, while the influence of charge per phase (at fixed pulse duration) is essentially infinite. Some authors have proposed that the ratio of safety to efficacy can be increased by shortening the pulse duration [98], but, if neural damage is indeed related to the induced neuronal activity, then the propensity to induce neuronal injury is probably related most closely to the efficacy of the stimulus, rather than individually to the charge per phase or to the pulse duration, which only together determine the effectiveness with which the stimulus will depolarize a particular neuron or cell process. However, if the objective is to excite only neurons or axons with short chronaxies, then short stimulus pulses may increase the margin of safety by reducing the depolarization and excitation of neurons and neuronal processes with longer chronaxies, thus reducing the totality of the neuronal “mass activity”. This selectivity for elements with short chronaxies is enhanced by immediately following the depolarizing pulse (phase) of the stimulus with the second phase, which achieves charge balance [99].
Thus, pulse duration must be considered as an independent factor in neural damage when we use a pulse duration equal to or greater than the chronaxies in the stimulated tissue, but, it is not clear that short pulses necessarily provide greater margin of safety-to-efficacy. This will probably depend on the particular neural substrate, and the objective of the stimulation protocol.

As the pulse duration is shortened, the stimulus current, and hence the current density at the electrode interface, must be increased. With microelectrodes, the high current densities, in conjunction with their high ohmic “access impedance” into the tissue, may cause the voltage transient induced by the current pulse to be very high [100]. These voltages are imposed across the insulation covering the electrode shaft or matrix, and also the interconnecting leads. Also, the controlled current stimulator must have sufficient compliance to operate over this range of access voltages, a possible problem with implantable devices utilizing “on-chip” microelectronic technology. At high current densities, the overpotentials of the faradaic reactions at the metal electrolyte interface may become very high due to finite kinetics (exchange current density) of the charge injection reactions, and this in turn leads to increased dissolution of the electrode or to formation of other toxic products due to recruitment of undesirable electrochemical reactions. The theoretical charge capacity of an activated iridium microelectrode may be very high, but the charge per phase that is accessible for pulsatile stimulation is limited to a few percent of this total charge, by the kinetics of the charge transfer reactions that occur in the rather thick iridium oxide film. Iridium oxide becomes unstable when operated at very high current densities, probably due to continuous generation and spalling of the oxide [95].
The combination of stimulus pulse duration and pulse amplitude necessary to excite an action potential in a particular neuron, at a particular distance from the stimulating electrode, is given by the Hill equation [101,102]:

\[ I_t = I_r / [1 - e^{-\tau t}] \]

The two parameters in the Hill equation are the rheobase current, \( I_r \), (the current required to excite an action potential when the pulse duration is very long), and the excitation time constant \( \tau \), is related to chronaxie, defined as the minimum pulse duration that is capable of eliciting an action potential when pulse amplitude is twice the rheobase current. \( I_t \) is the threshold current and \( t \) is stimulus pulse duration.
**Goal and Approach**

Taking into consideration, all the limitations of metallic and ceramic electrodes, Carbon has shown potential as a highly stable microelectrode material but it lacks mechanical and electrical robustness in its present design. The goal of this research was to overcome the design limitation of current carbon electrodes and utilize the excellent electrical and electrochemical properties of carbon for bioelectrode applications. A composite fiber microelectrode (140 µm) was developed, with two conducting carbon elements, well insulated and separated with Silicon Carbide (SiC), such that conducting elements are used for stimulation and simultaneous recording, depending on tip geometry and electrical coupling. The SiC-C fiber constitutes turbostratic carbon with nano sized blocks on its surface, which increases its real surface area by several fold, and ultimately lowers the impedance, enabling higher charge densities with the smaller geometrical surface area available. Carbon being a diamagnetic material (magnetic susceptibility, $\chi_m = -1.6$) is far more MRI compatible than paramagnetic platinum ($\chi_m = 26$) and tungsten ($\chi_m = 6.8$). Therefore, developing carbon into a dual function stimulating and recording electrode will definitely improve the outcome of various brain stimulation procedures. There are several advantages to having such an electrode. It will reduce the number of tracks necessary to locate the appropriate area for implantation, thereby reducing the risks to the patient and streamlining the procedure. It will improve the ability to check the location of the electrode without having to do MRI, thus reducing cost.
Chapter-2
Experimental

2.1 Materials and Compounds

2.1.1 SiC-C Fiber:
The particular fiber used is commercially available under trade name SCS-6. It is the cheapest fiber among SCS family of fibers. It is a round fiber with filament diameter of 5.6 mil (140µm). It is manufactured and marketed by Specialty Materials, Inc., Lowell, Massachusetts in various lengths depending on the customer requirement.

2.1.2 Electrode Assembly Components:

Above discussed fiber was inserted into a prototype model of an electrode probe device which was fabricated with non-metallic components. This ceramic and polymer based probe device could be an alternative for metallic guiding tube and drug delivery system. This device has following components:

2.1.2.1 Ceramic Tube: Ceramic tubes containing two and four holes were purchased from Omega. Ceramic tube has an outside diameter of 1/32 inch and inside hole diameter is .005 inch. This tube is made of impervious, recrystallized, pure alumina ceramic containing 99.8 % Alumina. It has the most superior gas and vacuum tightness and superior insulating properties. It is non-reactive and highly biocompatible. Table 2.1 summarizes the typical properties of ceramic tube used.

<table>
<thead>
<tr>
<th>Constitution</th>
<th>Water Absorption</th>
<th>Specific Gravity</th>
<th>Compressive Strength</th>
<th>Tensile Strength</th>
<th>Dielectric Constant</th>
<th>Dielectric Strength</th>
<th>Volume resistivity</th>
<th>Hardness (Mohs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.8 % Alumina</td>
<td>0.00</td>
<td>3.85</td>
<td>300,000 (psi)</td>
<td>30,000 (psi)</td>
<td>9.29</td>
<td>230 Volts/mil</td>
<td>$10^{13}$ Ohm cm</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 2.1 Typical properties of Alumina tube used

2.1.2.2 Delrin Tube: Acetal (Delrin) is the common name for a family of thermoplastics with the chemical name "PolyOxy-Methylene". Acetal is available in a general purpose
copolymer grade, a homopolymer version (Delrin ®), and several filled Delrin ® grades. Acetal provides high strength and stiffness, enhanced dimensional stability, and is easy to machine. As a semi-crystalline material, acetal is characterized by a low coefficient of friction and good wear properties. Due to the excellent mechanical strength and machining characteristics of Delrin it was preferred as a supporting holder for ceramic tube and gold pin connections. A delrin tube of outer diameter .27 inch and inner diameter .09 inch was purchased from Boedeker Plastics, Inc. Another thinner Delrin rod of .19 inch diameter was used. A hole was drilled in this rod to fit the ceramic tubing at one end and the other end was inserted into the thicker delrin tube.

2.1.2.3 Platinum Wire: A very fine grade polyester insulated platinum wire was used as a connecting wire between the Silicon Carbide fiber and the gold pins. The conductor diameter is 0.025 mm and the insulation thickness is 0.005 mm. The wire has 99.99 % purity. It was purchased from GoodFellow.

2.1.2.4 Gold Pins: Gold plated brass Printed Circuit pins were used as connectors for electrical connections between the circuitry and the carbon composite fiber electrode. Pins have .043 inch mounting hole diameter and .402 length. They are bought from Newark Electronics.

2.1.2.5 Silver Paste: A conducting silver epoxy was used to connect the fine platinum wire with the fiber electrode. It has excellent adhesion and conductivity.

2.1.2.6 Epoxies: A biocompatible 2 component epoxy was purchased from Epotek Technologies. This is a spectrally transparent epoxy and was used to seal the electrode tip with the ceramic tibe. It has viscosity around 100-200 cPs. It was cured overnight. Its index of refraction is 1.539 and volume resistivity is around $10^{13}$ ohms-cm. Another
epoxy glue from Harcos Chemicals was used to join the different components of the electrode probe together.

2.1.3 Chemicals:

Microelectrodes were tested for biochemical sensing of various neurotransmitters present in the human brain. Dopamine is the most important neurotransmitter and it is available as dopamine chloride.

Dopamine, is the naturally occurring immediate precursor of norepinephrine. Dopamine hydrochloride is a white to off-white crystalline powder, which may have a slight odor of hydrochloric acid. It is freely soluble in water and soluble in alcohol. Dopamine HCl is sensitive to alkalies, iron salts, and oxidizing agents. Chemically it is designated as 4-(2-aminoethyl) pyrocatechol hydrochloride, and its molecular formula is C8H11NO2•HCl. The molecular weight is 189.64. It was purchased from Sigma-Aldrich Inc.

Another Neurotransmitter used was Vitamin C. Ascorbic acid acts as an inhibitory transmitter in the brain to inhibit stimulated hormone release by scavenging nitric oxide. Vitamin C (ascorbic acid) is a water-soluble antioxidant that is responsible for maintaining iron in its reduced state thus preserving activity of the hundreds of enzymes that contain iron at the catalytic site. Molecular formula for Ascorbic Acid is C6H8O6 and it has molecular weight of 176.12 gms/mole. It was purchased from fischer scientific.

Ferrocene was used as an electrolyte for testing microelectrodes for their ability to detect one electron oxidation and reduction process. Ferrocene is the chemical compound with the formula Fe(C5H5)2. Ferrocene is the prototypical metalloocene, a type
of organometallic chemical compound consisting of two cyclopentadienyl rings bound on opposite sides of a central metal atom. Such organometallic compound are also known as sandwich compounds. Ferrocene is an air stable orange solid that readily sublimes in vacuum. As expected for a symmetric and uncharged species, ferrocene is soluble in normal organic solvents, such as benzene, but is insoluble in water. It was also purchased from fischer scientific.

**Phosphate buffered Saline** was used as an electrolyte in testing media and as a solvent for the biochemicals. It was purchased as tablets from sigma-aldrich. 1 tablet was dissolved in 200 ml of water to obtain 0.01 M Phosphate buffer, 0.0027 M Potassium Chloride and .137 M sodium chloride with pH 7.4.

Hydrofloric Acid and Chromic acid were used as etchant solution for electrolytic etching of Carbon composite fiber. These chemicals were purchased from Fisher Scientific.

### 2.2 Fiber Etching Setup

SiC-C composite fiber is originally in round shape. To make a microelectrode tip from this fiber it was electrolytically etched such that a sharp taper of carbon core with SiC insulation can be obtained. To monitor the etching process, the electrode is observed under optical microscope after every 1-2 minutes to inspect the geometry and shape. The process was also monitored using the oscilloscope. Another factor contributing to the controlled etching of the fiber is electrolytic current which was monitored during etching. This method gives very close control of the electrolytic reaction occurring in the vicinity of the electrode tip while, it is being etched [105] (MS Thesis, Singh). An improvement
to above discussed method was introduced using a specially designed etching setup as follows.

A reliable and easily implemented method was adapted for etching the SiC-C composite fiber such that a sharp taper of carbon core with SiC insulation was obtained. An in-house setup was designed such that SiC-C fiber is positioned at an angle of 90 degrees with a rotating disc powered by a motor (figure 2.1). An arrangement was made such that rotary motion of the disc was converted into vertical motion of the SiC-C fiber. Beneath the fiber a container with etchant solution was placed. The rotary speed of the motor adjusted the time of fiber insertion in etchant solution. The experimental setup is designed in such a way that voltage, current, pulse and frequency could be monitored to control tip geometry and morphology. A HP 3310A function generator was used to apply square wave pulses of frequencies ranging from 50-500 Hz. Voltage was controlled by the function generator as well. A bridge circuit was connected to monitor the input and output pulses separately. A HP 54600B oscilloscope was used to study the pulses and record them with the help of HP Benchlink Software on the connected computer. Keithly 617 programmable electrometer was connected in series to the circuit to monitor the etching current. A Pt wire ring was used as counter electrode as shown in figure 2.1. The ring shaped electrode was used so that the etching proceeds uniformly around the fiber and a sharp taper can be obtained.

The etchant solution used consists of 3 ml of HF acid and 10 ml of Chromic acid. HF is a widely used etchant for SiC and Chromic acid have been used for carbon etching. The voltage applied was 5 – 10 volts AC, which generated 500 – 1000 micro amps of current. Etching time was varied from 2 – 10 minutes depending on the size and shape of the tip.
desired. Different shapes and size tips were obtained depending on current and frequency as well as on depth of insertion in the etchant.

Figure 2.1 Apparatus for electrolytic etching of SiC-C composite fiber

2.3 Scanning Electron Microscopy:

Etched SiC-C fiber was studied for the tip geometries and morphologies using Scanning Electron Microscopy technique. Hitachi S3200 and Cambridge S-90 scanning electron microscopes were used to take the micrographs. The magnification of the SEM micrographs was varied from 1000X to 50,000X. The fiber was mounted on a round shaped sample mount such that around nine fibers could be mounted at the same time and be inserted into the vacuum chamber. The fiber was stuck to the mount using a carbon tape such that good electrical connections are obtained and no surface charging occurs because of the electron bombardment. Micrographs were obtained with a non-destructive technique as fibers being conducting themselves were not metallized to prevent excessive charging. Surface morphologies were studied for roughness at high magnification (50-100 KX).

2.4 Test Cell for Electrical Measurements

Test cells were fabricated to evaluate the corrosion, impedance and biocompatibility of the electrodes. Since the electrode will be functioning in the brain
and not in air, several different test cell environments were used to ensure appropriate probe analyses is done. Test cells were scaled from simple saline solutions to common food gelatin with electrolytes to simulate brain matter. 0.9 % Molar saline solution is the most common electrolyte used for biological purposes. Phosphate Buffered Saline has the closest chemical composition to the bodily fluids. Gelatin and natural sponge were soaked in the above discussed electrolyte solution. Even though gelatin does not have the same behavior as brain matter, it gives an initial and better approximation of the electrical behavior than that in air. Highly sensitive receivers and electrical measurement bridges available in our labs were used to establish resistivity, impedance, charge density, capacitance, etc., characteristics of the electrodes within the test medium.

2.4.1 Impedance and Capacitance Studies:

Figure 2.2 shows test cell apparatus for in-vitro testing of the electrodes. Ohmic Impedance, Capacitance and Loss characteristics were measured by HP 4276 LCZ meter. A Pt foil counter reference electrode was used with very high surface area as compared to the microelectrode. The testing media is 0.9 % Saline solution soaked in special natural sponge that imitates a tissue environment. Measurements were done at different frequencies ranging from 100-20,000 Hz. Different tip sizes were tested and compared with commercially available carbon fiber electrodes. As seen in figure 2.2 a sample holder was used that could monitor the depth of insertion of the electrode probe system into electrolyte.

2.4.2 Impedance Stability Testing:

An Omega-Tip-Z megohmeter from WPI Scientific was used especially for measuring impedance in the etched SiC-C fiber microelectrodes. The meter’s AC impedance-
measuring circuit is designed in such a way that it is unaffected by electrode offset or tip junction potentials. This instrument was also used to measure DC electrode tip potentials developed in adjacent tips in a multielectrode design, while stimulating from one of the tips.

Figure 2.2 Test cell for in-vitro testing of electrode assembly.

2.5 Cyclic Voltammetry

Cyclic voltammetry (CV) is an electrolytic method that uses a working electrode (SiC-C fiber), a standard electrode (Ag/AgCl) and a counter electrode (Pt foil) in an unstirred solution so that the measured current is limited by analyte diffusion at the electrode surface. The electrode potential is ramped linearly to a more negative potential, and then ramped in reverse back to the starting voltage. The forward scan produces a current peak for any analytes that can be reduced through the range of the potential scan. The current
will increase as the potential reaches the reduction potential of the analyte, but then falls off as the concentration of the analyte is depleted close to the electrode surface. As the applied potential is reversed, it will reach a potential that will reoxidize the product formed in the first reduction reaction, and produce a current of reverse polarity from the forward scan. This oxidation peak will usually have a similar shape to the reduction peak.

Cyclic Voltammetry is a powerful tool for the determination of formal redox potentials, detection of chemical reactions that precede or follow the electrochemical reaction and evaluation of electron transfer kinetics. If a redox system remains in equilibrium throughout the potential scan, the electrochemical reaction is said to be reversible. Cyclic voltammetry is a standard technique used to reveal surface contamination, estimate relative surface area and roughness, and evaluate electrolyte leakage at electrode-insulator interfaces. It also helps in estimating potentials at which reduction-oxidation reactions occur and determine charge storage capacity.

A potential ramp between –1.5 to 1.5 V vs. Ag/AgCl was applied using EG&G PARC 273A Potentiostat/Galvanostat and the scan rates were varied. Figure 2.3 shows the special electrochemical cell used in these experiments. The two important parameters in a cyclic voltammogram are the peak current and the peak potential. Potential scans were run in PBS and ferrocene solutions. Electrochemical reversibility phenomenon and reaction kinetics were studied by oxidation and reduction of ferrocene ions. Double layer charge injection mechanism was studied and capacitance was calculated from reversible voltage scans.
2.6 Electrochemical Impedance Spectroscopy (EIS)

The electrochemical cell for an EIS experiment conventionally consists of three electrodes, which are immersed in an electrolyte. The working electrode is the SiC-C carbon fiber in this case. The working electrode is grounded, usually through an operational amplifier that is part of a current to voltage converter. The counter electrode is made from an inert metal (graphite electrode was used in this experiment). The counter electrode is connected to a potential control device called a potentiostat. The potentiostat applies to the counter electrode whatever voltage and current are necessary to maintain the potential that is desired between the working and reference electrodes. The reference electrode is constructed so that it has a negligible contact potential regardless of the environment in which it is placed. The reference electrode is connected to a potentiometer.

To make an EIS measurement, a small amplitude signal, usually a voltage between 5 to 50 mV, is applied to a specimen over a range of frequencies of 0.001 Hz to
100,000 Hz. The EIS instrument records the real (resistance) and imaginary (capacitance) components of the impedance response of the system. Depending upon the shape of the EIS spectrum, a circuit model or circuit description can be developed for electrochemical processes occurring at electrodes. This technique also aids in evaluating suitability of a microelectrode for low-noise recording and low-power charge injection. It is highly useful in fingerprinting electrode for benchmarking and quality control.

2.7 Determining Safe Charge Injection Limits

2.7.1 Current and Potential Transient Measurements: Constant voltage and constant current square wave pulses (Unipolar and Bipolar) were applied to the microelectrodes in PBS solution using HP 3310A function generator and resulting stimulus waveforms were studied on HP 54600B oscilloscope which was further captured on computer using HP benchlink software. Effect of a series capacitor in the circuit with unipolar pulse stimulation was also studied. Direct information about charge injection process was obtained by monitoring electrode potential during stimulation and a comparison was made between carbon and tungsten.

2.7.2 PH shifts and Temperature Change: An Omega PHB-62 pH meter was used to monitor pH shifts in the PBS solution while passing various charge densities using SiC-C electrodes over a period of time and it was compared with tungsten electrodes of similar impedance. Charge densities were measured with a Keithly 6517 electrometer, and a safe value was established that gave minimal pH change in solution. High charge densities from a micron sized tip can cause local temperature rise. A comparison was done for temperature change near the tip between SiC-C and tungsten electrodes using K-type thermocouple, thus establishing safe charge densities.
2.7.3 Direct Observation: Microscopic observation of H₂ and O₂ gas bubbles on electrodes during in vitro stimulation was also used to compare charge injection limits of the SiC-C and tungsten electrodes.

2.7 Biochemical Sensing Apparatus

Dopamine and Ascorbic acid were used as neurotransmitters to verify the biochemical sensing features of the composite electrode probe. Different Concentrations of these chemicals in the PBS solution were prepared. Figure 2.4 shows molecular formulas of dopamine and ascorbic acid. Dopamine Hydrochloride was used which has molecular weight of 189.60 gms and Vitamin C (Ascorbic Acid) has molecule weight of 176.12 gms. Table 2.2 shows different amount of chemicals required to be dissolved into 100 gms of PBS to obtain various molarities of the solution. Very small amounts were difficult to measure, so higher concentration solutions were diluted according to the dilution equation \( M_1V_1 = M_2V_2 \).

![Molecular Structure of Dopamine and Ascorbic Acid](image)

<table>
<thead>
<tr>
<th>Amount of Dopamine Hydrochloride dissolved in 100 ml of PBS</th>
<th>Molar Concentration Dopamine Solution Obtained</th>
<th>Amount of Ascorbic acid dissolved in 100 ml of PBS</th>
<th>Molar Concentration Ascorbic Acid Solution Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0189 mg</td>
<td>1 ( \mu )M</td>
<td>0.0176 mg</td>
<td>1 ( \mu )M</td>
</tr>
<tr>
<td>0.189 mg</td>
<td>10 ( \mu )M</td>
<td>0.176 mg</td>
<td>10 ( \mu )M</td>
</tr>
<tr>
<td>0.945 mg</td>
<td>50 ( \mu )M</td>
<td>0.880 mg</td>
<td>50 ( \mu )M</td>
</tr>
<tr>
<td>1.896 mg</td>
<td>100 ( \mu )M</td>
<td>1.76 mg</td>
<td>100 ( \mu )M</td>
</tr>
<tr>
<td>18.96 mg</td>
<td>1 mM</td>
<td>17.61 mg</td>
<td>1 mM</td>
</tr>
<tr>
<td>189.6 mg</td>
<td>10 mM</td>
<td>176.12 mg</td>
<td>10 mM</td>
</tr>
<tr>
<td>945.3 mg</td>
<td>50 mM</td>
<td>880.0 mg</td>
<td>50 mM</td>
</tr>
<tr>
<td>1896.6 mg</td>
<td>100 mM</td>
<td>1761.2 mg</td>
<td>100 mM</td>
</tr>
</tbody>
</table>

Table 2.2 Amount of neurotransmitter to be dissolved in 100ml of PBS to get different molarities solution.
Sensitivity of the carbon fiber electrode to the different concentrations of neurotransmitter solution was studied by the apparatus shown in figure 2.5. A 2 volt signal was applied using HP 6214 C power supply, between the carbon composite electrode and a counter platinum electrode which were inserted into the neurotransmitter solution. A Keithly 6517 Electrometer was connected in the series to the circuit to measure the current produced by different concentrations. Different current values were obtained for different concentrations.

Another method to measure biochemical sensitivity of carbon composite fiber electrode included the chronoamperometry technique. A potential of 1V vs. Ag/AgCl reference electrode was applied using EG&G PARC 273A Potentiostat/Galvanostat and current was monitored over a period of time. Initially the current was recorded with some concentration of dopamine in the electrochemical cell. After the current value stabilized, a measured amount of high concentration dopamine solution was added, which increased...
the concentration by a controlled amount and a sudden peak in the current was observed. After the current gets stable again some ascorbic acid was added and the shift in the current-time plot was observed.

2.8 In-Vivo Recording

In-vivo experiments were performed by Dr. Michael Behbehani, electrophysiologist, (college of medicine, University of Cincinnati), and his research assistant Mr. James Lee. For testing the dual function electrodes, recordings were made from the cerebral cortex and spinal trigeminal nucleus of the anesthetized rat to assess whether the electrode can record action potentials. Electrodes were also inserted into motor cortex after removing dura mater, and electrical current was passed and resultant stimulus effect in the motor functions was observed. Rats were anesthetized with sodium pentobarbital (50 mg/kg, ip) and placed in a stereotaxic instrument. The recording electrode in the dual assembly was used to isolate the activity of a single neuron. Data was obtained on a computer using a program that could record action potential spike intensity and also firing rate from a neuron for a given period. The stimulating electrode was connected to a constant current, stimulus isolation unit. Currents were delivered via a WPI (World Precision Instrument) constant current stimulator. Different pulse duration and pulse intervals were used. These electrodes were fit through a microdrive. A special coupling was used such that the guidance system can be advanced into the brain without turning of the system. The distance of the advance is controlled by the turn of the microdrive. By calibrating the pitch of the screw, one could advance or retreat the tip of the electrode system a known distance. The non-twisting motion is important because any twisting motion of the system could cause excess damage to the brain.
2.9 SCS-6 Fiber:

In this research Silicon Carbide-Carbon composite fiber was studied as an improved electrode material. The particular fiber used is available under trade name SCS-6. These fibers are used in metal, ceramic and polymer matrix composites and are extremely effective for high-temperature applications. Silicon carbide reinforced titanium, aluminum and ceramic composites are high in strength and stiffness, lightweight, and maintain their properties at high temperatures. SiC fibers do not react with typical matrix alloys. Other properties of these fibers include low cost, high strength, wettability for metals, high heat resistance, and corrosion resistance/chemical stability. 

This fiber is originally 140 micron thick. It has a 33 micron carbon core as shown in figure 2.6. This carbon core is surrounded by SiC layer to provide stiffness. The outermost layer is again Carbon which makes fiber conducting throughout. Table 2.3 summarizes the typical properties of SCS-6 fiber.

![Figure 2.6](http://www.specmaterials.com/silicarbsite.htm)
The SiC coating on the carbon fiber provides various advantages that are very useful in the microelectrode application. A very fine electrical insulation in the form of SiC layer right at the edge of the tip is essential for highly localized charge transfer effects and moreover it provides stiffness. SiC has exceptional chemical stability and has high biocompatibility. Silicon Carbide coatings are found to protect the carbonaceous materials against oxygen and air oxidation for extended periods of time and temperature. They act as a diffusion barrier, providing protection by inhibition of oxygen diffusion, thereby slowing down the carbon gasification [103].

Fibers with micrometer dimension require insulation along the length of the cylindrical conducting element to measure with great sensitivity, increased temporal resolution, low charging currents and greater signal to noise ratio. In the microelectrode development research there is still a requirement for enhanced fabrication techniques such that the microelectrode is insulated with durable coatings, which exhibit excellent adhesion to the conductor substrate [104]. SCS-6 fiber used in this research incorporates advanced properties of silicon carbide insulation around the conducting carbon core. Other mechanical advantages of this fiber are summarized below:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistivity</td>
<td>250 Ω/mm</td>
</tr>
<tr>
<td>Filament Diameter</td>
<td>5.6 mil (140µm)</td>
</tr>
<tr>
<td>Tensile Strength</td>
<td>500 ksi min. avg. (3450 MPa)</td>
</tr>
<tr>
<td>Tensile Modulus</td>
<td>55 msi (380 GPa)</td>
</tr>
<tr>
<td>Density</td>
<td>0.11 lb./in³ (3.0 g/cc)</td>
</tr>
<tr>
<td>Coefficient of Thermal Expansion</td>
<td>4.1 ppm/°C at RT 2.3 ppm/°F at RT</td>
</tr>
</tbody>
</table>

Table 2.3 Typical Properties of SCS-6 fiber
a) Larger diameter fiber is easy to handle. The original fiber diameter is 140 micron, thus easily noticeable by the naked eye.

b) Silicon Carbide SiC layer, being a ceramic, provides stiffness and toughness. The whole fiber is a composite material so it gives better strength. This layer also provides insulation to inner core carbon conductor.

c) The fiber can be etched to desired size using a very simple and convenient method.

d) A sharp taper can be obtained with the tip diameter of less than 5 micron which helps in penetrating the cell with better ease.

e) Outer carbon layer makes the whole fiber conducting. Wire can be connected at the other end to make end connections to the electrical circuit.

f) Noise level is lower as signal travels through thick carbon rod (33 um) as compared to 8 micron. As carbon fiber already in use has uniform diameter of 8 micron throughout its length till it is connected to end wire but Silicon carbide fiber has 33 micron thick conducting core which can withstand higher charge transfer resulting in an excellent signal.

g) This fiber can be easily inserted in the guidant tubes used for implantation of electrode assembly as compared to glass pipette design.

2.10 Electrode Configuration and Design:

SiC-C electrodes were fabricated into multiple configurations considering various spatial requirements of neural recording and stimulation. A maximum of four electrode tips were inserted into a tapered alumina tube which ends up into a 200 µm diameter probe. Issues like electrical crosstalk including impedance and charge developed were studied while passing current through one of the adjacent electrodes. A Pt bead as a
counter electrode was also inserted in the vicinity. Various stimulus waveforms (sine, triangle, square) were tested and electrodes were optimized for volts, current and frequency combinations. Discussion on single and multiple design configurations follows:

2.10.1 Single Electrode Design:

For a single electrode device, etched SiC-C fiber was packaged into a fused silica / polyimide tubing (figure 2.7), with attached leads for electrical connections. The overall diameter of the device is 350 µm. A 348 µm fused silica tubing has a 18 µm polyimide coating on it. Unique mechanical (tensile strength 15,000 psi) and electrical (Volume Resistivity $10^{16} \Omega$-cm) properties of polyimide, coupled with the high purity of synthetic fused silica, make this tubing an excellent casing for SiC-C microelectrodes.

2.10.2 Multi-Electrode design:

A guidance delivery system is an essential component to implant recording and stimulating electrodes in the brain. Current electrode guidance systems are made of stainless steel, which have problems of corrosion and MRI incompatibility. Our novel electrode design can implant several electrodes into the same area of the brain. This will allow the stimulation of different areas of the brain within given vicinity (figure 2.8). It will also allow us to deliver substances to that area of the brain. We have used a very thin ($1/32''$) tapered ceramic tube as a shield for stimulating and recording electrodes. This tube can have upto 4 holes. A cross-sectional view of this ceramic tubing is depicted in
figure 2.9. We can use different holes for different kind of electrodes to give bipolar or quadripolar stimulation or recording. In case of bipolar stimulation we can use remaining holes for drug delivery. A Delrin plug is used to surround the ceramic tube such that it provides enough toughness to the ceramic.

This Delrin plug is again inserted into another thicker teflon tube, which helps in pin connections and handling. Gold plated pins are used as the end connections to the outer circuitry. The electrode tip is sealed with ceramic tubing using insulating epoxy. Efforts have been made to connect the electrode tip with the connecting wires inside the ceramic tube using conducting epoxy or silver paste.
The Ceramic tube is tapered in such a way that there remains a very thin ceramic insulation between recording and stimulating electrodes, which prevents cross-talk between two signals (figure 2.10). The Ceramic tube is expected to provide a shield to electric and magnetic field interactions with the metallic electrodes, which can be helpful in MRI imaging. The size of tip of the tube is lesser than 100 um. The surface is very smooth which helps in safe tissue propagation. Figure 2.11 shows all the fabrication steps undertaken to design this prototype electrode probe.

![Figure 2.10 Optical picture of tapered Ceramic tube](image)

**2.11 MRI Compatibility of SiC-C Microelectrode**

To determine whether SiC-C composite electrode system is suitable for use in the MR environment, a review was done. The implants made from ferromagnetic materials undergo magnetic field related translational attraction and torque and are known to present hazards. To compare magnetic behavior of different electrode systems we reviewed and compared magnetic susceptibilities of various materials with carbon and silicon carbide. The magnetic susceptibility of the diverse forms of carbon strongly
depends on the presence or absence of aromatic-like $\pi$ electrons. Diamond has a reported susceptibility of $\chi = -4.9 \times 10^{-7}$ emu/g, due to diamagnetic contributions from core and valence electrons, and a Van Vleck paramagnetic term [105]. Graphite has an anisotropic diamagnetic susceptibility. The susceptibility parallel to the planes is about equal to the free atom susceptibilities of $\chi = -5 \times 10^{-7}$ emu/g. The $c$-axis susceptibility of graphite is diamagnetic and very large ($\chi = -30 \times 10^{-6}$ emu/g), is due mainly to free electron configuration and has been extensively modeled by McClure and co-workers [106]. It is very sensitive to the details of the band structure, and therefore to the degree of graphitization, and has been used as a tool to characterize graphitic materials [107]. So considering above references, carbon is diamagnetic or paramagnetic and has very small magnetic susceptibilities as compared to other metallic electrode materials whose magnetic susceptibilities are given in Table 3.1. Carbon composite electrode system is expected to be MRI compatible and should not produce any artifacts during imaging.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Tantalum</th>
<th>Carbon Diamond</th>
<th>Carbon Graphite</th>
<th>Copper</th>
<th>Gold</th>
<th>Iridium</th>
<th>Iridium oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>Ta</td>
<td>C</td>
<td>C</td>
<td>Cu</td>
<td>Au</td>
<td>Ir</td>
<td>IrO$_2$</td>
</tr>
<tr>
<td>Mass Susceptibility ($10^6$ c.g.s. units)</td>
<td>+154</td>
<td>-5.9</td>
<td>-6.0</td>
<td>-5.46</td>
<td>-28</td>
<td>+32.1</td>
<td>+224</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>Iron Oxide</th>
<th>Molybdenum</th>
<th>Palladium</th>
<th>Platinum</th>
<th>Silicon carbide</th>
<th>Titanium</th>
<th>Tungsten</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>FeO</td>
<td>Mo</td>
<td>Pd</td>
<td>Pt</td>
<td>SiC</td>
<td>Ti</td>
<td>W</td>
</tr>
<tr>
<td>Mass Susceptibility ($10^6$ c.g.s.units)</td>
<td>7200</td>
<td>-96.5</td>
<td>+567.4</td>
<td>+201.9</td>
<td>-12.8</td>
<td>+150</td>
<td>+59</td>
</tr>
</tbody>
</table>

Table 2.4: Magnetic Susceptibilities of various electrode materials [107]
Figure 2.11 Step by Step fabrication method of the electrode probe design
Chapter-3
Results & Discussion

3.1 Electrolytic Etching

Figure 2.1 and section 2.2 described the detailed electrolytic etching apparatus. In the advanced etching process, the concentration of etchant solution, the depth of maximal immersion, the angular speed of the wheel, and the number of revolutions was varied and their effects on resultant microelectrode geometries were studied. A concentration of 10/3 ratio of chromic and hydrofluoric acids with a voltage of 5 volt, yielded straight tip profiles. Figure 3.1 is a very good representation of the fiber etching process. It is a half-etched fiber, taken out of the etchant solution. It details the etched surface (SiC Coating) slowly being etched from carbon core fiber. Discussion on the etching parameter values and the resultant tip profiles are given in next section.

Figure 3.1) A half-etched fiber taken out of the etchant solution.

3.2 Electrode Tips and Geometries

Figures 3.2 a & b show SEM pictures of two different very fine electrode tip sizes. Figure 3.2.a is a carbon tip of ~ 1 µm diameter and figure 3.2.b shows an even smaller tip diameter i.e in nano size range (500 nm). Using an advanced etching technique developed in this research, it was possible to etch the SiC-C fiber into the nano
size diameter. Tip size and taper is controlled by the number of revolutions per minute and total number of revolutions that the etching wheel makes (figure 2.1). Etching at an rpm of 20-25 for 4 minutes gave finer tips of around 0.5-1 μm thickness, but etching at lower rpm (i.e 10-12) for similar times has produced 20-30 μm tip diameter. The angular speed could be conveniently adjusted to obtain the desired tip diameter. Use of a very fine tip is essential to prevent any tissue damage, but the higher current density at the very fine tip can cause localized heating. A balance must, therefore, be established depending on the kind of application for which the tips used. For intracellular recording electrode’s tip size should be around 1 μm, as a neuron size is in the same size range, but for extracellular recording or for tissue stimulation thicker tips can be used. Hence the close control of the tip diameter obtained by adjusting a combination of rpm value and etching time, is a very useful outcome of this research.

Figures 3.3 a & b show SEM micrographs of two different carbon microelectrode tips with varying tapers. In figure 3.3.a carbon tip with taper value of around 10° is shown and figure 3.3.b shows a taper of around 25°. It was observed that smaller taper values < 10° was obtained when the wheel speed during electrolytic etching is high (~ 35 revolutions per minute) where as thicker tapers (~ 20°) are obtained at relatively low rpm values (~15). This can be explained by considering the exposure time of SiC-C fiber to the etchant solution, which ultimately affects the reaction time. At higher rpm the very end of the tip stays in contact with the solution for a minimum time i.e. the time for which the reaction takes place is much less than the time at lower rpm values. At lower rpm, the fiber moves through the electrolytic solution slowly, thus the time of immersion in the solution increases. This ultimately increases the time for which reaction takes
Figure 3.2.a) A carbon electrode etched down to 1 µm sized tip

Figure 3.2.b) A carbon electrode etched down to 500 nm sized tip
place, due to which the very tip of the carbon fiber is exposed to the chemical reaction for a longer period of time which results in etching away of sharpness of the taper. This is a very efficient way of controlling taper of a microelectrode tip. Moreover, it is very important to optimize the taper of the electrode tip as convex tip profiles or strong tapers make the layer covering the nervous tissue of the chiasm, stick to the electrode and concave tip profiles, on the other hand, make the tip of the electrode too vulnerable.

Figures 3.4 a & b show SEM pictures of two microelectrodes with different tip lengths. Figure 3.4.a shows an electrode with 600 µm tip length which is obtained by keeping the immersion depth of ~750 µm and figure 3.4.b shows an electrode with 1 mm tip length which is obtained with 1200 µm immersion depth. By using this electrolytic etching method, tip lengths ranging from 10 µm to 3 mm were obtained depending on the depth of immersion of fiber into the etchant solution. Longer tips are preferable in some of the applications where deep insertion is required. But they also lack spatial resolution, because the conducting area is much larger. They will record not from a single tissue but from a number of tissues surrounding the long electrode tip. Epoxy insulation is then required to insulate the tip to increase their spatial resolution. When dealing with such lengths it is also important to consider the stiffness of the fiber microelectrode. To test the stiffness, the fiber was bent at an angle of 45° and fixed on a carbon tape and then studied under SEM. Figure 3.5 shows the bent carbon fiber which did not break after bending at 45°, indicating great flexibility of the fiber. This is a prime concern for preventing tissue damage, a characteristic generally lacking in metal microelectrodes. The above discussed results on tip geometries and morphologies, are extremely relevant to the electrode’s electrochemical performance, since they are shown to be interdependent (section 1.9).
Figure 3.3 a) A sharp taper of microelectrode obtained at higher rpm.

Figure 3.3 b) A thick taper of microelectrode obtained at lower rpm
Figure 3.4.a) A microelectrode with tip length of 800 µm controlled with immersion depth.

Figure 3.4.b) A microelectrode with tip length of 3mm controlled with immersion depth.
Figure 3.5) SiC-C fiber bent at an angle of 45°, showing fiber’s stiffness and flexibility

3.3 Electrode Surface Morphology

The SEM analysis was done at very high magnifications to study the surface morphology of SiC-C fibers. Figures 3.6 a & b show surface features of SiC-C fibers at 50,000X and 100,000X. These SEM micrographs show the nano-sized troughs and ridges present on the fiber’s surface. Such a surface causes ultra-fine surface roughness effect, which enhances the real surface area of the electrode. Higher real surface areas deliver higher charge densities for a smaller electrode. These electrodes are excellent for use where higher charge densities and smaller sizes are involved. This is an advantage of SiC-C composite fibers over single carbon fibers because the single carbon fibers are very smooth. Also single carbon fiber being extremely small in size throughout their length; it is very difficult to control surface roughness. For the same reason single carbon fiber can not be used for stimulating a tissue as it can not deliver high current densities.

On the contrary, SiC-C composite fibers developed in this research exhibit higher real surface area and hence can withstand higher local charges, and can deliver current to
the tissue without any damage due to tip overheating, which will be presented in later sections. The presence of nano size features on the SiC-C fiber can be explained from its structural details. These fibers are fabricated by CVD and the 33 µm carbon core consists of 1-5 nm blocks of randomly oriented turbostratic carbon which are rotated randomly about the c-axis. The Carbon core is then coated with a 1.5 µm carbon layer consisting of 30-50 nm TC blocks. During electrolytic etching procedure the grain boundaries between these 30-50 nm sized blocks are etched and form a ultra-fine rough surface, which gives a morphology as shown in figure 3.6 a & b.

3.4 Real Surface Area Calculation:

To verify this surface area increase, attempts were made to calculate the apparent stimulation area based on the polarization voltage during electrolysis. When a small voltage pulse is applied to two inert electrodes in a solution of NaCl, a short current pulse will flow, and hydrogen will be formed at the cathode and chlorine at the anode. These gases form a voltaic cell \( \text{Cl}_2 \mid \text{NaCl} \mid \text{H}_2 \) that inhibits the current flow. According to the Nernst equation, the EMF of this cell can be calculated from the formula:

\[
E = (E_{\text{Cl}_2} - E_{\text{H}_2}) - \frac{RT}{F} \ln \left( \frac{a_{\text{NaCl}}^2}{p_{\text{Cl}_2} \cdot p_{\text{H}_2}} \right)
\]

If the outside pressure equals 1 atm, then \( p_{\text{Cl}_2} = p_{\text{H}_2} = 1 \) at maximum and since \( E_{\text{Cl}_2} = 1.358 \) V and \( E_{\text{H}_2} = 0.000 \) V(by definition) this formula reduces to \( E = 1.358 - 2 \cdot 0.05914 \log a_{\text{NaCl}} \). For a dilute solution \( a_{\text{NaCl}} \approx c_{\text{NaCl}} \). So if the commonly available 0.9 percent NaCl solution is used, then \( c_{\text{NaCl}} = 0.154 \) mol/l and electrolysis will not occur if a voltage less than 1.454 V is applied (at 25 °C). So if, for instance, a 600 mV constant voltage pulse is used, electrolysis will not occur. After formation of the voltaic cell, the current flow will stop. This is not completely true, however, since some small amount of
Figure 3.6.a) Electrode’s surface morphology at 50,000X.

Figure 3.6.b) Electrode’s surface morphology at 100,000X.
gas will dissolve in the solution and will be transported away from the electrode by diffusion, a very small current will flow to make up for this effect. When the current flow is integrated over a long enough time for the current to sink to a very low, practically constant level, the charge will be proportional to the stimulation area of the electrode. It was experimentally confirmed that the charge;

\[ Q = \int_0^t I_i \, dt \]  

is proportional to the stimulation area of the electrode under test if the other electrode chosen is very large. In our experiments, a large (3 cm\(^2\) area) electrode was used. The charge \( Q \) (2.76 mC, 6.04 mC, 9.68 mC, and 11.2 mC) was calculated for a series of platinum electrodes with known stimulation areas (0.15 cm\(^2\), 0.32 cm\(^2\), 0.88 cm\(^2\), and 1.21 cm\(^2\) respectively). The relative (apparent) surface of unknown inert electrodes was calculated from the charge \( Q \) obtained and the mean charge of known electrodes. For practical purposes, an apparatus was designed supplying a constant voltage pulse of 600 mV to the electrodes (section 2.4.1). The current pulse obtained was integrated over a period of 20 sec.

Using the above discussed technique, we calculated real surface areas of carbon fiber electrodes from their charges. It was found that an electrode with geometrical area of \( 4.5 \times 10^{-3} \text{ cm}^2 \) has \( 9.03 \times 10^{-3} \text{ cm}^2 \) of real surface area. This two fold increase in area is attributed to the nano-blocks of carbon present at the surface which enhances real surface area of SiC-C electrodes.

### 3.5 Novel dual-functional electrode design

An electrode design with dual functionality, i.e. stimulating and recording to avoid use of separate electrodes and smaller size for highly localized response, is highly desirable.
Smaller size of the microelectrodes for stimulation is also desired because steady state for a faradic process is attained very rapidly and the ohmic potential drop, $IR$, is decreased, as the measured currents are very low. The innovation of this new SiC-C fiber microelectrode lies in the composite structure of this fiber. As shown in figure 3.7.a, the etched surface clearly reveals three different components of this structure. Inner carbon core which can be etched down to 1 µm tip (fig 3.2.a) act as a recording electrode and a thick layer of highly adherent SiC insulation separates it from a ring shaped outer conducting carbon coating which makes it a suitable stimulation surface. As seen in the drawing in figure 3.7.b, the above mentioned two conducting elements can be joined to connecting wires at the other end which are further in the circuit for stimulation and recording. This particular design gives the convenience of recording from the carbon tip, thus locating the right area and then stimulating it with outer circumferential carbon ring electrode.

Figure 3.7.a) SEM picture of etched electrode tip showing various components of composite fiber
3.6 Electrochemical Characterization

The electrochemical behavior of SiC-C composite fiber was studied to establish the feasibility of this novel recording as well as stimulating electrode to be implanted in a single tissue.

3.6.1 Cyclic Voltammetry:

Figure 3.8.a shows the cyclic voltammogram for the reversible oxidation (forward sweep) and reduction (reverse sweep) for hydroxy-ferrocene. Figure 3.8.b shows the scheme by which hydroxy-ferrocene undergoes a simple outer sphere one-electron redox process in aqueous buffered electrolyte. The electrochemical behavior at the SiC-C fiber in figure 3.8.a exhibits the characteristic sigmoidal and peak shaped responses, for a reversible, one electron oxidation. It should be noted here that peak shaped response is prevalent, even at slow scan rates, due to a greater contribution from planar diffusion at an electrode.
of larger surface area. This signifies exceptionally high electrochemically active surface area of SiC-C fibers. The well characterized electrochemical behavior of ferrocene, in combination of cyclic voltammetry has proven to be a diagnostic probe to test the functionality of the fabricated SiC-C microelectrode.

Figure 3.8.a) Cyclic Voltammogram for a 10 µm SiC-C fiber in an aqueous solution of ferrocene with Phosphate buffer saline solution (pH 7.3)

Figure 3.8.b) Redox scheme for ferrocene

The initial electrode potential ($E_i$) was set in a region where no reaction occurs, i.e. 0V. The potential is then scanned in the forward direction at a given scan rate of 100 mV/sec, such that it can be determined at any given time $t$ by the relationship:
\[ E(t) = E_i - \nu t \]  

(2.3)

As the voltage becomes more positive (oxidizing), value is reached, i.e. 800mV Vs Ag/AgCl, where ferrocene carboxylic acid (reduced form) is converted to the oxidized ferricinium species. This results in the appearance of the anodic peak (figure 3.8.a). Assuming that the reaction kinetics are very fast compared to the scan rate, the equilibrium involving the concentrations of reduced and oxidised species at the electrode surface will adjust rapidly according to the Nernst equation;

\[ E = E^o + \frac{RT}{nF} \ln \frac{C_O}{C_R} \]  

(2.4)

Where \( C_O \) and \( C_R \) represent the surface concentrations of oxidised and reduced species. The magnitude of the observed signal, i.e. current density at the peak (210 \( \mu \text{A/cm}^2 \)), will be very much a function of diffusional properties of the system. Intuitively, the current intensity (i.e. the flow of electrons) is expected to depend on the surface area of the working electrode (9.03 \( \times 10^{-3} \) cm\(^2\)) and the concentration of the electro-active species (20mM). The relationship for the reverse scan in the cyclic voltammogram is given by;

\[ E = E_i - 2 \nu \lambda + \nu t \]  

(2.5)

where \( \lambda \) (8 secs) is the time at which the potential is reversed. The shape of the reverse scan is therefore dependent on \( E_i \) (the switching potential, 980 mV) and the kinetics of the actual system under test. The above discussion features that SiC-C fiber microelectrode is effective in probing the mechanics of redox and transport properties of a system in solution, through ferrocene oxidation experiment. It also proves the suitability of SiC-C microelectrodes for biochemical sensing, as the electroactive neurotransmitters also oxidize with a one electron process similar to ferrocene and their chemical concentration can be determined using the above discussed equations.
Figure 3.9.a shows cyclic voltammogram for SiC-C composite microelectrode in Phosphate Buffered Saline solution (pH 7.3). The cyclic voltammogram obtained is completely reversible as there is no anodic or cathodic peak signifying oxidation or reduction at the electrode’s surface between potential -1.0 V to 1.0 V. These CV’s are featureless voltammograms. Tissue stimulation must be accomplished without causing neural damage by the formation of irreversible faradic reactions such as water electrolysis, metal dissolution or oxidation of organics. These cyclic voltammetry experiments in Phosphate Buffered Saline solution (pH 7.3) prove the electrochemical reversibility of Carbon composite fiber. These almost rectangular shaped cyclic voltammograms over a wide range of scan rates is the ultimate goal in electrochemical double layer capacitors. Capacitance is a crucial factor in electrochemical experiments because it gives rise to current during the charging of the capacitor. To calculate the magnitude of this current, we differentiate equation

$$Q = CV$$  \hspace{1cm} (2.6)

with respect to $t$,

$$\frac{dQ}{dt} = C \cdot \frac{dV}{dt}$$  \hspace{1cm} (2.7)

and assume that capacitance is constant. Recognizing that;

- $dQ/dt$ is an expression for current
- $dV/dt$ is the potential scan rate $v$,

We obtain;

$$I = C \cdot v.$$  \hspace{1cm} (2.8)

From this very simple derivation, we have an expression for the charging current at steady state when applying a ramping voltage. Thus by measuring the charging current at
a given scan rate, we can determine the capacitance of the system. A SiC-C microelectrode with area $2.2 \times 10^{-3} \text{ cm}^2$ (figure 3.9.a), with charging current of 80 nA obtained at scan rate of 100 mV/sec, gives double layer capacitance of 3.6 $\mu$F/cm$^2$.

Figure 3.9.b shows the cyclic voltammogram for the same SiC-C microelectrode at different scan rates. This experiment was done to study the effect of scan rate on charging and discharging of double layer. The scan rate in cyclic voltammetry affects the rate at which molecules diffuse to the electrode. It is found that higher scan rates increased the charging current. A scan rate of 50 mV/sec gave charging current of 20 nA for a $2.2 \times 10^{-3} \text{ cm}^2$ area electrode, but as the scan rate is increased to 300 mV/sec charging current also increased to 90 nA for the same microelectrode. This is attributed to the fact that radial diffusion from SiC-C microelectrodes gives very high rates of mass transport to the electrode surface, due to which a strong response to scan rate in the form of charging current is observed.

Figure 3.9.a) Cyclic Voltammetry plot of SiC-C electrode at a scan rate of 100mV/s with potential sweep from -1V to 1V, in PBS solution.
3.6.2 Electrochemical Impedance Spectroscopy:

Resistive and capacitive characteristics of the SiC-C microelectrode and its electrochemical interface were studied by analysis of the Electrochemical Impedance Spectroscopy spectra, and a physical model and an equivalent electric circuit describing the system. Figures 3.10.a and b show the Bode and Nyquist plots for SiC-C electrode respectively. In figure 3.10.a, bode plot, the imaginary component of impedance ($Z_{\text{mod}}$) is plotted against a frequency range from 10Hz to 100 KHz. In figure 3.10.b, Nyquist plot, imaginary component of impedance($Z'\prime$) is plotted against real component($Z'\prime$). The interpretation of this data exhibits that SiC-C electrode showed almost purely capacitive behavior at low frequencies, but clearly did not have a semicircular region in Nyquist plot at high frequencies, signifying absence of a RC time constant. The absence of the semicircular region suggests that, prompt electrochemical responses occurred on the electrode surface and also a phase angle of 60 degrees instead of 90 degrees (for purely
capacitive) indicates that electrode does not behave like an ideal capacitor. A non vertical line in Nyquist plot also signifies presence of some Warburg impedance. The Warburg impedance element arises physically from mass transport limits of ions in the solution. The reaction rate at the electrodes is assumed to be very fast, leaving mass transport as the limiting factor. Since a fluid boundary layer around the electrode shields these ions from convection, and low electric fields (due to high fluid conductivity) limit migration, diffusion is the primary mass transport method.

EIS spectra obtained in figure 3.10 is typical for a situation where semi-infinite diffusion is the rate determining step. The electrode-electrolyte interface can be characterized in terms of two major components: charge coupling due to ionic accumulation at the surface of the electrode, and faradic current from chemical reactions. In an attempt to model this behavior we start from an equivalent circuit model as shown in figure 3.11.a. The $C_{dl}$ term is due to formation of an electrolytic double layer at electrode- electrolyte interface. It depends on electrode’s effective area, and increases with effective area. $R_{ct}$ or $R_p$, Polarization resistance, depends on effective cross sectional area, tip length, and surface condition of electrode. It also represents current flow via redox reactions at the electrode fluid interface. $R_s$ is lumped resistance term including resistance of the electrolyte in bath, and resistance of electrode - wire contact. $W$ is a Warburg impedance element which is only relevant at low frequencies. For the stimulation pulses used for neural stimulation, Warburg impedance merely rounds the corners of the waveforms, producing almost negligible effect. So to simplify the circuit model according to stimulation conditions, $W$ can be neglected. Similarly, as confirmed with cyclic voltammetry that redox was absent in the electrode-solution interface, and
also bode plot does not show a shift in line due to polarization resistance, $R_{ct}$ or $R_p$ could also be neglected. This simplifies circuit to a reasonable model as shown in figure 3.11.b, with $C_{dl}$ in series with $R_S$. $C_{dl}$ and $R_S$ can be easily be calculated from nyquist plot in figure 3.10.b. The calculated value of $R_S$ is 974 ohms. Rs value is calculated by magnifying the high frequency region in the Nyquist plot, as shown in the inset of figure 3.10.b. The point where Nyquist line touches the x-axis, represents Rs value. By observing the low-frequency regions in Nyquist plot of Figure 3.10.b and using the equation

$$Z'' = 1/2\pi fC_{dl}$$

(2.9)

where $Z''$ is the imaginary component of the complex impedance, $f$ is the frequency, and $C_{dl}$ is the associated double layer capacitance, corresponding capacitance value of $= 2.7 \mu F / cm^2$ was obtained for SiC-C electrode ($2.2 \times 10^{-3} cm^2$) in PBS solution (pH 7.3). This result from impedance analysis confirmed that obtained through cyclic voltammetry.

![Figure 3.10.a](image.png)

Figure 3.10.a) Bode plot for SiC-C microelectrode($2.2 \times 10^{-3} cm^2$ area) in PBS solution (pH 7.3)
Figure 3.11.a) Equivalent circuit model for the EIS data obtained.

Figure 3.11.b) Simplified equivalent circuit model

For an electrode an increase of capacitance will decrease the impedance and increase the sensitivity of the interface. Optimum recording of a nerve signal is achieved for low impedance electrodes that have a constant behavior over the appropriate frequency range for the nerve signals, 10-10,000 Hz. The small signal generated by a firing nerve fiber makes optimization of the electrical characteristics of the electrode more important. The
EIS results discussed above show constant electrical behavior for SiC-C microelectrodes in the frequency range of interest, thus makes them suitable to detect small electrical signals.

3.7 Calculating Safe Charge Injection Limits

Different neural tissues require different stimulation charge densities through implanted electrodes. It is very important to establish safe charge injection densities and applied potential limits. We measured charge densities for a SiC-C electrode (4.1 × 10^{-3} cm^2 geom.), with impedance value of 20 KΩ in PBS solution, under different potentials applied at 100 Hz square wave. Electrode tip was monitored under microscope for any gas evolution. As shown in figure 3.12.a at 522 µC/cm^2 and 4V potential, irreversible faradic reaction was observed, that sets up the upper limit of charge that can be injected safely. The generation of oxygen or hydrogen is potentially toxic because of both, the tissue pH shifts that can accompany the reactions and the tissue-disruptive effects of gross gas bubbles. Charge injection capacity was also compared with a tungsten microelectrode of similar area (figure 3.12.b) and impedance and it was found that a tungsten microelectrode has lower injection limits than carbon, as bubble formation was observed near the tip at lower potential (2.7 V) and charge density (250 µC/cm^2). This behavior is attributed to the metallic nature of tungsten and also tungsten is unacceptable with biphasic current pulses due to surface reactions and rapid changes in impedance. Tungsten and other non-noble metal electrodes rely on thin passive films for corrosion protection and inject charge primarily by faradaic processes involving reduction and oxidation of their passivating films. Problems occur with these metals when their potentials are driven too far anodic into the transpassive region where breakdown of the
passive layer occurs, e.g. 3V for tungsten. In the transpassive region charge injection occurs at least in part, by irreversible processes involving metal dissolution and water electrolysis. Tungsten was chosen for a comparison because microelectrode stimulation with electrodes of the size of single neuron is generally done with tungsten.

Figure 3.12.a) Charge density response of SiC-C composite microelectrodes to different potentials applied.

Figure 3.12.b) Safe charge injection comparison between SiC-C and tungsten microelectrode
3.8 Optimizing stimulation parameters

The time varying pattern that constitutes a stimulation waveform is probably the most important parameter governing both the efficacy and the safety of neural stimulation. We used three basic waveforms; sinusoidal (sine), pulsatile (square) and triangular at three different frequencies to study the efficacy of these different stimulation protocols in eliciting the desired physiological response. As established in previous section above, the safe potential window for SiC-C electrodes is 3.5 V, so here we applied 2 V potential at 1, 100, 1000 Hz, with different waveforms and studied the charge transfer response. Figure 3.13 shows the charge density measured for SiC-C microelectrodes with the combination of different frequencies and waveforms at 2V potential applied. As shown in this plot, square waveform gives highest charge densities for all the frequencies studied. Pulsatile waveforms consist of a periodic train of pulses usually having equal amplitude. The waveform is described completely by specifying the pulse amplitude and its duration, t, the polarity of the signal, and the period, T, or pulse repetition frequency. For figure 3.13 pulse amplitude, period and polarity of signal was same. So it is the duration of pulse, t, which makes a difference, and for a square wave, during pulsing, potential stays at a maximum of a polarity for the whole duration of pulse, t, which is not true for sinusoidal waveforms such as sine and triangular. Moreover charge, q, per pulse is proportional to pulse current and duration according to

\[ q = it \]  

(2.10)

and there is an inverse relation between duration and frequency. Due to this relationship, as shown in figure 3.13, a decline in charge delivered was observed with rising frequencies. At higher frequencies pulse duration gets smaller and thus the charge
delivered per pulse which is directly proportional to the pulse duration (eq. 2.10), also reduces.

![Effect of frequency and stimulus waveform on charge delivered](image)

Figure 3.13) Charge density obtained for SiC-c electrodes at different stimulation frequencies and waveforms.

### 3.9 Multi-Electrode Properties

The novel electrode design discussed in section 2.9.2 can implant several electrodes into the same area of the brain. Figure 3.14.a shows the SEM micrograph of four SiC-C electrode tips inserted in the tapered ceramic tube, in a total vicinity diameter of 200 µm. A sketch of this design is also shown in figure 2.9. A very thin (700 µm) tapered ceramic tube as a shield for stimulating and recording electrodes has been used. Ceramic tube is tapered to a tip of less than 100 µm diameter (figure 3.14.a). This tube can have up to 4 holes. The electrode tip is sealed with ceramic tubing using biocompatible insulating epoxy. It is very important to seal these electrodes efficiently with the ceramic tube as it improves insulation and provides enhanced spatial resolution. Figure 3.15 shows the SEM picture of a frontal view of the multi-electrode design. It can be clearly observed that the biocompatible epoxy used has no porosity and there are no
voids left. This configuration can be used for multiple stimulation depending upon the desired spatial requirements for the tissue to be stimulated. This will allow the stimulation of different areas of the brain within given vicinity. The activation of different parts of the nucleus may circumvent the adaptation of the brain circuitry that in turn reduces the efficacy of the chronic stimulation. Having four functional electrodes in proximity offers the potential that if the one electrode fails, e.g., breakage of leads, one can substitute its function with the other electrode. This will reduce the time and cost of total replacement. Another arrangement of electrodes is shown in figure 3.14.b, here a platinum bead with approx. diameter of 150 µm is used in vicinity of carbon electrodes. This particular configuration is helpful when stimulation intensity requirement is beyond safe charge injection limits of carbon microelectrodes.

Figure 3.14.a) A multielectrode design with four SiC-C carbon tips protruding out from an alumina tube

When using multiple microelectrodes in vicinity and in a limited area, it is very important to consider the issue of electrical cross talk between electrodes. A charge delivered
through electrodes produces diffusional flux which can influence properties of adjacent electrodes. If the gap between the electrodes is wide compared to the diffusion layer thickness then diffusion layers for different electrodes do not overlap and electrodes do not affect one another. But if they overlap partially, then the overall current is smaller than the currents passing through the electrodes when they operate independently. To investigate this, the impedance and DC electrode tip potentials, developed in adjacent electrode tips were measured. Figure 3.16 is a plot of impedance and DC potential developed in one of the electrodes in a four electrode assembly, while increasing the charge density delivered through an adjacent electrode in the vicinity. As shown in figure 3.16, while stimulating through one electrode, neighboring electrode’s impedance stays constant at 40 kohm upto charge densities of 454 µC/cm², but further rise in charge density effects impedance...
considerably. Similarly DC potentials developed on adjacent tip does not change much until charge delivered is below the 454 µC/cm² limit in adjacent electrode tip.

![Figure 3.16](image)

Figure 3.16) Impedance and potential developed in adjacent electrodes while stimulating under various charge density conditions for a multielectrode configuration.

This indicates that while using a multielectrode configuration using SiC-C electrode with electrodes ~200 µm apart, the diffusional flux generated by 454 µC/cm² charge density is safe. Any charge density beyond that causes diffusion layer to widen and overlap with adjacent electrodes thus affects their properties. A pictorial representation of this effect is depicted into a drawing as seen in figure 3.17. In this drawing the colored circular lines represent the charge flux density surrounding the tips and as the charge density increases these flux lines widens and intersect each other representing an overlap of charge density flux thus causing an electrical cross-talk between neighbouring electrodes, which changes their impedance and develop DC potentials. Above discussion has covered electrical cross-talk issues in multiple-electrode design, and it can be said that for a spatial area of 200 µm, SiC-C microelectrodes can inject charge densities as high as 450 µC/cm² without causing any electrical property change in the adjacent electrode.
3.10 Temperature and pH monitoring

Safe charge injection for stimulation also requires that there is minimal damage or change in the surrounding tissue environment. Some exothermic reactions near the tip can lead to temperature rise, thus can affect tissue. The measurement setup for this experiment is discussed in section 2.7.2 in detail. A k-type thermocouple was used at a distance of 1 mm from the tip and temperature was monitored for one hour in both SiC-C and tungsten microelectrode, while injecting charge densities near the upper limit of electrode’s safe injection limits. Figure 3.18.a shows a plot of temperature rise in 1 hour near the SiC-C microelectrode tip when 450 µC/cm$^2$ charge density was passed through the electrode. The temperature change observed was almost negligible (0.3 °C) for SiC-C electrodes and around 1.0 °C for tungsten microelectrode. Tungsten electrodes showed a little higher (~ 2.0 °C) rise as the reactions taking place near the tungsten tip are more intense because of its metallic nature. Similarly pH change was also monitored in the Phosphate Buffered Saline solution (pH 7.4), under similar conditions for 24 hours as discussed above. Figure 3.18.b shows a plot of pH change in PBS solution (initial pH 7.4) when 450 µC/cm$^2$ of
charge is passed for 24 hours. SiC-C microelectrodes did not show any significant pH change, whereas in the case of tungsten it dropped below 7.0, which clearly is undesirable. Reactions that take place at tungsten surface do produce or consume protons or hydroxyl ions as charge compensating counter ions to maintain electrical neutrality in the passive oxide films. One of the very common reaction is H-atom plating reaction on

![Temperature change comparison near the tip of microelectrode](image)

Figure 3.18.a) Temperature rise in 1 hour near the microelectrode tip when 450 µC/cm² charge density was passed through the electrode.

![pH change comparison of microelectrodes](image)

Figure 3.18.b) pH change in PBS solution (initial ph 7.4) when 450 µC/cm² of charge is passed for 24 hours
tungsten surface:

\[
\begin{align*}
W + H^+ + e^- & \rightarrow W-H \\
W + H_2O + e^- & \rightarrow W-H + OH^-
\end{align*}
\]

These reactions alter the concentration of protons and hydroxyl ions around the electrode surface. Therefore, the immediate layers of fluid next to the electrode undergo pH shifts. The magnitude of the pH change depends on the current density, the rate of fluid exchange and the buffering capacity of tissue fluid.

3.11 Potential and Current Transient Measurements:

Neural damage has more to do with the stimulation process than with the electrochemical reactions. It is very important to monitor various stimulation parameters and study evoked responses from them. Monitoring the electrode potential during stimulation can provide direct information on charge injection process. An experimental setup to do these measurements is discussed in section 2.7.1. The SiC-C electrodes were tested and compared with current and voltage source as stimulants. It was found that current source is far more stable than a voltage source. Figure 3.19.a shows an oscilloscope picture captured on a computer with the voltage stimulant square wave pulse as input (top) and current response as output (bottom). The shape of current waveform delivered to the tissue is not rectangular, but resembles the charging current of an RC circuit. However, the electrode-electrolyte interface is a complex RC network for which the impedance changes during stimulation. The lack of control over the resulting fluctuations of current and charge is a major drawback to voltage stimulation. Finally, it is impossible to relate the voltage applied between two electrodes to the actual potential difference across the electrode/electrolyte interface, and to ensure the absence of irreversible charge injection.
processes during the voltage pulse. This limitation is there because electrode/electrolyte interface consists of capacitive and resistive components, which makes it a separate circuit element altogether other than the actual circuit. When a potential drop is calculated between the actual electrodes, it ignores the potential drop at local electrochemical interface. Thus when any irreversible reaction takes place at the interface, then the potential drop related to that is not reflected in the actual drop. Due to this artifact in voltage stimulation, current stimulus is preferred. Figure 3.19.b shows the oscilloscope picture as captured on the computer, plotting input current stimulus (top) and resultant voltage response (bottom). Here a charge balanced, biphasic current pulse at charge density of 350 µC/cm² was used as a stimulation waveform, and resultant potential excursion was analyzed as shown in figure 3.19.b. The resultant potential excursions are quite symmetrical with input charge pulses, which signal a safe charge injection without much of faradic reactions taking place at the electrode’s surface. To study this response at an extrapolated scale, the lower end of the resultant voltage pulse was extrapolated in figure 3.20. This is the charge transfer region for the SiC-C electrode. A voltage drop of ~400 mV is observed and the shape resembles to capacitive discharging of the double layer of the adsorbed water molecules on the electrode surface. This also confirms results from CV and EIS experiments (section 3.6)

A simple monophasic waveform with a pulse train of single polarity is unacceptable for neural stimulation since it delivers a dc signal, which results in water electrolysis and both electrode and tissue damage. Stimulators that deliver monophasic waveforms have some kind of charge retrieval circuit built into them to prevent a net dc leakage.
Figure 3.19.a) Stimulation response with voltage pulse input.

Figure 3.19.b) Stimulation response with Current pulse input
The most effective charge retrieval mechanism is a capacitively coupled circuit in which a series capacitor delivers a reverse current through the stimulation electrode between pulses and prevents accumulation of charge on the stimulation electrode. SiC-C microelectrode was tested under similar condition with 1 µF capacitor in series. The resultant response obtained is shown in figure 3.21. After a cathodal current pulse, the potential of SiC-C electrode was negative with respect to the counter electrode and was discharged through the exhausting circuit until the potential difference between them is zero. This way, interpulse potential can be moved both ways, in the same as well as opposite to the polarity of the pulse delivered to the stimulation electrode. The new interpulse potential is advantageous for SiC-C stimulation cathode because it extends the potential window for charge injection.

An attempt was also made to stimulate with tungsten electrode of the same surface area as SiC-C and with a charge balanced biphasic current pulse as shown in
figure 3.21. a. Potential response obtained is quite asymmetric, which signifies that there are reactions that occurred during pulse stimulation. Since the biphasic current pulses were charge balanced, the asymmetry of the potential waveform reflects inequalities in the rates at which anodic and cathodic reactions occur. Biphasic potential transients tend to become asymmetric if a reaction, such as H$_2$ or O$_2$ evolution, which occurs in the first phase of pulse pair, can not be quantitatively reversed in the second phase. Similar effect was observed after a continued stimulation with tungsten microelectrode. As seen in figure 3.21.b, the resultant potential excursions become more asymmetric and artifacts are observed in both anodic and cathodic parts of the pulse.

![Figure 3.21.a)](image)

**Figure 3.21.a) Stimulation with Tungsten electrode gives asymmetric resultant pulse.**

### 3.12 Biochemical Sensing of Neurotransmitters

In this research, SiC-C composite electrodes were tested for biochemical sensing of neurotransmitters. As explained in section 1.8, at a given voltage any monoamine present at the electrode surface oxidizes and surrenders electrons to the surface.
These electrons form a current proportional to the concentration of monoamine at the electrode surface. This phenomenon was observed in the experiments involving electrochemical detection of Dopamine Hydrochloride and Ascorbic Acid. Figure 3.22 shows a plot of current response from SiC-C electrode for different Molar Concentration of Dopamine and Ascorbic Acid. The increase in concentration of dopamine and ascorbic acid has given an increase in oxidation current. The plots for dopamine and ascorbic acid follows the same behavior because ascorbic acid has adjacent labile hydroxyl groups on an unsaturated five-carbon ring and thus exhibits a similar oxidation reaction to the dopamine (Catecholamine). The oxidation reaction scheme for both of these compounds is discussed in section 1.8.

It is also noted in figure 3.22 that the increase in current varies differently for smaller and larger concentrations of these neurotransmitters. For example for lower dopamine or ascorbic acid concentrations (1 to 1000 μM) oxidation current varies linearly from 100 to 700 nAmp and for higher concentrations (1000 to 100000 μM) it...
varies by larger amounts ranging from 700 to 4500 nAmp. This behavior can be explained from the assumption that anodic current is the sum of the oxidation of adsorbed Dopamine or Ascorbic Acid and oxidation of DA or AA around the sample. At higher concentrations the adsorbed DA or AA should be saturated and therefore the intensity increases linearly according to the linear increase of DA or AA concentration around the electrode. But at lower concentration, adsorption process continues due to which change in concentration around the electrode is less evident.

![Current sensitivity for different Neurotransmitter Concentration](image)

Figure 3.22) Plot showing current response from SiC-C electrode for different Molar Conc. of Dopamine and Ascorbic Acid

The carbon composite electrode’s biochemical sensor response to the addition of ascorbic acid and dopamine was also detected by the potentiostat. Chronoamperometry technique was used where a constant potential of 1 volt was applied between the carbon composite electrode and standard Ag/AgCl electrode and the current was monitored between the working electrode and platinum counter electrode. The concentration of
electrolyte was changed and the change in the current response was observed. As shown in figure 3.23, current is plotted over a period of time. The initial concentration of the electrolyte (ascorbic acid) was 10mM, and it gave a constant current in the range of 5 nAmp. After 90 seconds, measured amounts of higher concentration ascorbic acid was added such that the final concentration of the electrolyte reached 20 mM which resulted in a peak in the current Vs time plot. The current obtained was around 12 nAmp. After 180 seconds when the process was stabilized another measured quantity of AA was added such that the final concentration of the electrolyte became 100 mM. This high concentration rise gave a very sharp peak in the current plot. This particular plot shows that the SiC-C electrode tip is highly sensitive to the concentration changes and it has a highly sensitive response.

As seen in the plot 3.23, it takes only 1 second to sense the concentration rise. Similar behavior was tested for Dopamine Hydrochloride addition to the electrolyte and the current vs time was plotted as shown in figure 3.24. It is seen that there is a current rise on addition of 100mM concentrations. This current rise is smaller and less sharp as compared to the current rise in ascorbic acid sensing. This could be based on the ease of diffusion of the electrons in different electrolyte. Besides the fact that Ascorbic acid and Dopamine have similar oxidation reactions, the molecules are different, in that dopamine hydrochloride has a complex molecular structure. This would likely lead to slow diffusion of electrons to the working electrode and hence gives weaker response as compared to ascorbic acid.
Figure 3.23 Chronoamperometry plot of Ascorbic Acid (Vitamin C) sensing showing current rise with the addition of higher molar concentrations as detected by SiC-C composite electrode.

Figure 3.24 Chronoamperometry plot of Dopamine sensing showing current rise with the addition of higher molar concentrations as detected by SiC-C composite electrode.
This phenomenon is very crucial to study the release of neurotransmitters in intact animals, without the use of complicated radioactive labeling. Moreover, selective sensing of different neurotransmitters is also possible with advanced electrochemical techniques including Fast Cyclic Voltammetry (FCV) and Differential Pulse Voltammetry (DPV), which differentiate Neurotransmitters based on their oxidation potentials. But these techniques are complex and are beyond the scope of this research.

3.13 In-Vivo Results

In vivo experiments were done at the University of Cincinnati’s Physiology Department by Dr M. Behbehani and his research assistant Mr. James Lee. The electrode prototype assembly was implanted stereotaxically into the various sections of the rat’s brain. Figure 3.25 shows action potential spikes recorded from cerebral cortex of the rat’s brain. Cerebral cortex plays a central role in many complex brain functions including memory, attention, perceptual awareness, "thinking", language and consciousness. The cerebral cortex is made up of neuron and supporting cells (glial cells) and functions to correlate information from many sources. Recording from such an area can provide much diagnostic information. Each line in figure 3.35 represents recording over a time of 60 seconds. This figure is densely plotted because a single spike has duration of 2 milliseconds. Figure 3.26 depicts the action potential intensity on the y co-ordinate, which suggests that action potential spike recorded from these particular neurons generated ~30-60 mV of signal. To verify above claim the data was expanded and plotted on a smaller scale. As shown in figure 3.27, a typical action potential signal lasts for about 2 msecs. These traces show continuous recordings. Over 10 minutes of continuous recording was done using SiC-C microelectrodes. Figure 3.28 show action potential
spikes recorded using a tungsten microelectrode. This recording was done for comparison between results from SiC-C and tungsten microelectrodes. When compared with recordings obtained from SiC-C, it was observed that a significant reduction in the noise level was obtained using SiC-C electrode. The Signal to Noise Ratio (SNR) observed in these recordings was in range of 20-25 for SiC-C electrode whereas 4-5 for W electrode.

Figure 3.25) Figure showing recording from cerebral cortex of rat brain, each line depicts 60 secs of recording

High SNR for SiC-C microelectrode is attributed to the exceptional electrochemical properties of carbon and its tendency to form a low noise interface with electrolytic
solutions. These recording plots show very smooth recording without any significant noise.

The SiC-C electrodes were also used to record the sensory response in neurons of spinal trigeminal area. The trigeminal nerve is responsible for sensation in the face. The spinal trigeminal nucleus receives information about light touch, pain, and temperature from the ipsilateral face. Spinal trigeminal nucleus is composed of three parts: the oral part and interpolar part and the caudal part in the Medulla Oblongata. In this experiment recording was done from the caudal part. Firing rate of sensory neurons changes if the receptive field (face; in this case) sees some touch, pressure etc. Firing response shown in figure 3.29 represents the action potential spike rate, recorded with SiC-C electrodes that are generated in the spinal area. There is a baseline with around 10 spikes/sec. As a touch near rat’s face is applied, neurons fire at a higher rate (figure 3.29). This data shows that SiC-C microelectrodes can record these instant changes in the rate of firing of sensory neurons. Above discussed data confirms the in-vivo recording characteristics of the SiC-C microelectrodes.

**Stimulation:** The effectiveness of SiC-C composite microelectrodes, to electrically stimulate rat’s brain, was established. For initial feasibility testing the electrode was inserted in an outer muscle near the rat’s brain and muscle twitching was observed after passing of electrical currents. After this confirmation, dura was removed and cortex was exposed. Then the SiC-C composite electrode was inserted in the motor cortex of rat’s brain. Motor cortex is a region involved in the planning, control, and execution of voluntary motor functions and it can be divided into the primary motor cortex and the secondary motor cortices. The primary motor cortex is one of the principal brain areas
and is located in the frontal lobe of the brain. The role of the primary motor cortex is to
generate neural impulses that control the execution of movement. Signals from primary
motor cortex activate skeletal muscles on the opposite side of the body, meaning that the
left hemisphere of the brain controls the right side of the body, and vice versa. If an
electrical stimulation is applied in this area the resultant stimulus effect will evoke
change in particular motor functions.

To do so, SiC-C composite electrode penetrations were made perpendicular to the
cortical surface. The penetration depth was kept around 1.5 mm below the pia. The
stimulating electrode was connected to a constant current, stimulus isolation unit.
Currents were delivered via a WPI (World Precision Instrument) constant current
stimulator. The initial stimulus parameters were a 100 ms train of 0.40 ms cathodal (-)
pulses. Application of this electrical stimulus in the RFL (Rostral Forelimb Region)
evoked contralateral movements in the forelimb. Also when the location of SiC-C
composite microelectrodes was changed, i.e, they were inserted in a region rostral to
RFL, rat’s nose twitching was observed and this twitching lasted for the complete period
of stimulation. Rat’s brain mapping experiments by various researchers have shown that,
with perpendicular electrode insertion it is very occasional to locate a rhinarium (nose
twitch) region which is either rostral or caudal to the RFL [108]. In our experiments it
was possible to do so, as 140 µm sized SiC-C microelectrodes, produced highly efficient
and much localized microstimulation. For these stimulation experiments different pulse
durations (400-600 µsec.) and pulse intervals (100-1000 msec.) were used and
movements evoked were compared and it was found that only the duration of the
movements changed, not the movement produced.
Figure 3.26 Plot showing volts intensity of action potential spikes recorded from cerebral cortex of rat’s Brain

Figure 3.27 A single action potential spike (2 mSec) plotted from the data obtained for 5 sec recording.
Figure 3.28) In-Vivo Recording from cerebral cortex of anesthetized rat’s brain using tungsten (W) microelectrode

Figure 3.29) Firing response from sensory neurons in caudal spinal trigeminal area on touching various areas on the rat’s face.
Chapter-4
Summary

In this research we have addressed various issues related to the materials science aspect of the medical electrodes. In particular we studied Silicon Carbide Carbon composite fiber as an electrode material for neuronal stimulus and activity sensing, and for biochemical detection of electroactive neurotransmitters. A novel microelectrode design was developed that constitute dual functionality. An advanced electrolytic etching method was designed and the etching process was monitored to obtain different sized, shaped and surface area microelectrode tips and quantifiably established relationship between these resultant tips and various etching parameters. Before testing the microelectrodes in in-vivo environment, feasibility was established in-vitro using various material processing, characterization and testing techniques.

Scanning Electron Microscopy technique was used to study different tip geometries, shapes and surface morphologies. Tip length, tip diameter and taper were carefully studied. Presence of nano-sized blocks on carbon surface was observed under high magnification SEM. These blocks increased the real surface area of these electrodes by several folds, as calculated by measuring polarization voltage during electrolysis. The well characterized electrochemical behavior of ferrocene, in combination of cyclic voltammetry was used as a diagnostic probe to test the functionality of the fabricated SiC-C microelectrode. The electrochemical behavior at the SiC-C fiber and ferrocene solution exhibited the characteristic sigmoidal and peak shaped responses, for a reversible, one electron oxidation. The cyclic voltammogram obtained was completely reversible as there was no anodic or cathodic peak signifying oxidation or reduction at the electrode’s surface observed. It also implied that the current generated was due to the
charging and discharging of the double layer. This signified exceptionally high electrochemically active surface area of SiC-C fibers. Electrochemical Impedance Spectroscopy was used to develop the electrochemical model of the electrode-electrolyte interface and to calculate double layer capacitance and resistance of the adsorbed water molecule capacitive layer. Safe charge injection limits of SiC-C microelectrodes were calculated and their dependence on different voltage applied was observed and compared with commercially available tungsten electrodes. SiC-C electrodes were observed to have higher charge injection limits than tungsten, because of their exceptionally high surface area and stable electrochemical behavior. Stimulation parameters were optimized, considering stimulus waveform shape, pulse amplitude, frequency and resultant current density and the best combination was achieved with a square wave at low frequency stimulus.

Issues of electrical cross talk and charge delivered diffusional flux overlap in a multi-electrode design were studied. Change in impedance and DC potential developed in adjacent electrodes on increasing charge densities in one electrode was calculated and an electrochemical relation between these parameters was established. Temperature and pH change in the electrolyte solution in vicinity of electrodes was monitored and compared with tungsten electrodes. Tungsten showed an undesirable pH change (< 7.0) and a temperature increase, given to the violent and exothermic corrosive reactions taking place in the vicinity of microelectrodes. Potential and current transient measurements showed stable response for a SiC-C microelectrode as compared to tungsten microelectrodes. Effect of a series capacitor in a stimulation circuit was studied and it was established that
higher charge densities can be injected with such arrangement as monophasic input stimulus can withstand higher currents.

Biochemical sensing of neurotransmitters including Dopamine Hydrochloride and Vitamin C was done with the SiC-C composite electrodes. Oxidation current for these electroactive chemicals was found to be varied linearly with the concentration. Excellent sensitivity towards the concentration change was observed and studied with the chronoamperometry technique. In-Vivo action potential recording from the cerebral cortex of the rat brain was successfully accomplished with very high signal to noise ratio. Effect of external stimulus (touch, pressure) on the sensory neurons of spinal trigeminal area was also studied in-vivo by observing firing rate of neurons. The effectiveness of SiC-C composite microelectrodes, to electrically stimulate rat’s brain, was established by inserting these electrodes into motor cortex of rat’s brain and studying evoked motor functions. A prototype electrode probe device was designed to incorporate the electrode such that it has provision for drug delivery in the vicinity of the electrode as well as it can implant several different electrodes in the immediate vicinity of each other. This particular design is totally non-metallic as compared to the commercially available stainless steel guiding tubes and hence it is expected to provide shield for electrical and magnetic fields.
Chapter-5
Conclusions

- Silicon Carbide/Carbon composite fibers were successfully etched in the shape of a microelectrode using electrolytic etching technique. The electrode tips with a size as small as 1 µm in diameter and 500 µm in length were obtained.

- The real surface area calculated with electrochemical techniques showed a two fold increase from geometrical area due to the presence of 30-50 nm Turbostratic Carbon blocks.

- A novel dual functional design was introduced such that inner carbon core is used as recording electrode and outer carbon ring as stimulating electrode.

- Functionality of the fabricated SiC-C microelectrode was tested using combination of cyclic voltammetry and the well characterized electrochemical behavior of ferrocene. Cyclic Voltammetry in PBS solution showed featureless voltammogram and a double layer capacitance value of 3.6 µF/cm² was calculated.

- An electrochemical model was developed using EIS that showed an absence of semicircular region in Nyquist plot at high frequencies, signifying absence of a RC time constant. A non vertical line in nyquist plot also signified presence of some Warburg impedance. Capacitance of capacitive double layer was calculated to be 2.7 µF/cm² and polarization resistance (Rs) of 974 ohms was also calculated from nyquist plot.

- Safe charge injection limit of 522 µC/cm² was calculated for SiC-C electrode as compared to 250 µC/cm² for tungsten microelectrode. It was also found that square waveform stimulus gives higher charge densities than sine and triangular pulses.
- SiC-C electrodes can be used in multielectrode configuration such that the distance between electrodes is 200 µm and charge density applied is less than 450 µC/cm².

- SiC-C microelectrodes do not show a pH and temperature change when stimulated with charge densities near the upper limits of safe charge injection, over a period of time, where as a tungsten electrode show a undesirable pH drop under similar conditions.

- Contrary to tungsten microelectrode a symmetric potential response was observed for SiC-C in potential transient measurements when a charge balanced biphasic current pulse is applied.

- Neurotransmitters including Dopamine and Vitamin C were successfully detected using SiC-C composite electrodes. The oxidation current increases linearly with the concentration. SiC-C electrodes have shown excellent sensitivity and high resolution for biochemical sensing. For 10 µM concentration of dopamine and ascorbic acid, a stable current of 500 nA is obtained. A 10 mM addition of Vitamin C and Dopamine Hydrochloride gives a significant peak in the current curve.

- Action potential spikes were successfully recorded from a rat's brain using SiC-C microelectrodes. The recordings obtained were continuous for 10 minutes and have a very high signal to noise ratio of 20-25 for SiC-C electrode compared to 4-5 for tungsten electrode. In-vivo stimulus (touch, pressure) response of sensory neurons of spinal trigeminal area was successfully detected with SiC-C microelectrodes.

- The effectiveness of SiC-C composite microelectrodes, to electrically stimulate rat's brain, was established by inserting these electrodes into motor cortex of rat’s brain and studying evoked movements in contralateral forelimb and nose.
Chapter-6
Future Direction

- The SiC-C composite fiber system can be designed and developed as a prototype DBS electrode device for clinical testing. This would include use of advanced micro-fabrication technology for electronic packaging and adjusting device parameters according to FDA regulations.

- The charge density profile as for particular, related to the electrode design and tip geometry can be established further. To do this one can study patterns of neural excitation as function of electrode geometry by measuring the electric field generated in the simulated tissue medium. These tests can be carried out in growth medium supplemented with serum, under sterile conditions, to emulate the in vivo condition.

- A three dimensional finite element model can also be developed, to quantify the current density distribution around the stimulation surface with varying surface area and thickness.

- The real surface area of the tip, as reflected by surface roughness, can be further enhanced by activation of carbon, or by deposition of a conducting polymer or other thin film coatings on the electrodes. This would increase charge carrying capabilities of microelectrodes with minimum surface area.

- Various In-Vivo tests can be further performed in guinea pigs to study long term effects and stability as their brain can sustain longer implantation as compared to rat’s brain. Explanted electrodes after prolonged tests can be examined, to determine deterioration of the electrode surface or any growth of cells on the
surface. Also the location of the electrode in the brain can be examined for tissue damage.

- A real time MRI experiment can be performed to verify the claims, as carbon has low magnetic susceptibility and has been claimed to give enhanced images as compared to blurred images obtained for a metallic electrode.

- For biochemical sensing enhanced selectivity, sensitivity, and resolution for different neurotransmitter chemicals can be obtained by activating the carbon surface or by using ion selective coatings like nafion. Advanced electrochemical techniques including Fast Cyclic Voltammetry and Differential Pulse Voltammetry can also be employed for this purpose.
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