2.1 PREPARATION OF GLASSES

In this work, to prepare bioactive glasses two different methods are adopted namely:

- Melt Quench Method
- Sol-Gel Method

2.1.1 Melt Quench Method

Bioactive glasses are typically prepared from high-purity raw materials as the quality strongly influences the end product. Accurately weighed amount of raw materials are melted in a Pt or Pt/Rh crucibles and then it is homogenized as required according to the composition. The melting temperatures can range from 1200 to 1450°C, depending on composition. The melt is cast in stainless-steel moulds, which do not contaminate or adhere to the glass. The glass is then annealed to suppress the thermal stress generated during casting and cooling, depending on the composition. The bioactive glasses are then cut into desired shape and size by using diamond wheels under coolant.

2.1.1.1 Materials used

High purity reagent grade, Na₂CO₃ (sodium carbonate), CaCO₃ (calcium carbonate), SiO₂ (Silicon-dioxide powder) and (NH₄)₂HPO₄ (di-ammonium hydrogen phosphate), were used precursor without further purification.

2.1.1.2 Synthesis

Initially, the reagents Na₂CO₃ and CaCO₃ were kept in oven and dried at 393K in order to remove any traces of water and adsorbed gases. Then the stoichiometric amounts of the starting materials were thoroughly ground in an agate mortar for 50min or using the planetary ball mill. The mixture was then placed in platinum crucible and melted in an electric furnace for 5 hours at 1773 K. After complete homogenization, the melt was poured into preheated stainless steel molds. The obtained bulk glass samples were then annealed, to remove the internal stresses, 30K
below their respective glass transition temperature for 5 h. The molar compositions of samples prepared by melt-quenching technique are listed in Table 2.1

Table 2.1: Molar Composition of melt-derived bulk glasses

<table>
<thead>
<tr>
<th>Composition (mol%)</th>
<th>SiO$_2$</th>
<th>Na$_2$O</th>
<th>CaO</th>
<th>P$_2$O$_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>45S5</td>
<td>46.1</td>
<td>24.4</td>
<td>26.9</td>
<td>2.6</td>
</tr>
<tr>
<td>55S4.3</td>
<td>55.1</td>
<td>20.1</td>
<td>22.2</td>
<td>2.6</td>
</tr>
<tr>
<td>60S3.8</td>
<td>60.1</td>
<td>17.7</td>
<td>19.6</td>
<td>2.6</td>
</tr>
<tr>
<td>73S10C</td>
<td>73.4</td>
<td>14</td>
<td>10</td>
<td>2.6</td>
</tr>
<tr>
<td>63S20C</td>
<td>63.4</td>
<td>14</td>
<td>20</td>
<td>2.6</td>
</tr>
<tr>
<td>53S30C</td>
<td>53.4</td>
<td>14</td>
<td>30</td>
<td>2.6</td>
</tr>
</tbody>
</table>

2.1.2 Sol-Gel Method

Sol-gel glasses were obtained by hydrolysis and polycondensation of organic precursors, in aqueous environment, with an acidic or basic catalyst. Ethanol was added to the starting solution, to help mixing organic and aqueous phases. Solution was continuously stirred for a long time, and organic precursors were hydrolyzed. For example a typical precursor for SiO$_2$ is TEOS (tetraethyl orthosilicate) that hydrolyses as Si(OH)$_4$ and ethanol (Figure 2.1).

Figure 2.1: Chemical Reaction in sol-gel synthesis [1]
In parallel with Si-OC₂H₅ bond hydrolysis, a process of recondensation occurs between the silanol groups. A polymerized network is obtained, which finally becomes a gel. After gelation, the sample is aged usually at room temperature, and then dried at 120-150°C, in order to eliminate excess water and the alcohol obtained from the hydrolysis. Dried gels can then be stabilized at higher temperatures [2]. For preparing Mesoporous bioactive glasses (MBGs), a modified sol-gel method was applied with polymer or surfactant used as template or structure directing agent (SDA) to generate mesopores in the resulting glass matrix. The synthesis of MBGs involved the addition of surfactant as SDA to the conventional sol-gel glass synthesis using evaporation induced self assembly (EISA) process (Figure 2.2), which plays a keystone for successful preparation of this new generation bioactive glasses.

2.1.2.1 Evaporation induced self assembly

**Self Assembly:** A general definition of self-assembly is the spontaneous organization of atoms/molecules through non-covalent interactions (hydrogen bonding, Van der Waals forces, electrostatic forces, π–π interactions, etc.) without any external intervention. Self assembly typically employs asymmetric molecules that are pre-programmed to organize into well-defined supermolecular assemblies. The most common are amphiphilic surfactant molecules or polymers composed of hydrophobic and hydrophilic parts. In aqueous solutions, above critical micelle concentration (cmc), surfactants assemble into micelles, spherical or cylindrical structures that maintain the hydrophilic parts of the surfactant in contact with water while shielding the hydrophobic parts within the micelle interior. Further, increasing the surfactant concentration results in the self-organization of micelles into periodic hexagonal, cubic, or lamellar mesophases.

**Evaporation induced Self Assembly:** It starts with homogeneous solution of precursors and surfactants prepared in ethanol/water solvent with the critical concentration, \( c_0 \), i.e. \( c_0 << \text{cmc} \). The concentration of the system is progressively increased by ethanol evaporation which derives to self-assemble of silica surfactant micelles and further organization into liquid crystalline mesophase. It is possible that by the variation of initial alcohol/water/surfactant molar ratio to obtain a different trajectories in the composition space and to arrive at different final mesostructures [3].
2.1.2.2 Non-ionic block co-polymers

When mesoporous silica was synthesized, several types of surfactant have been used e.g. cationic CTAB [4,5], non-ionic PEO surfactants [6] or Pluronics [7]. In this work Pluronic P123, a non-ionic amphiphilic triblock co-polymer has been used as surfactant. There are several non-ionic triblock co-polymers under the trademark Pluronics [8]. They all consist of hydrophilic polyethylene oxide chains (PEO) and hydrophobic polypropylene oxide chains (PPO). There are several Pluronics with varying molecular weights as well as different PEO/PPO ratios \((\text{EO}_x\cdot\text{PO}_y\cdot\text{EO}_x)\). The notation for Pluronic triblock copolymer starts with a letter followed by two or three digit numbers. The letter describes the appearance of the polymer: F (flake), P (paste) or L (liquid). The first one or two numbers multiplied with 300 indicates the molecular weight of the PPO block and the last number gives the PEO weight fraction [9]. Hence, P123 is a paste with \(~3600\) g/mol PPO and 30 wt% PEO while F127 is solid flakes with the same weight of PPO but 70 wt% PEO. These differences give rise to the variation of pore structures observed.
in the mesoporous materials, e.g. F127 is used for synthesizing spherical pores in a body centred cubic structure while P123 is used for hexagonally ordered cylindrical pores.

The non-ionic amphiphilic triblock copolymer (Pluronic, P123) that has following sequence: EO$_{20}$PO$_{70}$EO$_{20}$, where EO corresponds to poly (ethylene oxide) and PO corresponds to poly (propylene oxide) (MW=5800), were used as surfactant or SDA represented in Figure 2.3. The non-ionic block copolymers are chosen because they exhibit excellent stabilization properties, low cost, non-toxic and biodegradable. It is well known that non-ionic triblock copolymers of poly (ethylene oxide) (PEO) – poly(propylene oxide) (PPO) – poly(ethylene oxide) (PEO) in water form micelles in which the core and shell are composed of PPO and PEO respectively. The hydrophobic blocks of the block copolymers (PPO) form the core of these micellar aggregates, whereas the hydrophilic ones (PEO) (Figure 2.3), with the surrounding water molecules, form the corona. In the solution with low pH, PEO chains were associated with cationic silica species through hydrogen bonding while hydrophobic PPO is trapped in the core. It is known that during the calcinations process the surfactant used as template would be removed to produce porous structure.

![Figure 2.3: Chemical formula and properties of surfactant P123](image)

2.1.2.3 Materials used for MBGs synthesis

The precursors used were TEOS for SiO$_2$, TEP (triethyl phosphate) for P$_2$O$_5$, sodium acetate (NaAc) (CH$_3$COONa) or sodium nitrate (NaNO$_3$) for Na$_2$O and calcium acetate (CaAc) (CH$_3$(COOCa)$_2$) or Calcium nitrate tetrahydrate
(Ca(NO₃)₂·4H₂O) for CaO. In addition, acetic acid (CH₃COOH) or Hydrochloric acid (HCl) was used in the synthesis, to catalyze TEOS and TEP hydrolysis.

2.1.2.4 Synthesis of MBGs

In a typical synthesis of MBG, the molar ratios of reactants were designed according to the glass compositions required. In order to achieve clear sol P123(30 wt%), precursor compounds and acids were dissolved in ethanol and stirred at room temperature for 24 hrs and the corresponding molar ratio of TEOS/TEP:ethanol = 1:4; TEOS/TEP:water = 1:4 and weight ratio of water: acid=1:6. All the synthesis was carried out with 2 gm of P123 dissolved in 30 gm of ethanol and ratio of TEOS/P123 ratio was kept constant. The resulting sol was then introduced into the petridish to undergo EISA process. Following this, as evaporated gel was aged for 3 days and then dried at 100°C for 48 hrs. After aging and drying to remove the template, acid treatment was carried out. The molar ratios of composition prepared by modified sol-gel samples is listed in Table 2.2

Acid Treatment

For the treatment with sulphuric acid, 1.0 gm of material was mixed with 100 ml of 48wt% H₂SO₄ solution and heated at 95°C for 24 hrs. After the prolonged refluxing, the resulting admixture was washed with water using a suction pump until eluent became neutral then again washed with acetone and then dried at 80°C for 36 hrs. Subsequently, the resulting material was calcined at 350 °C for 4hrs to obtain the final product. Calcinations were done after acid treatment because ether cleavage at quite low temperature (95°C) and generates mesopores by removal of the PO groups. The EO chains which are less accessible to acid could be decomposed by the subsequent thermal treatment. In this study, 17 MBGs with the compositional ranges along with codes are given in Table 2.2.
Table 2.2: Molar Composition of sol-gel derived mesoporous glasses

<table>
<thead>
<tr>
<th>Composition (mol%)</th>
<th>SiO₂</th>
<th>Na₂O</th>
<th>CaO</th>
<th>P₂O₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSSG-30</td>
<td>70.0</td>
<td>30.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MSSG-25</td>
<td>75.0</td>
<td>25.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MSSG-20</td>
<td>80.0</td>
<td>20.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MSSG-15</td>
<td>85.0</td>
<td>15.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MSSG-10</td>
<td>90.0</td>
<td>10.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MSSG-5</td>
<td>95.0</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MBG-67SQ</td>
<td>67.4</td>
<td>25.0</td>
<td>5.0</td>
<td>2.6</td>
</tr>
<tr>
<td>MBG-62SQ</td>
<td>62.4</td>
<td>25.0</td>
<td>10.0</td>
<td>2.6</td>
</tr>
<tr>
<td>MBG-57SQ</td>
<td>57.4</td>
<td>25.0</td>
<td>15.0</td>
<td>2.6</td>
</tr>
<tr>
<td>MBG-52SQ</td>
<td>52.4</td>
<td>25.0</td>
<td>20.0</td>
<td>2.6</td>
</tr>
<tr>
<td>MBG-75SNP</td>
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<td>MBG-70SNP</td>
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<td>10.0</td>
</tr>
<tr>
<td>MBG-75SNC</td>
<td>75.0</td>
<td>20.0</td>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td>MBG-70SNC</td>
<td>70.0</td>
<td>20.0</td>
<td>10.0</td>
<td>-</td>
</tr>
<tr>
<td>73S10C</td>
<td>73.4</td>
<td>14</td>
<td>10</td>
<td>2.6</td>
</tr>
<tr>
<td>63S20C</td>
<td>63.4</td>
<td>14</td>
<td>20</td>
<td>2.6</td>
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<tr>
<td>53S30C</td>
<td>53.4</td>
<td>14</td>
<td>30</td>
<td>2.6</td>
</tr>
</tbody>
</table>

2.2 CHARACTERIZATION

Various characterization techniques were used for characterizing the melt-derived and sol-gel bioactive glasses.

2.2.1 X-Ray Diffraction (XRD)

The basic principles of X-ray diffraction and the analysis of the same is well known in the literature. The observation of diffraction peaks is an indication of the crystalline behavior of a material under study. These peaks corresponds to the basic Bragg reflection belonging to a particular family of planes, which is named after the scientists W.L.Bragg and W.H.Bragg who simplified the basic diffraction phenomena of x-rays by lattice planes present in a crystal. The Bragg equation which relates the lattice spacing and the observed 2θ in a diffraction pattern is given below.

\[ n\lambda = 2d_{hkl} \sin\theta \]
where \( d_{hkl} \) is the lattice spacing of a particular \((h k l)\), \( \theta \) the glancing angle, \( \lambda \) the wavelength of the X-ray source (Cu Ka, \( \lambda = 1.541\text{Å} \)) and \( n \) the order of diffraction. The diffraction pattern was collected in a \( \theta - 2\theta \) mode and was scanned in the \( 2\theta \) range of 10°-80° at a scanning rate of 3 °/min. The intensity of a diffracted beam is determined by the positions of atoms within the unit cells of the crystal while the diffraction direction is related to the shape and size of the unit cell. A detector which can be rotated to any angular position would measure the intensity of diffracted x-ray. The lattice spacing and the unit cell parameters of the crystalline samples are calculated from the observed diffraction pattern and were compared with the JCPDS data [10].

**Small Angle X-Ray Scattering (SAXS)**

SAXS or small angle x-ray scattering is the technique based on the elastically scattered x-rays from the sample. It is a fundamental method for structural analysis of condensed matter. The sample is illuminated by x-rays and the scattered radiation is registered by a detector. X-ray is a kind of electromagnetic radiation with a higher energy than ultraviolet radiations this is absorbed by core electrons (electrons that do not participate in the bonding). Based on this technique, the scattered radiation is recorded using a low range of angles, i.e. between 0.1° to 10°. SAXS technique is commonly used for probing large length scale structures such as high molecular weight polymers, biological macromolecules (proteins, nucleic acids, etc.), and self-assembled superstructures (e.g. surfactant templated mesoporous materials) [11].

**Instrument Specifications**

*Powder X-ray diffraction experiments were performed with Bruker D4 X-ray diffractometer equipped with Cu Ka radiations (wavelength 1.5406Å). Additionally, XRD patterns were collected in 2\( \theta \) range between 0.8° to 6° and 10° to 60° with step size of 0.02° and counting time of 5 s per step.*

**2.2.2 Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA)**

DSC is the thermo analytical technique in which the difference in the amount of heat required to increase the temperature of the sample and the reference are measured as function of temperature. DSC is commonly used to measure glass transitions, phase
changes, melting behavior, crystallization phenomenon etc. TGA measures the amount and the rate of change in the weight of the material as a function of temperature or time in a controlled atmosphere. This technique characterizes the materials that exhibit weight loss or gain due to decomposition, oxidation or dehydration etc. [12].

**Instrument Specifications**

*Differential scanning calorimetry studies were done with Mettler Toledo STARe simultaneous TGA/DSC at the heating rate of 10 °C/min with nitrogen as the purge gas in the temperature range of 30°-1000°C.*

### 2.2.3 Specific Surface Area Analysis and Porosity Determination by Gas Adsorption

The concept of the theory is an extension of the Langmuir theory [13], which is a theory for monolayer molecular adsorption to multilayer adsorption with the following hypotheses:

(a) Gas molecules physically adsorb on to solid surfaces in layers of infinite thickness;

(b) There is no interaction between each adsorption layer;

(c) The Langmuir theory can be applied to each layer;

Gas adsorption measurement is widely used for determining the surface area and pore size distribution of variety of solid materials. Adsorption is the enrichment of one or more components in interfacial layer. The reverse process of adsorption, i.e. the process in which adsorbed molecules escape from solid surfaces, is called Desorption. Physiosorption is a general phenomenon, it occurs whenever an adsorbable gas (the adsorptive) is brought into contact with the surface of a solid (adsorbent).

*Adsorption Isotherms:* The energetics of adsorption depends on the extent to which the available surface is covered with adsorbate molecule. The fractional coverage of a surface is defined by the quantity:
At any temperature, the adsorbate and the surface come to a dynamic equilibrium, that is, the chemical potentials of the free adsorbate and the surface bound adsorbate are equal. The chemical potential of the free adsorbate depends on the pressure of the gas, and the bound adsorbate depends on the coverage \( \theta \). Thus the coverage at a given temperature (T) is a function of the applied adsorbate pressure (P). The variation of \( \theta \) with P at a given T is called an adsorption isotherm.

An adsorption isotherm is obtained by measuring the amount of gas adsorbed across a wide range of relative pressures at a constant temperature (typically liquid N\(_2\), i.e. 77 K). Conversely desorption isotherms are achieved by measuring the amount of gas removed as pressure is reduced. It is important to define the pressure variables, which is commonly used in these experiments:

\[ P: \text{Vapor pressure or equilibrium vapor pressure is the pressure of a vapor in thermodynamic equilibrium with its condensed phases in a closed system. All liquids and solids have a tendency to evaporate into a gaseous form, and all gases have a tendency to condense back to their liquid or solid form.} \]

\[ P_0: \text{Saturation pressure is the pressure for a corresponding saturation temperature at which a liquid boils into its vapor phase.} \]

In monolayer adsorption all the adsorbed molecules are in contact with the surface layer of the absorbent. In multilayers, the adsorption space accommodates more than one layer of molecules so that not all adsorbed molecules are in direct contact with the surface layer of adsorbent. Capillary condensation is the “process by which multilayer adsorption from the vapour phase into a porous medium proceeds to the point at which pore spaces become filled with condensed liquid from the vapour phase”.

*The Classical Isotherm types described by Brunauer, Deming, Deming and Teller (BDDT) [14].*
Type I: Characteristic of either chemisorption or physisorption on a material that has extremely fine pores (micropores) (Figure 2.4).

![Figure 2.4: Type I Isotherm](image)

Type II: Characteristic of non-porous or possibly macro-porous materials (Figure 2.5).

![Figure 2.5: Type II Isotherm](image)

Type III: This is characteristic of a material, which is not porous, possibly macro-porous and has a low energy of adsorption (Figure 2.6).

![Figure 2.6: Type III Isotherm](image)

Type IV: This is characteristic of a material, which contains mesoporosity and has a high energy of adsorption. These often contain hysteresis attributed to the mesoporosity (Figure 2.7).
Type V: This is characteristic of a material, which contains mesoporosity and has a low energy of adsorption. These often contain hysteresis attributed to the mesoporosity (Figure 2.8).

Type VI: This type of isotherm typically categorized into chemisorptions and is attributed to several possibilities. The observed characteristics are due to the epitaxial growth or multiple pore sizes (Figure 2.9).

Figure 2.7: *Type IV Isotherm*

Figure 2.8: *Type V Isotherm*

Figure 2.9 *Type VI Isotherm*
Chapter 2

Characterization of Hysteresis Loops

H1 loop (Figure 2.10) signifies regular even size pores without interconnected channels.

![H1 hysteresis loop](image1)

**Figure 2.10: H1 hysteresis loop**

H2 loop (Figure 2.11) signifies pores with narrow and wide sections and possible interconnected region.

![H2 hysteresis loop](image2)

**Figure 2.11: H2 hysteresis loop**

H3 loop (Figure 2.12) signifies the slit-like pores for which adsorbent-adsorbate pair would yield a type II isotherm with out-pores.

![H3 hysteresis loop](image3)

**Figure 2.12: H3 hysteresis loop**
H4 loops (Figure 2.13) denote us about slit-like pores for the type I adsorbent-adsorbate pair.

![Figure 2.13: H4 hysteresis loop](image)

**Surface Area Analysis and Pore Size Measurement**

Before performing a surface area analysis or pore size measurement, solid surfaces must be freed from contaminants such as water and oils. Surface cleaning (degassing) is most often carried out by placing a sample in a glass cell and heating it under a vacuum, or a flow of dry, inert gas. Once it cleaned, the sample is brought to a constant temperature by means of an external bath, typically a dewar flask containing a cryogen like liquid nitrogen. Then, small amount of gas (the adsorbate) is admitted in steps into the evacuated sample chamber. To determine the surface area, solid samples are pre-treated by applying some combination of heat, vacuum and/or flowing gas to remove adsorbed contaminants acquired from atmospheric exposure. Subsequently, the sample is cooled, under vacuum usually at cryogenic temperature. An adsorptive (typically nitrogen) is admitted to the solid in a controlled increment. After each dose of adsorptive, the pressure is allowed to equilibrate and the quantity of gas adsorbed is calculated. The gas volume adsorbed at each pressure (at one constant temperature) defines an adsorption isotherm, from which the quantity of gas required to form a monolayer over the external surface of the solid and its pores are determined. With the area covered by each adsorbed gas molecule known, the surface area can also be calculated.
The surface area determinations involve creating the conditions required to adsorb an average monolayer of gas molecules onto a sample. By extending this process the gas is allowed to condense into pores, the sample’s fine pore structure can be evaluated. As pressure increases, the gas condenses first in the pores with the smallest dimensions. When the pressure is increased until saturation is reached and all pores are filled with liquid. The adsorptive gas pressure is then reduced incrementally by evaporating the condensed gas from the system. An evaluation of the adsorption and desorption branches of these isotherms and the hysteresis between them reveals information about the pore size, pore volume, pore area, and pore shape. The different stages associated with the surface area, pore diameter, volume and distribution for the determination are as follows (Figure 2.14):

**Stage 1:** Isolated sites on the sample surface begin to adsorb gas molecules at lower pressure.

**Stage 2:** As gas pressure increases, coverage of gas molecules increases to form a monolayer (one molecule thick). The BET equation is used to calculate the surface area.

**Stage 3:** Further increase of gas pressure will cause the beginning of multi-layer coverage. Initially, the smaller pores in the sample would get filled.

**Stage 4:** Additionally, with an increase of the gas pressure will cause complete coverage of the sample and fill all the pores. The BJH calculation can be used to determine pore diameter, volume and distribution.

Figure 2.15 represents the types of pores present in the samples. The Brunauer-Emmett-Teller (BET) theory is widely used for determination of the surface area of porous solids. Based on the BET analysis the gas absorption on clean particle surfaces at specific partial pressures with equilibrium between the vapor pressure and adsorbate, when the multilayer adsorption occurs, can be written as [15]:

**Figure 2.14**: Pore filling stages at different pressures

**Figure 2.15**: Types of Pores

\[
\frac{1}{W \left( \frac{P}{P_0} \right)} = \frac{1}{W_m C} + \frac{C - 1}{W_m C} \left( \frac{P}{P_0} \right)
\]

Where

- \( P \): partial pressure of adsorbate,
- \( P_0 \): saturation pressure of adsorbate at the experiment temperature,
- \( W \): amount of gas adsorbed at relative pressure \( P/P_0 \), and
- \( C \): BET constant relating to the adsorption enthalpy.
- \( W_m \): Monolayer capacity.
The above equation would generate a linear plot of $P/[W(P_0-P)]$ vs $P/P_0$ with a value of $W_m$. The amount of gas for a monolayer adsorption $W_m$ can be obtained from the slope $(s)$ and intercept $(i)$ of the BET plot.

$$W_m = \frac{1}{s + i}$$

The specific surface area ($S$) of the sample can be calculated as:

$$S = \frac{W_mN_As}{mM_a}$$

where

$W_m$: Monolayer capacity of particles (i.e., amount of gas molecules constituting a monolayer of surface coverage),

$N_A$: Avogadro’s number

$A_s$: Cross-section area of the adsorbate molecule,

$m$: Weight of powder sample, and

$M_a$: Molecule weight of the adsorbate.

Porosity of porous samples can be easily characterized using gas adsorption method and it can be described by determining the total pore volume and average pore diameter. The total pore volume is derived from the amount of vapor adsorbed at relative pressure close to unity, by assuming that the pores are then filled with liquid adsorbate. The volume of the nitrogen adsorbed ($V_{ads}$) can be converted to the volume of liquid nitrogen ($V_{liq}$) contained in the pores using the following equation:

$$V_{liq} = \frac{P_aV_mV_{ads}}{RT}$$

where

$P_a$: ambient pressure;

$T$: Temperature; and

$V_m$: molar volume of the liquid adsorbate.
The average pore size can be estimated from the pore volume because pores which would not be filled below a relative pressure of one have a negligible contribution to the total pore volume and the surface area of the sample. By assuming cylindrical pore geometry, the average pore diameter \( r_p \) can be expressed as:

\[
r_p = \frac{2V_{\text{liq}}}{S}
\]

where \( V_{\text{liq}} \): volume of liquid nitrogen; and \( S \): BET surface area. For other pore geometries, a knowledge of the shape of the hysteresis in the adsorption/desorption isotherm is required.

**Instrument Specifications**

The textural properties of the calcined samples were determined by nitrogen adsorption/desorption analyses at -196°C using Quantachrome Autosorb-1C TCD analyser (Model ASIC-X-TCD6) and with the absorptive gas of nitrogen, \( N_2 \) (cross sectional area 0.162 nm\(^2\)). Prior to the analysis, the samples were degassed under vacuum for 6 hrs at 200°C. The surface area was determined using Brunauer-Emmett-Teller (BET) equation on the nitrogen adsorption data obtained. The Pore-size distribution was determined by the Barret-Joyner-Halenda (BJH) method from desorption branch of the isotherm.

**2.2.4 Fourier-Transform Infra Red Spectroscopy (FTIR)**

Molecules are made up of atoms which are linked together by chemical bonds. These atoms and chemical bonds are in constant motions. These motions are composed of two components, stretching and bending vibrations. The vibrational spectra depend on the masses of the atoms, the strength of their chemical bonds and their geometrical arrangements. Infrared spectrometer is an important analytical tool to detect the vibration characteristics of chemical bonds in a material. Infrared and Raman spectroscopy provide complementary images of molecular vibrations, because in these spectroscopic techniques the mechanisms of the interaction of light quanta with molecules are quite different. Interaction of infrared radiation with a vibrating molecule is only possible if the electric vector of the radiation field oscillates with the
same frequency as does the molecular dipole moment. A vibration is infrared active only if the molecular dipole moment is modulated by the normal vibration,

\[
\left( \frac{\partial \mu}{\partial q} \right)_0 \neq 0
\]

where \( \mu \) is the molecular dipole moment and \( q \) stand for the normal coordinate describing the motion of the atoms during a normal vibration. When a material is irradiated by infrared light source, the chemical bonds can absorb an amount of energy \( h\nu \) to reach a vibrational excited state (\( h \): Planck’s constant, \( 6.626 \times 10^{-34} \) Js, \( \nu \): frequency). The frequency of an absorption band is proportional to the energy difference between the vibrational ground and excited states. Based on their wave numbers, infrared light can be divided into three different categories such as far infrared (4 – 400 cm\(^{-1}\)), mid infrared (400 – 4,000 cm\(^{-1}\)) and near infrared (4,000 – 14,000cm\(^{-1}\)).

Fourier transform spectroscopy is a measurement technique whereby spectra are collected based on measurements of the temporal coherence of a radiative source using an interferometer. In the interferometer, an infrared beam from the source is split into two beams by a half–silvered mirror, one is reflected off a fixed mirror and one off a moving mirror which subsequently recombined at the beam splitter. The optical path difference between the two beams gives rise to constructive and destructive interference. By measuring the signal at many discrete positions of the moving mirror, the spectrum can be reconstructed using a Fourier Transform. The final infrared spectrum plots absorbance (or transmittance) versus wave number [16].

**Instrument Specifications**

*Fourier Transform infrared (FTIR) spectroscopy was performed with Perkin Elmer FTIR system spectrum GX in transmittance mode. The FTIR spectra’s were run on KBr Pellets with weight ratio of sample to KBr of 1:100. The spectrum was recorded with the resolution of 4 cm\(^{-1}\).*
2.2.5 Raman Spectroscopy

Raman spectroscopy is a spectroscopic technique based on scattering of monochromatic light, usually from a laser source. When monochromatic radiation of wave number $\nu_0$ is incident on a substance like molecule or crystal, most of it is transmitted without change, but, in addition, some scattering of the radiation occurs. If the frequency content of the scattered radiation is analyzed, one would observe not only the wave number $\nu_0$ associated with the incident radiation but also, in general, pairs of new wave numbers of the type $\nu_s = \nu_0 \pm \nu_m$. In molecular systems, the wave numbers $\nu_m$ are found to lie principally in the ranges associated with transitions between rotational, vibrational, and electronic levels. Such scattering of radiation with change of wave numbers (or frequency) is called Raman scattering. Raman spectroscopy can be used to study solid, liquid and gaseous samples. The Raman effect is based on molecular deformations in electric field $E$ determined by molecular polarizability ($\alpha$). The laser beam can be considered as an oscillating electromagnetic wave with electrical vector $E$. Upon interaction with the sample it induces electric dipole moment $P=\alpha E$, which deforms molecules. Because of periodical deformation, molecules start vibrating with characteristic frequency $\nu_m$.

![Virtual states](image)

**Figure 2.16:** Energy level diagram for representing quanta of the energy $\nu_0$ hit the molecule, an elastic impact scatters the quantum $\nu_0$, inelastic impacts scatter quanta which have energies smaller or larger by the amount of the vibrational energy, $\nu$.

Amplitude of vibration is called a nuclear displacement. In other words, monochromatic laser light with frequency $\nu_0$ excites molecules and transforms them into oscillating dipoles. Such oscillating dipoles emit light of three different frequencies (Fig.2.16):
1. A molecule with no Raman-active modes absorbs a photon with the frequency $\nu_0$. The excited molecule returns back to the same basic vibrational state and emits light with the same frequency $\nu_0$ as an excitation source. This type of interaction is called an elastic **Rayleigh scattering**.

2. A photon with frequency $\nu_0$ is absorbed by Raman-active molecule which at the time of interaction is in the basic vibrational state. Part of the photon’s energy is transferred to the Raman-active mode with frequency $\nu_m$ and the resulting frequency of scattered light is reduced to $\nu_0 - \nu_m$. This Raman frequency is called Stokes frequency, or just “**Stokes**”.

3. A photon with frequency $\nu_0$ is absorbed by a Raman-active molecule, which, at the time of interaction, is already in the excited vibrational state. Excessive energy of excited Raman active mode is released, molecule returns to the basic vibrational state and the resulting frequency of scattered light goes up to $\nu_0 + \nu_m$. This Raman frequency is called Anti-Stokes frequency, or just “**Anti-stokes**”.

About 99.999% of all incident photons in spontaneous Raman undergo elastic Rayleigh scattering. This type of signal is useless for practical purposes of molecular characterization. Only about 0.001% of the incident light produces inelastic Raman signal with frequencies $\nu_0 \pm \nu_m$. Spontaneous Raman scattering is very weak and special measures should be taken to distinguish it from the predominant Rayleigh scattering. Instruments such as notch filters, tunable filters, laser stop apertures, double and triple spectrometric systems are used to reduce Rayleigh scattering and obtain high-quality Raman spectra.

**Instrumentation used**

A Raman system typically consists of four major components:

1. Excitation source (Laser).
2. Sample illumination system and light collection optics.
3. Wavelength selector (Filter or spectrophotometer).
4. Detector (Photodiode array, CCD or PMT).
A sample is normally illuminated with a laser beam in the ultraviolet (UV), visible (VIS) or near infrared (NIR) range. The scattered light is collected with a lens and is sent through interference filter or spectrophotometer to obtain Raman spectrum of a sample. More precisely, the major problem here is not the Rayleigh scattering itself, but the fact that the intensity of stray light from the Rayleigh scattering may greatly exceed the intensity of the useful Raman signal in the close proximity to the laser wavelength. In many cases the problem is resolved by simply cutting off the spectral range close to the laser line where the stray light has the most prominent effect. People use commercially available interference (notch) filters which cut-off spectral range of ± 80-120 cm\(^{-1}\) from the laser line. This method is efficient in stray light elimination but it does not allow detection of low-frequency Raman modes in the range below 100 cm\(^{-1}\). Stray light is generated in the spectrometer mainly upon light dispersion on gratings and strongly depends on grating quality. Raman spectrometers typically use holographic gratings which normally have much less manufacturing defects in their structure. More commonly, the stray lights produced by holographic gratings is about an order of magnitude less intense then from ruled gratings of the same groove density. Using multiple dispersion stages is another way of stray light reduction. Double and triple spectrometers allow taking Raman spectra without use of notch filters. In such systems Raman-active modes with frequencies as low as 3-5 cm\(^{-1}\) can be efficiently detected. In earlier times people primarily used single-point detectors such as photon-counting photomultiplier tubes (PMT). However, a single Raman spectrum obtained with a PMT detector in wave number scanning mode was taking substantial period of time, slowing down any research or industrial activity based on Raman analytical technique. Presently, often researchers use multi-channel detectors like photodiode arrays (PDA) or, more commonly, charge - coupled devices (CCD) are used to detect the scattered light. The sensitivity and performance of modern CCD detectors are rapidly improving. In many cases CCD is becoming the detector of choice for Raman spectroscopy [17].
Instrument Specifications

The temperature evolution of Raman spectra were recorded, using a 532nm excitation of a frequency-doubled Nd:YAG solid state laser (model GDLM-5015, Photop Suwtech Inc., China) and custom-built Raman spectrometer equipped with a SPEX TRIAX 550 monochromator and a liquid nitrogen cooled CCD. Laser power of the sample was 8mW, and spectral acquisition time was 2 min with the spectral resolution of 2 cm⁻¹.

2.2.6 Nuclear Magnetic Resonance (NMR) spectroscopy

NMR is based on the specific property of the atomic nucleus that it possesses a nuclear magnetic moment and can respond to an applied external magnetic field. Due to this interaction, a response signal can be detected. The majority of nuclei in the periodic table possess a nuclear magnetic moment, which is proportional to the nuclear spin angular momentum I:

\[ \mu = \gamma \cdot I \]

where the gyro-magnetic ratio \( \gamma \) is a constant, specific for each nucleus. The angular momentum of a nuclear spin is a vector and can point in any possible direction in the space. When a magnetic field is applied to the spins, they start to precess (rotate) around the magnetic field axis. The frequency of this precession is called the Larmor frequency \( \omega_0 \):

\[ \omega_0 = -\gamma \cdot B_0 \]

where \( B_0 \) is the external static magnetic field. The quantization axis in NMR is defined by the axis of the static magnetic field (usually taken as along the z-axis). The Hamiltonian \( \hat{H} \) for a nuclear spin in a static field is expressed as

\[ \hat{H} = -\mu \cdot B_0 \]

where \( \mu \) is the nuclear magnetic moment operator and \( B_0 \) is the magnetic field applied in the NMR experiment. The magnetic moment operator \( (\mu) \) can be expressed as follows:

\[ \mu = \gamma \hbar \cdot \hat{I} \]

where \( \hbar \) is the reduced Planck constant. As the magnetic field is applied along \( z \)-axis, the \( x \) and \( y \) contributions of \( \hat{I} \) will be 0 and thus
\[ \hat{H} = -\gamma \hbar \hat{I}_z \cdot \vec{B}_0 \]

A radio-frequency (rf) pulse is an oscillating magnetic field of specific frequency \( \omega_{rf} \) and duration, and it is applied to fulfill a resonance condition. The radio-frequency pulses are applied in a direction perpendicular to the magnetic field \( B_0 \). If the rf pulse is set to be on resonance, then

\[ \omega_{rf} = \omega_0 \]

An oscillating magnetic field, \( B_1 \), is associated with the applied radiofrequency pulse and is perpendicular to \( B_0 \). The rf pulse flips the magnetization vector into the \( xy \)-plane (transverse plane). Spins will keep precessing around the magnetic field \( B_1 \), as they did around \( B_0 \), at frequency

\[ \omega_1 = -\gamma \cdot B_1 \]

which is called the nutation frequency. After the application of the rf pulse, the spins return to their thermal equilibrium state. Time interval, needed for reaching the thermal equilibrium, denoted \( T_1 \), is called spin-lattice relaxation. The precession of the transverse magnetization induces oscillating electric currents that can be detected: this is an NMR signal, the so-called free induction decay, FID. This oscillating signal detected in the time domain can be transformed into the frequency domain by Fourier transformation. The peak maximum of the obtained spectrum corresponds to the Larmor frequency of the nuclei under observation. The main and most prominent interaction of the nucleus is the interaction with the static magnetic field. This external interaction is called the Zeeman interaction. Nuclei always experience local electric and magnetic fields that originate from the sample itself – internal interactions. There are a number of internal spin interactions: chemical shift, dipolar couplings, J-couplings, and quadrupolar couplings.

**Chemical Shift**

The electrons cause changes in the local magnetic fields around nuclei. The local magnetic fields experienced by nuclei at two various sites in the same molecule or crystals are different if the electronic environments are different. This interaction is called chemical shift; it is the most important interaction in NMR, since it allows
identification of the local nuclear environments. The chemical shift interaction contains two steps:

1. The external magnetic field $B_0$ induces circulating electric currents in the electron clouds in the atoms;

2. The induced electric currents generate a small magnetic field ($B_{\text{induced}}$). The nuclear spins sense the local magnetic field $B_{\text{loc}}$, which is the sum of the external magnetic field $B_0$ and the induced field $B_{\text{induced}}$ generated by the electrons:

$$B_{\text{loc}} = B_0 + B_{\text{induced}}$$

The induced magnetic field is usually very small, around $10^{-4}$ of the value of the external field. Its strength is directly proportional to $B_0$:

$$B_{\text{induced}} = \Delta B_0$$

Thus, the oscillating frequency during the free induction decay is changed from the Larmor frequency according to the change in the magnetic field:

$$\omega' = -\gamma B_0(1+\Delta)$$

As the result of the chemical shift interaction, NMR peak positions are shifted from the position of Larmor frequency. As was mentioned before, induced magnetic fields are proportional to the external magnetic field and so are the chemically shifted Larmor frequencies. To calibrate NMR frequencies and make measurements comparable, NMR spectroscopists use reference compounds for each NMR-active nucleus. These compounds are chosen so that they have a large signal intensity and low chemical reactivity; and their chemical shifts are set to 0 ppm. All the other spectral positions are measured with respect to the corresponding reference frequencies. Calculated with respect to references peak positions $\delta$ are very small values ($\approx 10^{-6}$), and therefore the unit of parts per million, ppm, is used.

**Magic-Angle Sample Spinning**

In solution NMR, high resolution is obtained due to fast isotropic tumbling of the molecules, which averages the anisotropic (orientation-dependent) interactions to zero. To achieve high resolution in solid-state NMR, the same approach should be
adopted: anisotropic interactions should be eliminated. Chemical shift anisotropy and dipolar coupling Hamiltonians contain second-rank tensors, which comprise the same term \( (3\cos^2\theta - 1) \), where the angle \( \theta \) describes the orientation of the crystallite or CSA ellipsoid. Thus, second-rank tensors can be averaged to zero if the orientation-dependent term vanishes. That can be achieved when the angle between inter nuclear vector or major axis of the CSA ellipsoid and the external magnetic field \( \theta \) is equal to \( \theta = 54.74^\circ \) (the magic angle). At this angle, the term \( (3\cos^2\theta - 1) \) term equals 0. In reality single crystals are very difficult to obtain, and spectroscopists deal with polycrystalline samples. Nevertheless, a solution has been found. Rotation of the solids has been shown to produce narrower lines [18]. If the spinning axis is chosen so as to create a 54.74° angle with \( B_0 \), the CSA and dipolar interaction are eliminated. This technique is known as magicangle spinning (MAS) [19]. The narrowing of lines at magic-angle spinning has proved experimentally [20].

A vector at an angle of 54.74° can be considered as a space diagonal of a cube (Figure 2.17). The \( x \)-, \( y \)-, and \( z \)-axes are symmetric with respect to this space diagonal. Under rotation, each crystallite will experience the average chemical shift.

\[
\delta_{iso} = \left( \frac{\partial_{xx} + \partial_{yy} + \partial_{zz}}{3} \right) = \left( \frac{\partial_{11} + \partial_{22} + \partial_{33}}{3} \right)
\]

i.e. an isotropic chemical shift. During the rotation of the sample, the static line shape splits up into a center band and spinning sidebands, which are separated by a value equal to the spinning frequency. At high spinning speeds the signal intensity is mostly concentrated at the center band, and its position corresponds to the isotropic chemical shift.

\[\text{Figure 2.17: The vector at the magic angle (angle } \theta = 54.74^\circ \text{ between the magnetic field and the rotation axis) as a space diagonal of a cube [21].}\]
Instrument Specifications

High resolution MAS-NMR spectra of glass and glass-ceramics were acquired at room temperature using Bruker DSX-300 spectrometer operating at 121.49 (\(^{31}\)P signal) and 59.62 MHz (\(^{29}\)Si signal) with field strength of 7.04 T. The \(^{29}\)Si MAS-NMR spectra were made of 2048 free induction decays (FID) with a 30° pulse of 2.5 \(\mu\)s and a relaxation decay of 5 s. The \(^{31}\)P MAS-NMR spectra were recorded at 512 scans with a 45° pulse of 3.5 \(\mu\)s and a relaxation decay of 5 s. The chemical environment of two different nuclei: silicon \(^{29}\)Si and phosphorous \(^{31}\)P were recorded with respect to tetramethylsilane (TMS) as reference for \(^{29}\)Si spectra and \(\text{H}_3\text{PO}_4\) (85%) solution as reference for \(^{31}\)P spectra. All the samples were spun at the magic angle of 54.7° and at the spinning rate of 5-8 kHz on finely ground powders filled in 5 mm zirconia rotors. The experimental errors in the chemical shifts were ±0.1 ppm for \(^{29}\)Si and ±0.5 ppm for \(^{31}\)P signals.

2.2.7 Broad band Impedance spectroscopy.

Response mechanisms in the presence of an applied external electric field

Although conductivity spectroscopy may be performed using a direct current (d.c.), or an alternating current (a.c.) external electric field, the present study is limited to the use of an a.c. field, and hence the following discussion will be limited as such. The mechanistic response of a material to an applied external field may reflect the simultaneous response of charged species which may be either local or long-range in nature. Experimental data gathered by means of conductivity spectroscopy often reflect a superposition of multiple response mechanisms, and it is often the task of the experimenter to separate the various response behaviors present. Of further interest is how response mechanisms evolve as the temperature, frequency, and pressure of the sample environment change. Fortunately, different response mechanisms often depend on these factors in different ways, and by systematically changing the measurement environment in a defined fashion it is possible to understand the individual behavior of multiple superimposed response mechanisms.

Generically, conductivity spectroscopy is a measure of how a material responds to an applied alternating external field. A sine wave of known amplitude and frequency is generated, and the response in the material takes the form of an induced current, of equal frequency, new amplitude, and shifted in phase, Figure 2.18.
The applied external field results in a potential of the form:

\[ \hat{U} = U_0 \cdot e^{i\omega t} \]  

(1)

where \( \hat{U} \) is the instantaneous voltage, \( U_0 \) represents the peak voltage, \( \omega = 2\pi\nu \) the angular frequency, and \( t \) representing time. Similarly, the form of the induced current is

\[ \hat{I} = I_0 \cdot e^{i(\omega t + \varphi)} \]  

(2)

With \( \hat{I} \) the instantaneous value of the current, \( I_0 \) the peak value of the current, and \( \varphi \) representing the phase angle between the external field and the internal current.

From the known instantaneous values of the voltage and current it is possible to calculate the sample impedance as defined by

\[ \hat{Z} = \frac{\hat{U}}{\hat{I}} \]  

(3)

Using equations (1) and (2) in combination with Euler’s Identity it is possible to extract the real and imaginary components of the impedance,

\[ \hat{Z} = \frac{U_0}{I_0} \cdot e^{-i\varphi} = |\hat{Z}| \left[ \cos \varphi - i|\hat{Z}| \sin \varphi = R - iX \right] \]  

(4)

In the above equation the real component is represented by \( R \), the resistance; \( X \), the reactance represents the negative imaginary component. Having determined the impedance it is possible to determine the admittance, \( \hat{Y} \), using the simple relation
\[ \hat{Y} = \frac{1}{Z} = \frac{R}{R^2 + X^2} + i\frac{X}{R^2 + X^2} = G + iB \] (5)

Where in equation (5) G represents the conductance, and B the susceptance. From equation (5) it is possible, by accounting for the geometry of the sample, to calculate the specific complex conductivity,

\[ \hat{\sigma}(\omega) = \sigma'(\omega) + i\sigma''(\omega) \] (6)

for which the real part is defined by

\[ \sigma'(\omega) = \left( \frac{R}{R^2 + X^2} \right) \cdot \left( \frac{l}{A} \right) = G \cdot \left( \frac{l}{A} \right) \] (7)

and the imaginary part is defined by

\[ \sigma''(\omega) = \left( \frac{R}{R^2 + X^2} \right) \cdot \left( \frac{l}{A} \right) = B \cdot \left( \frac{l}{A} \right) \] (8)

where l corresponds to the sample thickness, and A the surface area of the sample. While the complex conductivity is a useful and very popular way of expressing impedance data, often times it is also informative to analyze data using the complex dielectric function.

**Time temperature superposition**

In a general sense, in the context of ionic conductivity, time-temperature superposition is based on the principle that with increasing temperature there is a corresponding increase in the rate at which ions contributing to the conductivity hop from one site to another. In attempting to identify generalities pertaining to amorphous materials, it was observed that the frequency at which the conductivity starts to rise above the d.c. limit is roughly proportional to the d.c. conductivity times temperature. The quantity, \( \sigma_{dc} \cdot T \), is proportional to the coefficient of self-diffusion, \( D_o \), of the mobile ions via the Nernst-Einstein relation:
where \( N_V \), represents the number density of mobile ions, \( q \) the charge of the mobile ions, and \( k_B \) Boltzmann’s constant. Given the case where the mechanism by which ions diffuse remains unchanged with increasing temperature, the coefficient of self-diffusion will increase at the same rate as the frequencies involved will increase. Consequently, in a log-log plot of \( \sigma' \cdot T \) vs. \( \nu \), the onset of dispersion of the different isotherms is observed along a line with a slope of one. If the individual isotherms of \( \sigma'(\nu) \) (for a suitable range of temperature and frequency) are then shifted along this line, they will collapse to form a master curve. This observation led to a mechanism-independent empirical scaling law of the frequency-dependent conductivity, applicable to data gathered within the conventional experimental constraints of impedance spectroscopy. This law, commonly referred to as Summerfield scaling, depends only on the ratio of the frequency-dependent conductivity to the d.c. conductivity [22]. In its most general form conductivity data may be scaled using the following equation:

\[
\frac{\sigma'(\nu)}{\sigma_{dc}} \propto \left( \frac{\nu}{\nu_o} \right) \sigma_{dc} \cdot T
\]

Where the function \( F \) is independent of temperature and for which the value of the characteristic frequency, \( \nu_o \), has been defined in a variety of ways over time, each of which expresses a proportionality between this reference frequency and the “successful” hopping rate of the ions.

Empirical scaling of the frequency-dependent conductivity is also possible by adjusting for the relative increase in hopping frequency with increasing temperature thereby facilitating the collapse of independent isotherms of the frequency-dependent conductivity onto one master curve. When the number density of mobile carriers or the hopping distances involved in diffusive motion become temperature dependent the individual isotherms do not shift along a slope of one, and although the isotherms may still be collapsed into a master curve, as they do not shift along a line of a slope of one, the term Summerfield scaling does not apply. The general shape of the master curve is similar for glasses spanning a remarkably wide range of compositions, but
nevertheless it shows clear differences even among glasses of the same family [23]. The temperature and frequency range over which such scaling is possible is limited by experimental conditions which sustain the hopping mechanism responsible for the conductivity. As a counter example, in attempting to scale conductivity isotherms at ever decreasing temperatures, the scaling eventually fails when the material response is no longer dominated by the hopping mechanism responsible for long-range transport. At some point the d.c. conductivity becomes unmeasurably small and at any given frequency the material response is dominated not by a d.c. hopping response, but rather by localized displacements.

Early studies involving sodium borate glasses, having between 10 and 30 mol percent Na$_2$O, demonstrated the nature by which the conductivity isotherms of such glasses can be individually collapsed to form master curves, each strongly resembling one another in overall form. Although considerable similarity exists between the master curves of borate glasses with different sodium content, a systematic difference in the curvature arises in the dispersive region between the low frequency d.c. plateau and the high frequency universal behavior where the slope approaches a value of one, Figure 2.19. Differences which arise as a function of mobile charge carrier concentration, are also observed when the corresponding data from the permittivity are scaled [24].

![Figure 2.19: Simultaneous presentation of scaled conductivity master curves for xNa$_2$O.(1-x) B$_2$O$_3$ glasses. The master curves for three compositions nearly overlap one another after introducing a mobile ion dependent factor x, representing the sodium content, on the scaled frequency axis. Despite considerable similarity it is clear that with decreasing sodium content an increase in curvature results in the region lying between the d.c. plateau and the high frequency response.](image-url)
Further, without being overly critical of the dispersive region mentioned earlier, it is nearly possible to collapse the master curves of a variety of different glass formers by accounting for systematic changes in the permittivity which accompany a variation in modifier ion concentration. Use of the change in permittivity in scaling on the frequency axis, measured as the difference between the static and high frequency permittivity, accounts for length scale changes characteristic of the hopping processes which occur as the concentration of the modifier ion is varied [25]. By incorporating a mechanism to account for changes in the permittivity encountered across isothermal data sets to be scaled, it is possible to scale both the a.c. conductivity, permittivity, and the dielectric loss [26]. The combined analysis of such scaled data is a helpful approach in trying to understand how mechanisms contributing to the ionic conductivity change or even cease to exist, as a function of temperature.

Figure 2.20: Set-up of the Novocontrol impedance spectrometer with temperature controller.
Instrument Specifications

The complete setup of the Novocontrol impedance spectrometer is shown in Figure 2.20. Additionally, we also show the schematic diagram of the setup of the Novocontrol dielectric analyser in Figure 2.21. Conductivity Measurement were carried out by using Novocontrol A-S high resolution dielectric analyzer in a frequency range from 10mHz to 3MHz, and temperature range from room 283K to 633K.

2.2.8 Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a very useful imaging technique for obtaining information on various micro structural features of materials. The main components of a transmission electron microscope include an electron gun, a condenser lens system, a specimen chamber, objective and intermediate lenses, projector systems for producing images and diffraction patterns and a viewing screen. In the conventional TEM, the accelerating voltage of electron gun is in the range of 100 – 200 kV. TEM with
higher voltage i.e. 300 – 400 kV (medium voltage) and 600 – 3000 kV (high voltage) are employed to penetrate thick samples and to obtain higher resolutions. The condenser lenses would demagnify the electron beam and control the beam spot size and the convergence angle as the beam hits the specimen. In order to form the first intermediate image in the image plane and diffraction pattern in the back focal plane, an objective lens is then utilized.

More commonly three types of information can be offered by TEM. They are bright field image, dark field image and selected area diffraction. In bright field imaging, an aperture is inserted in the back focal plane of the objected lens to cut out the diffracted beams, therefore only the undeflected electrons contribute to the formation of image. In the absence of a sample, a bright background is observed, commonly known as bright field imaging. On the other hand, in dark field imaging, the aperture is displaced and a particular diffraction beam can be selected. In the absence of a sample, the background would appear dark, known as dark field imaging. The resulting image of dark field imaging is low resolution because all the imaging electrons are traveling far from the optical axis, where spherical aberration is large. In order to get a better resolution for dark field imaging, the primary electron beam is tilted so that the chosen diffracted beams travels along the optical axis and passes through the centered aperture. In selected area diffraction, both the transmitted and diffracted rays contribute to the formation of a diffraction pattern in the back focal plane of the objective lens. When the selected area diffraction aperture is inserted, only a small area enclosed by the aperture contributes to the diffraction pattern to reveal the crystallographic information on that small area of a sample [27, 28].

**Instrument Specifications**

The morphology of the sample were characterized by the FEI Tecnai™ Transmission Electron Microscope (TEM) (TECNAI G² T30 U-TWIN) and had been attached with a double-tilt holder (± 70°) along with an EDX analyzer under an accelerating voltage of 300kV (with the resolution of 0.19nm). The images were recorded using a CCD camera (Gatan) and Fourier transform (FT) patterns have been conducted using a digital micrograph (Gatan).
2.2.9 Scanning Electron Microscopy (SEM)

SEM is a widely used imaging technique for examining the surface and subsurface structures of solid materials [29]. There are two types of electron guns which are commonly used for generating beams of electrons in SEM. They are thermionic triod electron gun and field emission gun. In thermionic triod electron gun, a piece of tungsten acts as the cathode. The filament is heated by the passage of a current to about 2800 K. Electrons emitted from the filament are rapidly accelerated towards the anode and a beam of high energy electrons is emitted through the circular hole at its entry into the microscope column. On the other hand, field emission gun is used when a high brightness is required. In field emission gun, an extremely high electric field (>109 V/m) is applied to a metallic surface to enable an electron to leave before being provided with the amount of energy represented by the work function. This is known as the tunneling effect. As a result, many more electrons can be emitted from a piece of tungsten and the brightness can be increased by a factor of up to a thousand or more.

The main component of SEM includes an electron optical column, a vacuum system including the specimen chamber and signal detection, and a display system. Basically, monochromatic beam of electron produced by an electron gun would pass through two or more condenser lens upon which a demagnified beam is formed. Further demagnification of the electron beam is achieved by the objective lens producing a probe size of a few nanometers in diameter for a typical SEM, operating at 20 keV. This fine beam of electrons is rastered across the specimen surface, being deflected by the scan coils while a detector counts the number of low energy secondary electrons or other radiation given off from each point on the surface. The primary electron beam interacts with the specimen to produce secondary electrons (SE), backscattered electron (BSE), auger electrons, characteristic X–rays and photon of various energies. Each of these signals can be detected, amplified and used to control the brightness of cathode – ray tubes (CRT). The spot position in each of these CRTs is determined by the same scan generator which affixes the position of the primary beam on the specimen surface. The dependence of electron yield on the tilt angle of a surface element, the enhanced emission at edged and small particles and the shadow contrast are used to image the surface topography.
Instrument Specifications

SEM (table top mini SEM SNE-3000M nano eye) magnification up to 30,000x (Digital zoom 4x) and variable accelerating voltage from 5KV to 30KV.

2.3 IN VITRO BIOACTIVITY STUDIES ON SOL-GEL GLASSES

The importance of analyzing bioactivity by in-vitro prior to in-vivo analysis is quite clear. In vivo studies require animal sacrifices, are more costly and less easily reproducible, and involve ethic issues. For these reasons, before testing bioactivity of the materials in-vivo, in-vitro tests are necessary. The choice of the solution used to simulate in-vitro reactions occurring on the surface of the biomaterial is very important: simple solutions that mimic only the inorganic composition of human body fluids can be used or more complex solutions that contain biological moieties such as proteins. Moreover, cells-containing solutions can be employed, thus increasing both the similarity to real body fluids and the complexity of the test. The rate of ionic release and pH increase also depends on the dynamic or static method used to simulate biomaterial reactivity. Many studies are done in ‘static’, which means that the solution used to dissolve in-vitro, the biomaterial is never changed in the course of the experiment. In other studies, instead, the solution where the biomaterial dissolved is periodically changed and refilled with some fresh one [30].

In recent experiments, the solution is continuously re-circulated, so that new solution is in contact with the biomaterial at all times [31]. It is difficult to prove which experimental method best simulates the in-vivo situation. In fact, body fluids circulate at the interface with the wounded area, but the extent of this circulation is far to be well-defined. Experimentally, it has been shown that the static method quickly induces saturation of the solution, so that apatite precipitates faster, and the pH increases more than with the dynamic method [32]. Earlier studies concerning the bioactivity of Bioglass® were carried out in simple tris-(hydroxymethyl) aminomethane (TRIS) buffered solution [33]. Since this solution does not contain any ions other than the ones that are dissolved by the materials immersed into it, it can be very useful if one wants to analyze the basic steps involved in HA deposition on bioactive materials.
In 1990, Kokubo et al. introduced the use of simulated body fluid (SBF) to analyze bioactivity of different materials [34]. The SBF is an aproteic and acellular solution containing different salts that simulate the identical concentration and pH of human plasma (Table 2.3). Both SBF and human plasma are saturated with respect to hydroxyapatite. For this reason, only a few nucleation sites are sufficient to observe HA nucleation on the surface of some material. This allowed the analysis of bioactivity on simple materials that did not contain calcium and phosphorous in their starting composition. Moreover, the rate of HA deposition in SBF is much higher than in TRIS-buffered solution, obviously because the supersaturation degree with respect to HA, which is reached much more easily in SBF.

Table 2.3: Concentration (mM) and pH of simulated body fluid (SBF) and human plasma [35]

<table>
<thead>
<tr>
<th>ION</th>
<th>SBF (mM concentration)</th>
<th>Plasma (mM concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
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<td>142</td>
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<td>K⁺</td>
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<td>SO₄⁻</td>
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<td>pH</td>
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</tr>
</tbody>
</table>

2.3.1 Experimental setup for In Vitro Studies

During the in-vitro assays, the glass disks are soaked in SBF for different time durations. An each specimen (1mg) was immersed in 1mL of SBF and treated at 37°C under continuous orbital stirring in incubator under sterile conditions. The schematic depiction of In vitro bioactivity test is shown in Figure 2.22. After removal of the soaked sample from the incubation solution, were rinsed gently with first
deionized water and in acetone and then dried in air at room temperature. Finally, the SBF soaked samples were kept in desiccators for further characterizations.

**Figure 2.22: Schematic depiction of the in vitro bioactivity test**

### 2.3.2 Solution Analysis

#### 2.3.2.1 pH measurements

The glass dissolution or ion release rates after immersion of the sample in SBF were monitored by measuring the pH of the solution. The pH values were measured with a pH-meter, i.e. a glass probe that has two electrodes inside. One electrode is in contact with a liquid of fixed acidity, whereas the response of the second electrode depends on the pH of the sample solution. The difference in the potential between the two electrodes is measured as the pH value.

**Instrument Specifications**

*Evolution of the SBF concentrations was monitored by means of pH meter (Sigma instruments) caused by the ion exchange processes between bioactive glass and the surrounding medium.*
2.3.2.2 Ion Coupled Plasma Emission Spectroscopy

Ion coupled plasma emission spectroscopy (ICP-ES) allows the compositional analysis of aqueous solutions. The sample solution is nebulized (i.e., transformed into an aerosol), and carried by a gas carrier (usually Ar) through a torch, where a plasma (i.e., gas in which atoms are ionized) is ignited. When sample atoms are ionized, they emit radiation at some specific wavelength. These specific components are selected by a diffraction grating, and converted in electric signals by a photomultiplier. After the calibration, it is possible to determine the amount of each element present in solution by analyzing the intensity of the radiation emitted at the specific elemental frequency [36].

Instrument Specifications

*Inductive Coupled Plasma (ICP) atomic emission spectroscopy using Perkin-Elmer Optima 2000 DV spectrometer.*
REFERENCES


