CHAPTER 1

INTRODUCTION

Gel electrophoresis is a standard procedure to separate macromolecules, especially proteins or DNA/RNA based on their surface charge and molecular weight. The gel network allows movement of proteins under the applied voltage. The lower molecular weight (MW) molecules separate from higher MW and move farther distance. The separation of molecules in gel electrophoresis is called sieving. The most commonly used gels are agarose and polyacrylamide gel for proteins or DNA/RNA separation. The polyacrylamide and agarose form a solid, yet porous gel matrix. Therefore, these gels allow separation of proteins (Johansson 1972, Jeppson et al. 1979, Sameh Magdeldin 2012, Arora et al. 2013).

The standard electrophoresis setup is shown in Fig.1.1. The two electrodes provide the electric field, whereas the buffer provides the electron pool to carry current and also helps to maintain relatively constant pH and temperature. The gel slab is placed in the container and microwells are created at one end also called as loading sites where the test samples are loaded. Agarose gel is a crossed linked linear polymer of agarobiose. The gel is characterized by large pores volume and variable pore size. Therefore it has
a poor resolution for proteins and can be applied to proteins of MW > 200 kDa (Keutel 1964). SDS-PAGE provides better resolution as uniform pore size limits its application. SDS-PAGE is used to separate proteins of range (5-2000 kDa) (Johansson 1972, Arora et al. 2013). Moreover, acrylamide is neurotoxic and therefore must be handled carefully.

Although there has been a refinement of methodology since the inception of electrophoresis, the overall basic principle and protocols have not changed over the years. The elaborate protocol constraints, non-biodegradable and low bio-compatibility of gel matrix limits the use of these gels for any other analytical process or techniques (Keutel 1964, Krizek and Rick 2001, Sameh Magdeldin 2012, Arora et al. 2013). In this study, a novel technique sol-gel electrophoresis (SoGE) is developed using tetraethyl orthosilicate (TEOS) derived sol-gel silica matrix as a suitable platform for gel electrophoresis. The application of silicate gel as electrophoresis platform was first reported by Starosvetsky et al. (2008) for improvement of the concentration of proteins on the gel surface. Moreover, the use of sol-gel for separation and chromatography dates back to the 1970’s with the patenting of the use of sol-gel for chromatography by Kirkland (1973). The sol-gel processed columns for HPLC (high-performance liquid chromatography) for packing material, bonded phase chemistry and column construction have been a preferred material for both analytical and preparative separations for silica-based HPLC columns (Majors 2003).

Sol-gel processing is a century old technology for the synthesis of porous, transparent and amorphous silica gel at room temperature (Ebelmen 1844, Kistler 1931). The sol-gel process involves polymerization of the most commonly used precursor-like TEOS in the presence of water/ethanol and HCl/NaOH as a catalyst. The chemical steps are divided into hydrolysis and condensation, which leads to $\text{Si} - \text{O} - \text{Si}$ linkage and form the gel network. The reaction chemistry and properties of the sol-gel-derived silica materials are well described by Iler (1979) and Brinker and Scherer (1990). The molar ratio $(R)$ is the ratio of amounts in moles of $\text{H}_2\text{O}/\text{C}_2\text{H}_5\text{OH}$ to TEOS / TMOS. The molar ratio decides the physicochemical properties (gelation time, stability, structural morphology, and transparency), as well as network properties of sol-gel. For gel electrophoresis study, the physicochemical properties and network properties of sol-gel silica matrices, as well as dip-coated thin films, were characterized as a function of molar ratios ($\text{H}_2\text{O}/\text{TEOS}, R=4, 16, 32$ and $64$).

Sol-gel (without buffer) shows a less aggregation of gel particles as they are mutually repulsive. Stable sol-gel of large particle size with reduced gelation time can be prepared by addition of buffer. To neutralize sol of pH (2-4) and to analyze the effect of the buffer in the gelation time, sodium phosphate buffer (pH=7.4, 0.01 M) was used to prepare sol-gel. sol-gel showed a remarkable change in its properties after the addition of buffer. The sol-gel (R=4) showed rapid gelation (4-5 min) and quickly turned into a white gel whereas R=16 formed a translucent gel in a short time (20-30 min). The
sol-gel prepared at R=32 and 64 with buffer turned into gels within 40-60 min and 6-7 hrs respectively. Interestingly, the addition of buffer did not alter the transparency of the sol-gel prepared at R=32 or R=64. Transmission studies of sol-gel were carried out to find the absorption characteristics of sol-gel. The transmission profile from milky and opaque sol-gel (R=4 and R=16) showed lower transmittance values (76-78%) whereas R=32 and R=64 showed high transmittance values (95-97%). The modified sol-gel (R=32, pH=7.4) prepared by addition of buffer, showed some of the desired properties (pH=7.4, transparency = 97% and gelation time = 30-40 min).

The pore volume and pore size distribution were studied using \( N_2 \) adsorption for bulk sol-gel and thin films with all molar ratios (R= 4, 16, 32 and 64). The gel pore size and pore distribution were molar ratio dependent. The R=32 derived sol-gel showed better pore distributions compared to other R=4, 16 and 64. R=32 was characterized by the highest average pore volume (0.40 cc³/gm) and low average pore diameter (1.21nm). The average micropore volume was also observed to be high for R=32 (0.17 cc³/gm). The adsorption hysteresis curve which denotes the material consists of agglomerates of approximately uniform spheres and has a narrow pore size distribution. The pores are characterized by ink bottle pores where the neck is narrow compared by the cavity below. The addition of buffer also influenced the porosity profile of the sol-gel which increased the pore size to the broader spectrum (2 to 6nm). The modified sol-gel has complex pore structures with well-defined pores which are interconnected by capillaries. The thin film \( N_2 \) adsorption exhibited reduced pore size distribution and very low porosity.

Sol-gel thin films were prepared on glass coverslips using spray and dip coating technique. The dip-coated thin films were preferred due to a smooth and uniform thickness. The surface characters of sol-gel thin films were visualized under a microscope. The film quality for R=4 and R=16 was very rough and uneven. The adhesion to the surface was observed to be poor. The R=32 and R=64 showed essential characteristics like the smooth surface and uniform film throughout the surface, and The R=64 derived thin films showed cracks and uneven surface. The surface of the thin film was analyzed using a surface profiler. The thickness measurement gave an average thickness of 136.2 microns for R=16 whereas 104.51 microns for R=32. Atomic force microscopy (AFM) studies were performed on thin films to study the surface characteristics. The surface roughness \( (R_{sa}) \) was observed to be the lowest value (2.2m) for R=32. The interface width roughness \( (W) \) was observed low for R=32. Similarly, the positive values of surface skew \( (R_{sk}) \) observed for thin film derived from R=32 which confirms more peaks than the valleys. The Kurtosis \( (R_{kv}) \) was high for R=32.

The transmission electron microscopy (TEM) studies result showed that silica gel particles are grouped in the micron scale, and interstitial space is randomly distributed between the particles. The size of silica particles of modified sol-gel (R=32) was in the range of 30-60 nm. Similarly, the TEM image of sol-gel in the absence of buffer showed
dense smaller particles of size 15-25 nm. The calculated particle size of sol-gel in the lack of buffer is always smaller than the size of the modified sol-gel. The formation of larger particle size in modified sol-gel was due to the addition of buffer and surfactant Triton X-100 (TX-100). The internal gel network and surface morphology of bulk sol-gel (R=32) were examined by scanning electron microscopy (SEM). The SEM image depicted a large quantity of evenly distributed silica layers with slit-like openings on the gel surface.

The sol-gel prepared at R=32 has gelation time (24 hrs) and upon addition of buffer clear and transparent gel formed in a short period (45-60 min). The porosity analysis displayed a broad spectrum of pores and high pore volume which confirmed that gel is mesoporous and have complex pore structure. The AFM results of thin films showed low $R_a$, low W, positive $R_sk$ and high $R_kv$ values confirming the smooth and crack free surface with thickness (104 µ) of gel coating. The particle distribution and surface morphology suggested that gel was characterized by larger silica particles (30-60 nm) with evenly distributed silica layers with slit-like openings on the gel surface. These properties are desired for a gel system to be suitable for electrophoresis applications. Hence modified sol-gel R=32 was used as a gel matrix for electrophoresis studies.

Sol-gel electrophoresis (SoGE) was performed using a gel matrix derived using R=32. The molecular markers such as Bromophenol blue (BB), Thymol Blue (TB) and Ethidium Bromide (EtBr) were used to check the migration in a sol-gel matrix. The markers BB, TB and EtBr (10 µM, 5 µl) were loaded into the separate microwells created using gel-comb on the silica matrix. The gel was run for 45 minutes under a standard voltage (50V, DC). Out of the three markers, TB (+ve) and BB (+ve) migrated towards the cathode whereas EtBr (-ve) migrated towards the anode. The sol-gel silica matrix used for this study was transparent with near neutral physiological micro-environment (pH=7.4). The rate and extent of migration of markers in a silica gel matrix were compared with SDS PAGE matrix results and were found to be similar to SoGE.

The migration of molecular markers in SoGE strongly suggested that a modified sol-gel silica matrix prepared at (R=32, pH=7.4) can be a suitable platform for electrophoresis of proteins. Further studies with pre-stained protein markers (8-220 kDa) were undertaken as model proteins. During SoGE, The lower molecular weight proteins migrated to a longer distance (8-20 kDa: 3.65±0.06cm) at a faster rate compared to higher molecular weight proteins, (100-220 kDa: 2.28±0.38cm). The silica matrix allowed sieving of pre-stained proteins according to their MW (08-220 kDa). The significant drawback of the SoGE was overlapping of the closely related molecular weight protein bands. SoGE results in this study confirmed that the internal network of modified sol-gel (R=32) facilitated the migration of molecular markers as well as proteins based on their MW. The molar ratio (R=32, H2O/TEOS), phosphate buffer (pH=7.4, 0.01M), surfactant (TX-100) are the crucial factors in the preparation of modified silica matrix for
SoGE. The success of SoGE in sieving of proteins depending on their molecular weight prompted for the sol-gel based microfluidic device. The Fig.1.2 shows the schematic illustration of sol-gel electrophoresis (SoGE) process.

![Sol-gel electrophoresis process](image)

**Fig. 1.2 Sol-gel electrophoresis process (SoGE)**

The increasing number of researchers are opting for developing and improving microfluidics-based integrated single-chip medical devices. However, the materials currently used to manufacture these labs-on-a-chip and other microfluidic devices have critical impediments, including retention of small nonpolar and weakly polar molecules, adsorption of biomolecules, and the materials molecules leaching into the microfluidic channel. Therefore a silica and silica-based channels can be a suitable alternative for microfluidics applications. In this work initially, channels were designed on the glass surface using masking tape, and the sol-gel thin film was deposited to prepare channels. The proteins were loaded into the loading area and allowed to sieve under the influence of the voltage. This design has significant drawbacks as channels tend to crack and erode easily from the glass surface. Also, the migration of proteins was restricted due to adhesion to the film surface. Therefore a modified design which comprised of a thick glass slab of 5mm with channels grounded on the glass surface was attempted. This design allowed the control of thickness efficiently. The design also required a low volume of sol-gel to fill the channels. Channels were also fabricated on cellulose acetate sheets using precise laser cutting technology. The channel’s width and depth (5mm-1mm) were varied and the rate of protein movement was observed. Channel depth did not alter the rate of migration but the change in width altered the speed of migration, due to the increase in potential gradient resulted by narrowing of the channel. The results of the novel modified design are promising, and in the future, a novel technique using sol-gel microfluidic channel can be realized.
In this chapter, an outline of the research carried out is described in brief. The need for the silica sol-gel based microfluidic channel for development of Lab-on chip electrophoresis devices in the future. In the next chapter, we have discussed in detail the work carried out by different researchers on the sol-gel process, entrapment of proteins, porosity studies, electrophoresis and chromatography applications and the different methodologies and applications of microfluidics or lab on chip technology. The detailed discussions of methodology and methods have been discussed in chapter-3 which is followed by the exhaustive discussions on the results obtained in Chapter-4. The Chapter-5 briefly describes the conclusion and future scope of research.