MICROSTRUCTURAL ANALYSIS OF 58S BIOACTIVE GLASS WITH RELEASE STUDIES OF INCORPORATED BONE MORPHOGENETIC PROTEIN-2

by

Kelsey M. Gloss

A Thesis

Submitted in Partial Fulfillment

of the Requirements for

the Alfred University Honors Program

December, 2015

Under the Supervision of:

Chair:

Dr. Matthew Hall

Committee Members:

Dr. Anthony Wren

Dr. Garrett McGowan

ACKNOWLEDGEMENTS

I thank the faculty and staff of Alfred University, particularly those within the Inamori School of Engineering. They have helped me throughout the duration of my undergraduate education with regards to administration and instruction of various topics relevant to my degree.

I thank Dr. Hall for all of the time and effort he put into helping me understand the various concepts of research design and implementation. His guidance has helped me to develop into a better student, researcher, and professional. His help as my thesis advisor and academic advisor has better prepared me for graduate school and a job in research. I also thank Dr. Wren for his instruction throughout most of my Biomaterials Engineering classes here at Alfred University. He passed along valuable knowledge and experience working in the lab. Thank you Lana Placek and Katy Tierney for all of your help throughout this last semester.

Finally, I thank my parents for their never-ending love and support throughout my years in Alfred. My undergraduate experience would have been significantly more difficult without their advice and willingness to listen to me answer my own questions. I am so appreciative for all of the sacrifices my parents have endured to ensure my success in life. I thank Patrick for being there for me to ensure that I make time for fun and I also thank my Nannie for her prayers and sending me my daily scriptures.

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ABSTRACT

58S bioactive glass with a composition of $0.6SiO_2$ - 0.36CaO - $0.04P_2O_5$ was prepared using an acid-catalyzed sol-gel technique. This glass was then treated at temperatures of 500°C, 625°C, 675°C, 700°C, and 725°C for 24 hours. Each glass sample was characterized using x-ray diffraction (XRD) and the Brunaur, Emmett, and Teller (BET) method to observe any microstructural changes and to determine the surface area and porosity of each glass. XRD exposed the formation of crystalline CaO and SiO_2 phases. BET confirmed that the surface area of sol-gel glasses is large and it also revealed an inverse relationship between final heat treatment temperature, surface area, and pore volume. As the heat treatment temperatures increased, the surface area and pore volume of the glass decreased. The pore size was determined to be on the order of 7nm. Bone morphogenetic protein-2 was incorporated into the glass treated at 625°C and release products of the loaded glass over 1 hour, 3 hours, 6 hours, and 24 hours was observed using an enzyme linked immunosorbent assay (ELISA). While protein release was observed, it was unable to be properly quantified.

1 INTRODUCTION

Biomaterials are materials which have a reaction to the body or cause a reaction within the body. Their development thus far has enabled a broader and more in depth understanding of biological processes and materials science. Historically, the development can be divided into three non-mutually exclusive generations. The first generation is characterized by inert and tolerant biomaterials.[1] Inert and tolerant biomaterials are those which have and elicit a minimal response within the biological environment; these materials typically elicited the formation of a fibrous capsule when implanted.[1] Examples of such materials include alumina, titanium and its alloys, zirconia, thermoplastic polymers, and porous ceramics.[1,2] The second generation began at the advent of Dr. Larry Hench's Bioglass in 1969 and is distinguished for responsive or bioactive materials. Bioactive materials are those which bond to bone, have the ability to guide restoration of the natural bone tissue, and are resorbable.[1] The third, and current generation, is instructive materials. These materials have the characteristics of bioactive materials as well as the ability to promote the formation of new blood vessels, deliver biomolecules, and induce bone formation by acting on stem cells to influence their differentiation or maturation into bone-forming cells. Materials which fit into this generation are smart biomaterials, tissue engineered biomaterials, and biomimetic biomaterials. [1]

The purpose of this research is to propose and test a protein doped composition of an existing 58S bioactive glass to introduce a potential bone tissue scaffolding material. The goals of this thesis are to 1. Introduce bone morphogenetic protein-2 (BMP-2) into bioactive glass, 2. Examine the rate and quantity of dissolution from the glass, and 3. Determine the activity of BMP-2 released into solution.

1.1 BONE TISSUE ENGINEERING

Disease, injury, and trauma are the primary factors which lead to skeletal tissue degeneration.[3] Once the natural regenerative abilities of the body cease to correct tissue damage, tissue degeneration initiates a cascade of physiological failures; if left untreated, these will lead to necrosis at the site of trauma, impaired function of the affected area, and/or impaired quality of life. Current treatment options include transplanting tissues, natural bone repair, and implanting orthopedic devices.

1.1.1 Bone Grafts

Transplanting procedures are those which utilize bone grafts to treat fractures, joint replacements, and bone loss due to cancer or infection.[4]Autografts, also called autogenous grafts, are the most widely used type of graft with the highest probability of success. Autografts are grafts which are taken from the patient, and transplanted at the injury site. Despite being the most favorable method of transplantation and carrying the least risk of stimulating an immune response, autografts have several limitations; they are painful for the patient, expensive, carry a risk of donor-site morbidity, and the regions of the body which a graft can be taken from are severely limited depending on the amount of bone required for the graft.[5] Grafts must be taken from regions of the body which have a large enough surface area to ensure the mechanical and structural stability of the donor-site, typically the posterior iliac crest.[4] Donor-site morbidity defines any major or minor complication resulting from harvesting tissue from the patient; examples of such complications may be seen in Table 1.[5] Major consequences are those which require additional surgery, additional hospital admission time,

or those which result in disability.[5] Minor complications are those which do not require additional surgery and are manageable by post-operational treatments.[6]

Table 1. Complications of Harvesting Autografts

Time	Major	Minor
Early Complications	Deep infection	Hematoma
	Prolonged drainage	Wound drainage
	Large hematoma	Severe pain
	Reoperation	Temporary sensory loss
Late Complications	Sensory loss	Chronic mild pain
	Chronic severe pain	Superficial infection
	Chronic infection	Delayed wound healing
	Large bony defect/scar	Minor wound problems

Allografts are those which tissue is taken from an individual of the same species. Similar to autografts, they are able to repair bone defects, can be shaped to fit into a particular geometry, and have similar biocompatibility, however, they are a less favorable source of transplant material.[7] There is a limited supply of available and willing donors to supply tissue and there is also a higher risk of donor-patient disease transmission. [7] Since allografts are taken from a different individual, the proteins present on cellular surfaces of the donor tissue will vary slightly from those present within the patient cells. This slight difference at the least can cause a minor immunologic reaction and at most, cause graft rejection.[7]

1.1.2 Natural Bone Remodeling

Natural repair processes take time and have a systematic mechanism. The steps of bone repair are: inflammation, formation of a soft callus, formation of a hard callus, and a brief remodeling phase. Within moments of trauma, a hematoma forms around the injury site. Macrophages, leukocytes, and other inflammatory cells are sequestered within the injury site

where they attract other inflammatory cells via chemotaxis and work to remove cellular debris from the area.[9] Inflammatory cells secrete cytokines and growth factors to induce clotting, angiogenesis, and cell proliferation. Cytokines and growth factors involved in this process include: vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF- β), tumor necrosis factor-α (TNF- α), interleukins 1 and 6 , and fibroblast growth factor-2 (FGF-2), and various other factors which promote inflammatory and stem cell recruitment and cell division.[10] The soft callus is formed from the matrix of fibrocartilage secreted by proliferated chondrocytes; once formed, the soft callus is ossified into the hard callus. During the last stage of repair, the hard callus, made of spongey woven bone, is replaced by dense cortical bone.[10] While the body is capable of repairing minimal damages; therapeutic intervention is often needed for extensive traumas to ensure the success and speed of repair.[11]

1.1.3 Scaffolds for Tissue Engineering

Tissue engineering combines medicine and materials science to promote the regeneration of patient tissue via the use of scaffolds, cells, and biologically active molecules.[15] Tissue scaffolds, often seeded with cells or various growth factors, are used to mediate skeletal tissue repair by interacting with the tissue surrounding it and inducing formation of healthy tissue.[6] The properties of a good scaffold material include: controllable biodegradability, osteoconductivity, and the ability to deliver cells.[12] Biodegradability is the ability of a material to be chemically dissolved via biological means and is an important property of a scaffolding material because a high degree of biodegradability ensures that the scaffold will eventually be replaced by host tissue, which is a primary goal of tissue engineering.[11] While the ability to degrade within the body is important, the rate of that degradation is of equal interest as well; if

the degradation rate is too high, the material will dissolve before cells can attach to it and form their own networks. If the degradation rate is too slow, the prolonged presence of a foreign material within the body could cause an unwanted immunological response.[3] Osteoconductivity is the ability of a material to facilitate the attachment and proliferation of bone-forming cells.

1.2 BIOACTIVE GLASS

There have been many materials researched for potential scaffold use, including natural and synthetic polymers, glasses, and ceramics, however, the properties of bioactive glasses have made them a material of increasing promise. Bioactive glasses react with physiological fluids which results in the formation of a biocompatible layer of hydroxyapatite on the surface of the material.[12] This hydroxyapatite layer enables cell attachment and the resorption of the glass into the new tissue. Bioactive glasses also release soluble ions in solution which act on surrounding tissue which leads to rapid bone formation.[13]

1.2.1 Composition

The most prevalent type of glass available is soda-lime-silica glass and is composed of silica, soda ash, and limestone. It is used in windowpanes, food containers, and bakeware. The composition of glass has a large effect on the properties and correspondingly, the behavior of the glass. Composition has been the key variable when developing new glasses for specific applications and lends itself to the high versatility of glass. The modifications of traditional glass led to the discovery of bioactive glasses. Bioactive glass was first discovered at the University of Florida by Dr. Larry Hench in 1969. His specific composition of bioactive glass is called Bioglass and has a composition of $0.45SiO_2$ - $0.245Na_2O$ - $0.245CaO$ – $0.06P_2O_5$. He discovered that his

composition of glass was biocompatible and could bond chemically to bone when implanted in living tissues; the property which makes it a bioactive glass.[14] Biocompatibility is the ability of a material to elicit an appropriate biological response in a specific application. To accomplish this response, the composition of the glass plays the most important role. Compounds or minerals can be added to existing glass compositions in order to increase the biocompatibility of a glass.

Chemical or mineral additions and modifications to the original composition of Bioglass have yielded new compositions of glasses, 45S5 and 58S. 45S5 glasses closely resemble to original composition of Bioglass; they are comprised on silica, sodium oxide, calcium oxide, phosphorous pentoxide, and depending on the type of 45S5 glass, calcium fluoride, and boric oxide. Ions released by these compositions of glasses have been shown to cause the gene upregulation of bone-forming cells.[15]

58S bioactive glasses have the composition of $0.6SiO_2 – 0.36CaO – 0.04P_2O_5$. This composition differs in that it has significantly more silica and calcium oxide, no sodium oxide, and less phosphorous pentoxide. The bone-bonding properties of bioactive glasses are largely due to the silica content. A silica-rich gel layer on the surface of the glass facilitates the strong bond between the glass and bone.[16] Silica has also been shown to increase the activity of osteoblasts, the cells responsible for synthesizing new bone, which then hastens the creation of new bone tissues.[17]

1.2.2 Chemistry

The interaction of bioactive glasses and an aqueous environment such as the body has been described as a five-step process. This process, proposed by Filgueiras, is listed and described below: [18]

Stage 1: Ion exchange between ions within the glass and protons or hydronium ions in solution to produce silanols on the glass surface.

Stage 2: The silica structure of the glass breaks down due to interaction with water-based species within the solution. Soluble silica, $Si(OH)_4$, is released into solution leaving silanols on the surface of the glass.

Stage 3: The silanols on the surface of the glass condense which causes the polymerization of a porous silica-rich gel layer on the surface of the glass.

Stage 4: Ca^{2+} and PO_4^{3-} migrate to the surface of the silica-rich gel layer to form a CaO-P_2O_5-rich film on the surface of the silica-rich film.

Stage 5: Amorphous CaO-P_2O_5 film crystallizes upon the introduction of OH^-, CO_3^{2-}, or F^- anions from solution this forms a mixed hydroxyl, carbonate, or fluorapatite layer.

1.2.3 Fabrication

The primary techniques for bioactive glass fabrication are a melt-derived process or a sol-gel method. Melt glasses are made by combining the glass powder and oxide components and melting them together in a crucible at high temperatures; the exact temperature depends on the glass composition. This mixture is then poured into a mold and quenching.

Sol-gel is a process which is characterized by the transition of a glass system from a liquid “sol” into a solid “gel” phase and involves the chemical synthesis of inorganic materials

via preparation and gelation of a sol. [13] Sol-Gel glasses are synthesized via a hydrolytic reaction of an alkoxide precursor that forms a colloidal silica solution.[20] Tetraethylorthosilicate is added as a silica precursor, triethylphosphate is used to introduce phosphate, and a calcium nitrate salt adds calcium. A polycondensation reaction involving the silica in the solution occurs to form a silica network, which in turn, creates the network. The network is heated to burn off water, ethanol, and nitrate byproducts. This heating not only removes unwanted byproducts, it also further drives the polycondensation reactions within the gel.[20]

1.3 BONE MORPHOGENETIC PROTEINS

BMPs are multifunctional growth factors which induce bone formation and repair; specifically, BMP-2 has osteoinductive properties and can also cause osteoblast differentiation.[21] BMPs are present in many tissues but were named bone morphogenetic proteins because they were originally identified in bone-inductive extracts of demineralized bone. [22] BMPs are part of the transforming growth factor β (TGF-β) superfamily. The TGFβ superfamily is made up of many signaling molecules and growth factors which have a wide range of functions.[23]

1.3.2 Working Biology of TGF-β Superfamily

These functional pathways play central roles in tissue morphogenesis and homeostasis.[24] BMP plays a key role throughout the lifetime of a cell; the cellular processes in which BMPs play a role are as follows: cell growth, differentiation, and apoptosis.[24] BMP plays such a key role because it is capable of causing intracellular transcriptional responses based on extracellular signals.[24] BMP signal transduction is a downstream cascade and can be described as follows: ligands interact with transmembrane receptors via SMAD protein phosphorylation.[24]

SMAD proteins are a family of signal transducer proteins which regulate and facilitate BMP and other TGF-β ligand binding to serine-threonine kinase receptors on the cell surface.[25] Once the ligand forms a receptor complex, the specific binding of the ligand will induce a signal cascade by which cellular machinery will target genes within the nucleus.[24] At this point, these targeted genes will be expressed and the cell will either undergo growth, differentiation, or apoptosis.

1.3.3 Functional Biology of BMPs

There have been approximately twenty different types of BMPs identified and characterized, each having its own array of functions. Examples of the different types of BMPs can be seen in Figure 1.

BMP	Function
BMP-2	osteoinductive, osteoblast differentiation, apotosis
BMP-3 (osteogenin)	most abundant BMP in bone, inhibits osteogenesis
BMPs	
BMP-4	osteoinductive, lung & eye development
BMP-5	chondrogenesis
BMP-6	osteoblast differentiation, chondrogenesis
BMP-7 (OP-1)	osteoinductive, development of kidney & eye
BMP-8 (OP-1)	osteoinductive
BMP-9	nervous system, hepatic reticuloendothelial system, hepatogenesis
BMP-10	cardiac development
BMP-11 (GDF-8, myostatin)	patterning mesodermal & neuronal tissues
BMP-12 (GDF-7)	induces tendon-iliac tissue formation
BMP-13 (GDF-6)	induces tendon & ligament-like tissue formation
BMP-14 (GDF-5)	chondrogenesis, enhances tendon healing & bone formation
BMP-15	modifies follicle-stimulating hormone activity

* GDF = growth/differentiation factor.

Figure 1. Bone Morphogenetic Proteins and their respective functions

BMPs can induce the formation of bone and cartilage and can also contribute to non-osteogenic development.[21] While there are a number of BMPs that have osteoinductive properties; BMP-2 has been the most extensively studied for its specific properties. There have

been many preclinical models and clinical trials that have examined the ability of BMP-2 to heal bone defects and long bone critical-sized defects have been healed using BMP-2 in rats, rabbits, dogs, non-human primates, and sheep.[21] BMP-2 was chosen for this research because it has been shown, along with BMP-6 and BMP-9, to play an important role in mesenchymal stem cell differentiation into osteoblasts, bone matrix-secreting cells.[26]

2 EXPERIMENTAL PROCEDURES

2.1 Preparation of Glass

Glass samples with a composition of $0.6SiO_2$ - $0.36CaO$ - $0.04P_2O_5$ were prepared using an acid-catalyzed sol-gel method. The solution for the glass was prepared by mixing 227.2 ml of 0.1M nitric acid (Sigma Aldrich, USA) to maintain a 2.25:1 ratio of nitric acid to TEOS, 108.2 ml of tetraethylorthosilicate (TEOS; $Si(C_2H_5OH)_4$) (Sigma Aldrich, USA), 10.99 ml of triethyl phosphate ($P(C_2H_5OH)$) (Sigma Aldrich, USA), and 47.70 g of calcium nitrate ($Ca(NO_3)_2$-$4H_2O$) (Sigma Aldrich). The nitric acid and TEOS were combined and stirred for 30 minutes. The triethyl phosphate was then added and the mixture was stirred for 45 minutes. Finally, the calcium nitrate was added and the complete mixture was stirred for 1 hour to ensure complete hydrolysis. The solution was poured into sealed polyethylene tubes and incubated at 40°C for 72 hours. Four holes were punched into the tops of each tube and the gels were dried for an additional 5 days at 80°C and stored in a desiccator. Two batches of glass were made using the methods described above. Gels from the first batch were consolidated for 24 hours at 625°C while gels from the second batch were consolidated for 24 hours at 500°C, 625°C, 675°C, 700°C, and 725°C. The glass particles were ground using a mortar and pestle then sieved using a 75 µm sieve and a 63 µm sieve. The glass particles that were smaller than 75 µm but larger than 63 µm were used. Remaining glass powder was divided by size, collected and stored in a desiccator.

2.3 Characterization of Glass

2.3.1 BET Analysis

Brunauer, Emmett and Teller (BET) analysis was used to determine the surface area and porosity of the glass. The surface area of the resultant glass was then analyzed using a gas

absorption method. The sample was weighed and then degassed at 150°C for 60 minutes. The degassed sample was then weighed again then placed into the sample holder for analysis in a Micromeritics TRI Star II Surface Area and Porosity system using TriStar II 3020 software. (Micromeretics, GA, USA).

2.3.2 X-Ray Diffraction

X-ray Diffraction (XRD) was used to analyze the glass from both batches before and after the final heating treatments. Samples were loaded into a Bruker top-loading sample holder. A diffraction pattern for the glass was collected using a Siemens D5000 diffractometer (Bruker AXS, Inc., WI, USA) equipped with Cu radiation at measurement conditions of 15°2θ-65°2θ, a step size of 0.02°2θ, and a count time of 2 seconds. A generator of 40kV and a tube current of 30 mA were employed.

2.5 Activity Assay

2.5.1 Incorporation of BMP-2

Recombinant Human BMP-2 (ScienCell Research Laboratories, CA, USA) was reconstituted to a concentration of 1µg/mL using 0.067 M phosphate buffered saline (PBS). This stock solution was divided into smaller vials of 100µL. Each aliquot was stored at -80°C until use. Upon use, the aliquot was thawed and diluted using a 1:100 dilution. 1 mL of this solution and 0.025g of first batch glass were incubated at 37°C for 1 hour in Eppendorf tubes. After the hour, the Eppendorf tubes were centrifuged and the solution was poured off. The glass was rinsed and then incubated with 1 mL of phosphate buffered saline for 1 hour, 3 hours, 6 hours, and 24 hours. The eluate solution was then collected and stored at -80°C until its use.

2.5.2 ELISA

Release products of glass samples loaded with BMP-2 were tested using a standard enzyme-linked immunosorbent assay (ELISA) kit (ScienCell Research Laboratories, CA, USA) to test for the presence of human BMP-2. A standard curve was generated for the sample. The provided standards and eluate samples were added in duplicate to a 96-well plate along with a PBS control. The plate was incubated at 37°C for 90 minutes. The biotinylated antibodies were added to each well and incubated at 37°C for 60 minutes. The strips were washed three times with 0.01M TBS. The ABC working solution was added and the strips were incubated at 37°C for 30 minutes. The strips were washed five times with 0.01M TBS. The TMB color developing agent was added and the strips were incubated in dark at 37°C for 25 minutes. The TMB stop solution was added and the plate was read using a μQuant microplate spectrophotometer (BioTek Instruments, Inc., VT, USA) accompanied by KCjunior software for data analysis.

3 RESULTS & DISCUSSION

3.1 Glass characterization

3.1.1BET Analysis

BET analysis was conducted in order to observe a hypothesized change in porosity and surface area at varying temperatures and to determine an effective heating temperature for the consolidation step. The efficiency of the heating temperature is defined as the temperature which maintains the inherent pore size of the scaffold. As the gel is consolidated, the organics within the gel network are burned off, which results in a shrinkage of the gel. Effective consolidation is one in which the pore size does not shrink below the critical pore size. Sol-gel fabrication processes tend to be a desirable method of glass fabrication due to the porous glass nanostructure generated by the process. These micropores are produced during gelation and increase surface area for glass corrosion as well as act as nucleation sites for HA formation on the surface.[20] A higher surface area for glass corrosion means that sol-gel derived glasses can degrade more readily and the nucleation sites allow glasses to be more bioactive. These pores within the nanostructure also act as a region for tissue in-growth, which further increases the bioactivity of the glasses.[27] The surface area for the first glass batch consolidated at 625°C was found to be 213.5734m^2/g with an average pore volume of 0.444 cm^3/g and an average pore size of 66.06 Å. The absorption and desorption data can be seen in Figure 2 which shows the amount of nitrogen gas adsorbed to the glass. The shape of the figure indicates that the glass has both micropores and mesopores.

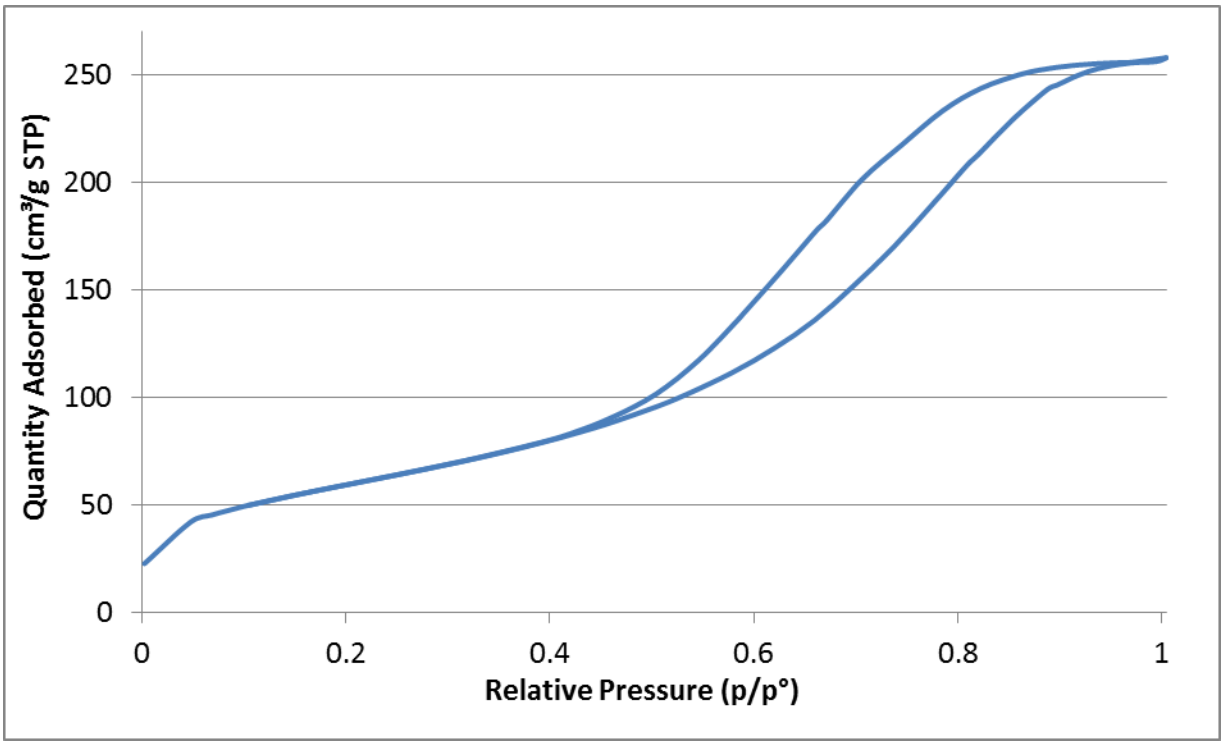

Figure 2. Nitrogen adsorption/desorption isotherm for glass heated to 625°C

A second set of experiments was done to explore the effect of consolidation temperature on the porosity and surface area of the glass. The results of BET analysis may be seen in Table 2. The adsorption and desorption data obtained for 500°C, 625°C, 675°C, and 725°C may be seen in Figure 3. The shape of the isotherm plot for the second batch of glass at a second temperature remained the same.

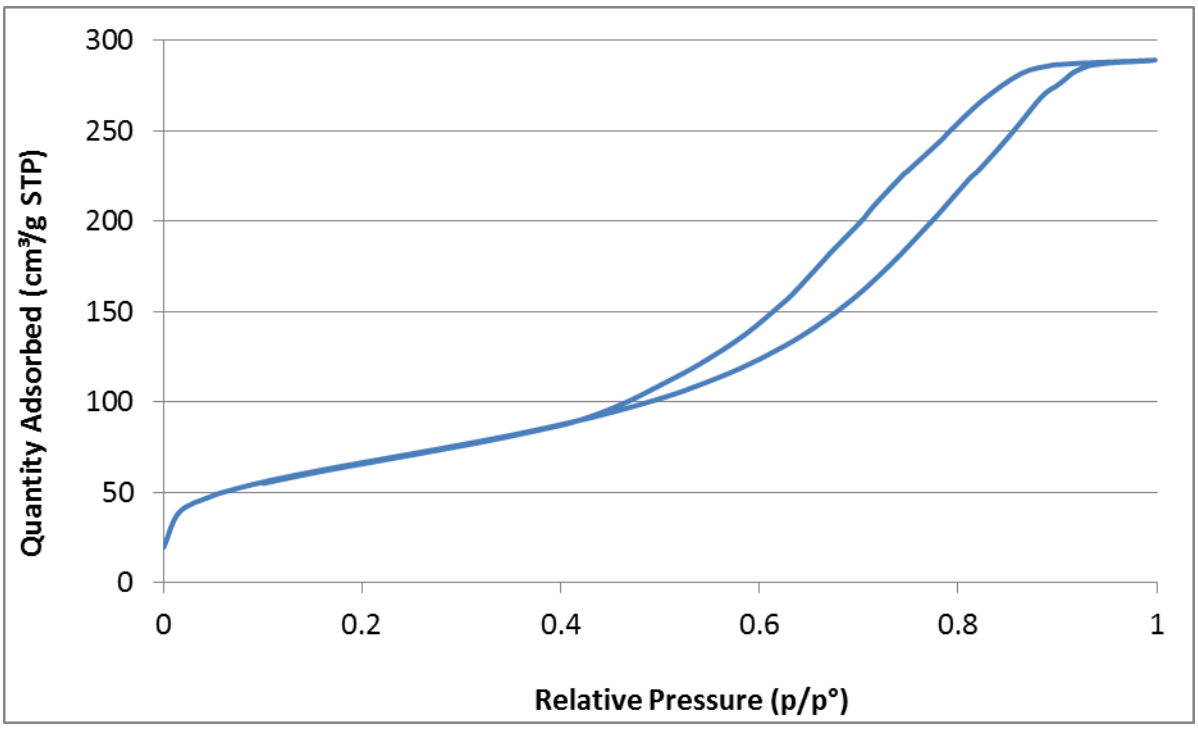

Figure 3. Nitrogen adsorption/desorption isotherm for glass heated to 500°C

The results of the porosity studies may be seen in Table 2 and Figure 4. Table 2 is the tabulated summary of the BET data. Figure 4 shows the relationship of temperature with surface area and pore volume. The average pore size for each of the heated glasses was found to be on the order of 7nm. The results of BET analysis revealed that as temperature increased, the surface area and pore volume decreased. However, as the temperature increased, the pore size increased. This means that the glasses consolidated at lower temperatures have a small pore size but a very large surface area and pore volume. In order to determine the ideal heating temperature for the consolidation step, a balance between surface area, pore size, and pore volume must be maintained. Based on the results obtained for temperatures of 500°C, 625°C, 675°C, and 725°C, the temperature which supports maximum allowable surface area, and pore volume is 500°C.

Table 2. Surface Area and Porosity Analysis Results

Temperature	Average Surface Area	Average Pore Volume	Average Pore Size
500 °C	236.72 m^2/g	0.488 cm^3/g	71.16 Å
625 °C	220.79 m^2/g	0.458 cm^3/g	72.31 Å
675 °C	210.76 m^2/g	0.446 cm^3/g	73.10 Å
725 °C	200.19 m^2/g	0.430 cm^3/g	73.23 Å

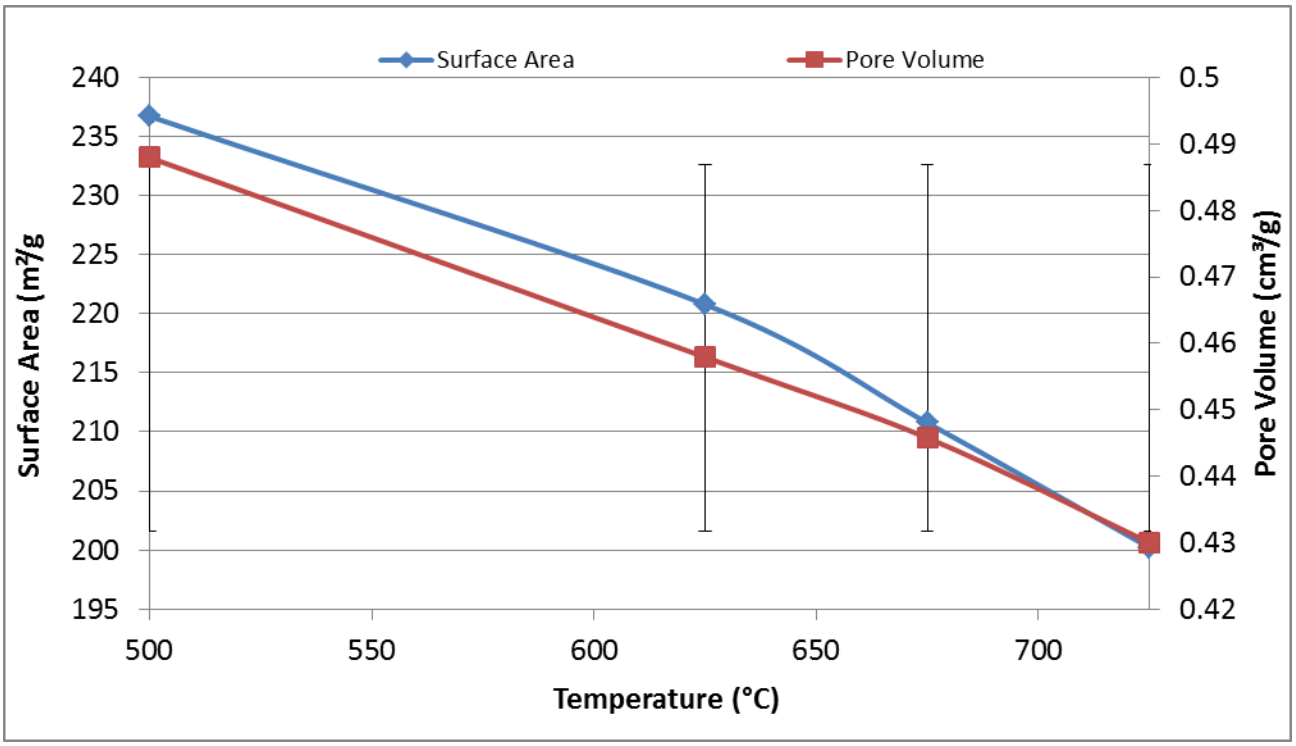

Figure 4. Surface area and pore volume at a) 500°C, b)625°C, c)675°C, and e) 725°C

3.1.2 X-Ray Diffraction

XRD was performed in order to qualitatively analyze the structure of both batches of glass before and after consolidation. Figure 5 shows the diffraction patterns obtained before and after heating for the first batch of glass. Figure 6 shows the XRD patterns for the second

batch of glass unheated and at 500°C , 625°C , 675°C , 700°C , and 725°C. The glass before heating is shown to have a characteristic amorphous structure whereas the glass after heating became more crystalline. The phase identified for the first batch of glass using ICDD is indicated by the stick pattern and was determined to be calcium oxide. This identification holds true for the second batch of glass as well. Calcium oxide was present in each of the temperature heated glasses. Two other phases, silicon oxide and lime were also identified in the diffraction patterns. The peaks for silicon oxide peaks can be seen to start developing at 625°C. As the temperature increases the intensity of the silicon oxide peaks increases to a maximum at 675°C. After 675°C, the silicon oxide peak intensities decrease. At 700°C and 725°C, another calcium oxide phase, lime, developed. The presence of calcium oxide is likely due to calcium remaining dissolved in the pore liquor of the gel. Calcium was not incorporated into the silicate network during the drying process.

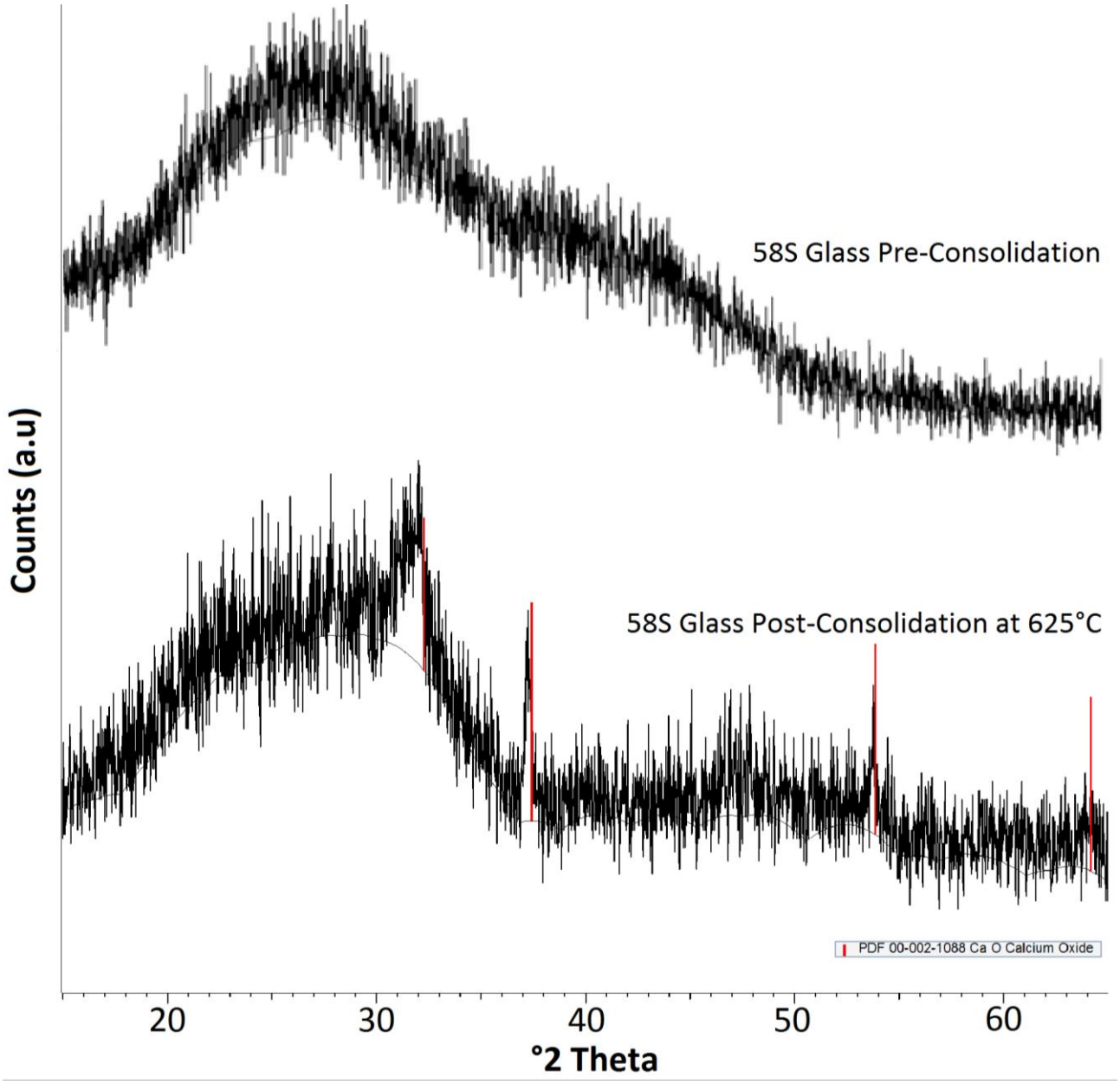

Figure 5. X-Ray Diffraction Pattern for 58S glass Pre-and post-consolidation

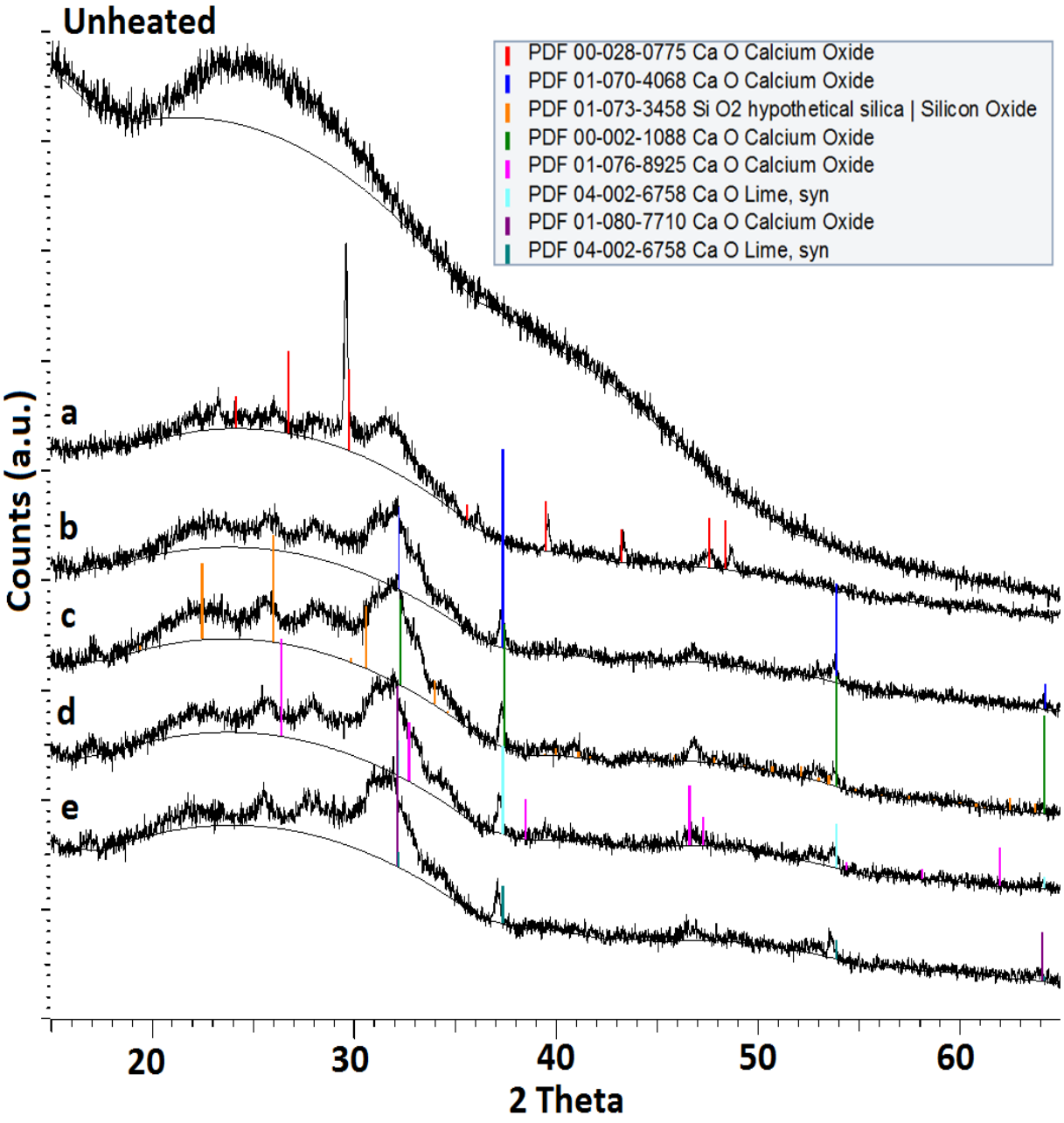

Figure 6. X-Ray diffraction pattern of glass pre and post-consolidation at a) 500°C, b)625°C, c) 675°C, d) 700°C, and e) 725°C

3.2 Activity Assay

Protein release was measured using an enzyme-linked immunosorbent assay (ELISA). This technique is designed to detect and quantify biological substances using antibodies specific to the substance, or antigen, being measured. ELISA is a colorimetric assay by which a tag, colored by conjugated enzyme activity, is observed using UV/VIS spectroscopy. The intensity of the absorption directly correlates to the amount of detected antigen. Figure 7 is the standard curve for the BMP-2 ELISA kit used and Figure 8 are the results of BMP-2 release studies over time and at varying concentrations of BMP-2. While there are no obvious trends in Figure 8, it can be noted that there was some degree of protein release measured from the glass. It is unclear whether or not this observed release is release of protein adsorbed to the surface of the glass or if it is true release from protein loaded into the pores. Future studies will need to be conducted in order to properly quantify BMP-2 release and to assess the validity of the data.

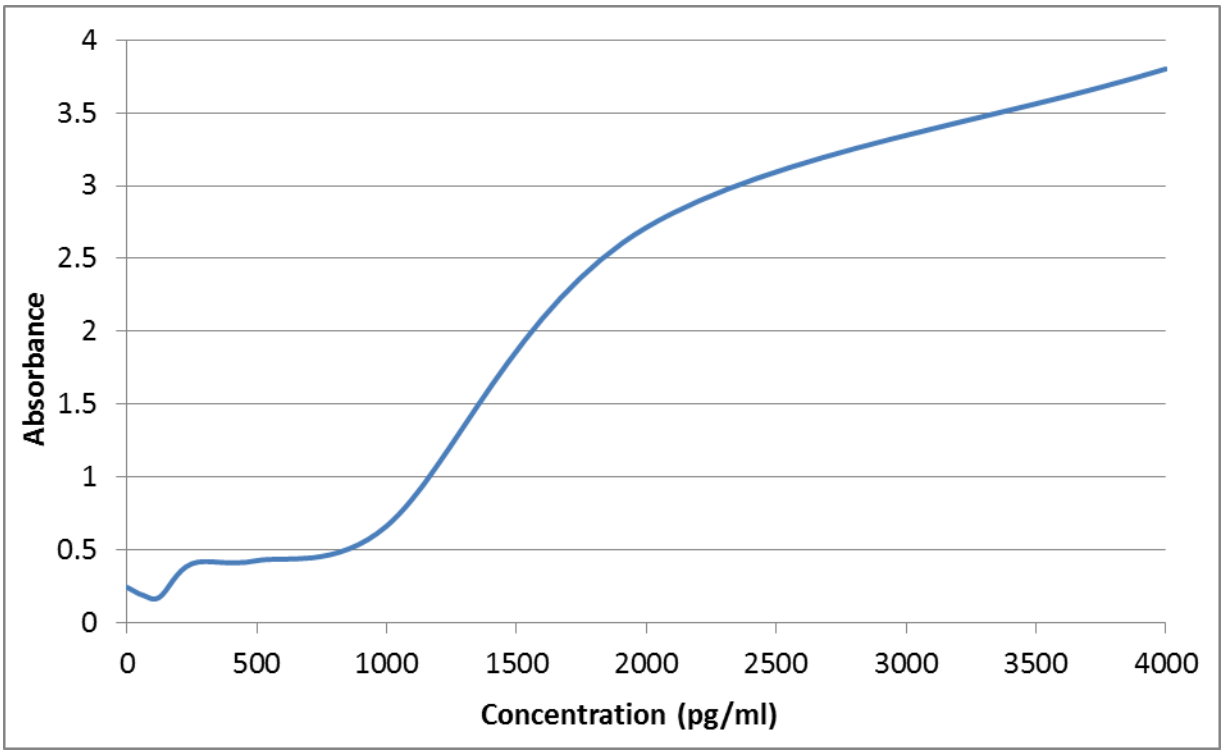

Figure 7. Human BMP-2 ELISA Kit Standard Curve

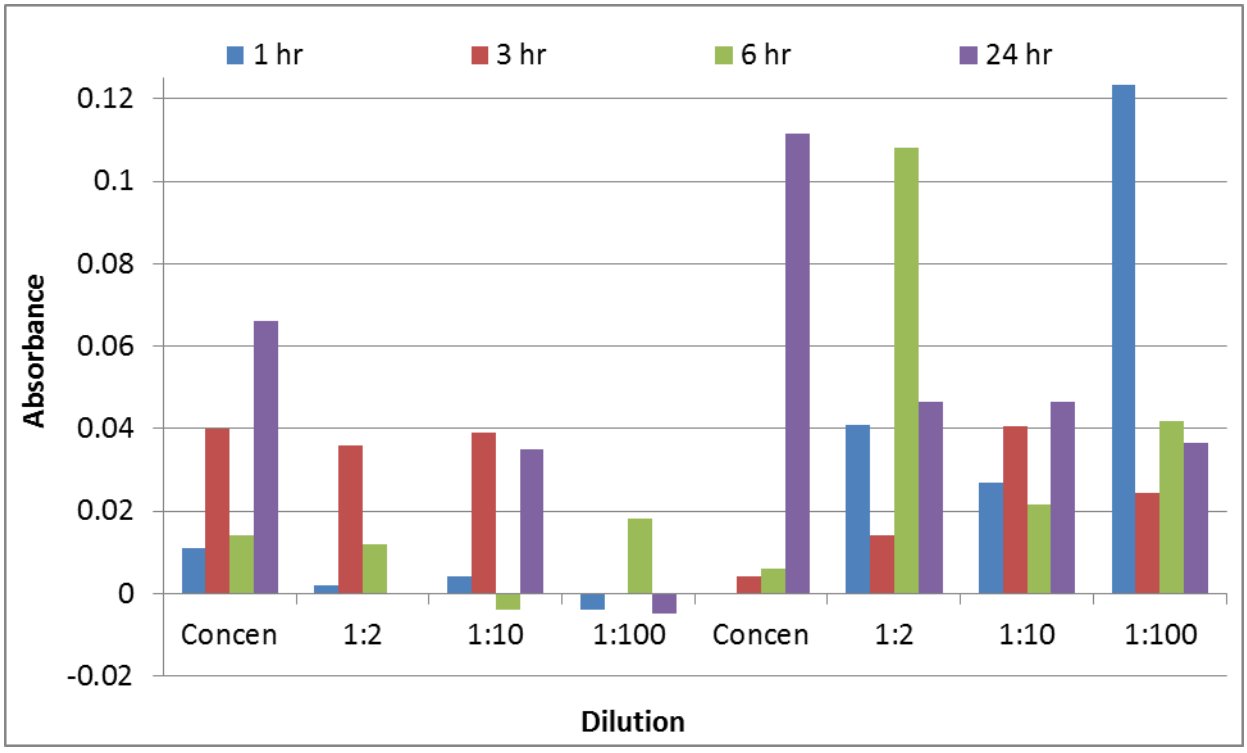

Figure 8. Human BMP-2 Absorbance vs. Concentration at 1hr, 3hr, 6hr, and 24hrs

4 CONCLUSIONS

Once heated, the glass formed calcium oxide and silicon dioxide phases. The hysteresis loop on the nitrogen adsorption and desorption isotherms indicates that this glass has a microporous and mesoporous structure. The prepared glass is affected by the final heat treatment temperature; the surface area and pore volume decrease as the temperature increases. The ideal heating temperature for consolidation is 500°C or below. BMP-2 was released from the glass, but unable to be quantified.

FUTURE WORK

In order to develop a greater understanding of the effect of final heating temperature on this composition of sol-gel derived glass and to narrow down the ideal temperature for glass consolidation, further heat studies must be done. Future studies should focus on incremental temperature changes between 450°C and 625°C. Each glass should be tested using x-ray diffraction and BET analysis. An effort to properly quantify BMP-2 release from the glass should also be made in future experiments.

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