TAMPEREEN TEKNILLINEN YLIOPISTO
TAMPERE UNIVERSITY OF TECHNOLOGY

PANU NOPPARI

SHELF-LIFE AND INJECTABILITY STUDY OF SYRINGES FILLED WITH A BIODEGRADABLE SILICA-SILICA COMPOSITE USED IN THE PARENTERAL ADMINISTRATION OF PHARMACEUTICAL AGENTS

Master of Science Thesis

Examiner: Professor Minna Kellomäki
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ABSTRACT

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This thesis was carried out with the collaboration of DelSiTech Ltd, which is a leading silica-based drug delivery technology and drug development company based in Finland. In the beginning of this study, spray dried silica gel microparticles, used as a carrier material for therapeutic agents, were mixed together with a sol-gel derived silica sol. It was paramount that the mixing was performed before the initial silica sol had been allowed to form a hydrogel. This ensured the creation of a homogenous mixture. In the end, the mixture was filled into syringes and allowed to form a stable and injectable silica microparticle-silica hydrogel composite product.

The continuous hydrogel matrix of the composite elicits excellent shear-thinning characteristics, and as a result, could facilitate the parenteral administration of therapeutic agents encapsulated within the silica microparticles. Throughout the study, the formulation of the sol-gel derived silica sol was kept constant at an R-value of 400 (molar ratio of water to silica alkoxide). In contrast, the volume concentration of silica microparticles ranged from 0.5 to 1 g/ml, which was noticed to affect the gelation rate of the silica-silica composite. This indicated that the microparticles act as nucleating agents in the formation of the three-dimensional gel structure.

In order to optimize the manufacturing process of the composite and to define the applicability of the final product, three objectives were set: determine the time-frame in which to mix the composite as well as fill the syringes, evaluate the shelf-life of the final product and to assess the injectability of the product through various needle gauges (23G, 27G, and 30G). The mixing and filling time-frames were evaluated by determining the gel-point of the composite under small angle oscillatory shear. Process parameters, such as temperature, evaporation and the age of the initial silica sol were examined to optimize the filling process. The broadest time-fame was achieved by utilizing unaged silica sols in sealed process systems at refrigerator temperatures (4-8 °C). Respectively, the shelf-life of the final product was also characterized by oscillatory measurements, which indicated that the composite retained its viscoelastic characteristics for at least 30 days, regardless of changing microparticle concentrations. Lastly, the final product was injected out of 1ml syringes through thin needles to evaluate the injectability of the product. Ultimately, a microparticle concentration of 0.75 g/ml exhibited the most prominent injectability characteristics out of all the concentrations investigated.

TIIVISTELMÄ

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Tutkielman toimeksiantaja oli DelSiTech Oy, joka on erikoistunut silikapohjaisiin lääkeannostelutekniikoihin. Työn tavoitteena oli valmistaa injektoitava silikamikropartikkeli–silikahydrogeelikomposiitti lääkeruiskuihin. Komposiitin tarkoitus on toimia lääkkeiden parenteraalisena annostelijana: silikamikropartikkelien sisälle on kapseloitu lääkeainetta ja partikkelit kuljetetaan hydrogeelissä haluttuun kohteeseen injektoitaessa. Mikropartikkelit oli valmistettu ennen työn aloittamista sumukuivaamalla silikasooleja, jotka oli tuotettu sooli-geeli-tekniikalla. Silikageelit valmistettiin tutkimuksen aikana sooli-geeli-tekniikalla. Silikageelien lähtöaineena käytettiin tetraetyyliortosilikaattia ja geelien R-arvo (veden suhde silikaattiin) vakioitiin arvoon 400.

Homogeenisen komposiitin aikaansaamiseksi oli tärkeää sekoittaa mikropartikkelit silikasooliin, joka muuttui geeliksi ajan edetessä. Soolin geeliytyminen kuitenkin nopeutui merkittävästi, kun siihen sekoitettiin silikamikropartikkeleita – toisin sanoen valmistettu komposiitti muuttui geeliksi nopeasti. Tämä aiheutti haasteita, sillä komposiitin tuli muuttua geeliksi vasta lääkeruiskujen sisällä. Näin ollen tutkimukselle asetettiin kolme tavoitetta.

Ensimmäinen tavoite oli optimoida komposiitin valmistusprosessi säätelemällä lämpötilaa, haihtumista ja sekoituksessa käytetyn soolin ikää. Prosessi optimoitiin reometrisilla oskillaatiomittauksilla, joiden avulla komposiittinäytteille määritettiin geelipiste. Geelipisteen avulla rajattiin aikaikkuna, jonka aikana ruiskut tuli täyttää komposiitilla. Tutkimuksessa havaittiin, että jos komposiitti valmistettiin tuoreesta silikasoolista, haihtuminen minimoitiin ja prosessilämpötila oli + 4 °C, komposiitin geeliytyminen hidastui merkittävästi. Näin ollen ruiskujen täyttöaika piteni. Toinen tavoite oli tutkia lopputuotteen (komposiitilla täytetty lääkeruisku) säilyvyyttä. Tätäkin tutkittiin oskillaatiomittauksilla, joilla kartoitettiin mahdollisia muutoksia komposiitin viskoelastisissa ominaisuuksissa varastoinnin aikana. Kuukauden kuluttua tuotteessa ei havaittu muutoksia. Kolmas tavoite oli tutkia komposiitin injektoitavuutta. Komposiittia injektoitiin erikokoisten neulojen (23G, 27G ja 30G) läpi. Viskositeettimittauksilla määritettiin ja osoitettiin komposiitin leikkausohenevuus. Lopuksi havaittiin, että mikropartikkelien pitoisuudella on suuri vaikutus komposiitin injektoitavuuteen. Lupaavimmat tulokset injektoitavuuden suhteen saavutettiin pitoisuudella 0,75 g/ml.

PREFACE

I would like to extend my gratitude to Lasse Leino, the CEO of DelSiTech Ltd, for allowing me to undertake this demanding project of optimizing the injectability of their silica microparticle-silica hydrogel composite.

With the aid of DelSiTech's research staff and Mika Jokinen, the Research Director at DelSiTech Ltd, I managed to accomplish the tasks given to me and finalize my thesis. I would also like to thank Professor Minna Kellomäki for acting as the examiner of this thesis and providing me with vital commentary with the intent of improving my work.

I am also grateful for the staff at Turku Clinical Biomaterial Center for their hospitality, guidance and for providing the facilities to conduct a majority of my experiments. Last, but not least, I want to extend my deepest gratitude for my significant other Laura, who has supported me with her undying appreciation and unwavering support during the entire course of this undertaking.

Helsinki, 25.5.2016

Panu Noppari

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GLOSSARY

23G	Needle gauge (0.6 x 30 mm)
27G	Needle gauge (0.4 x 20 mm)
30G	Needle gauge (0.3 x 13 mm)
G´	Shear storage modulus
G´´	Shear loss modulus
Gel-point	When tan $\delta = 1$ and begins to approach 0
HCl	Hydrochloric acid
Hydrogel	Water dispersed in a continuous solid phase
NaOH	Sodium hydroxide
R-value	Water to silica alkoxide molar ratio
Silica	Silicon dioxide (SiO_2)
Sol	A colloidal suspension of small particles dispersed in liquid
tan δ	loss tangent (tan $\delta = G''/G'$)
TEOS	Tetraethoxysilane ($Si(OC_2H_5)_4$)

1. INTRODUCTION

The objective of controlled drug delivery is to provide effective drug administration to a specific location in a patient (e.g. a specific organ or organic system). Controlling the delivery is of great concern, since indiscriminate drug delivery can lead to severe or lethal side effects. This in turn decreases the therapeutic value of a drug. Furthermore, effective drug administration implies the ability to control the distribution of a drug, not only in space, but also in time. [1, p. 322-323.] In other words, the drug delivery system should elicit a controlled release rate of the therapeutic agent for a predetermined period of time in a specific location in the body of a patient. Essentially, the controlled release of a drug is designed to maintain the drug concentration in the body within the optimum therapeutic range. This range is determined by the minimum therapeutic threshold (lower limit) and the toxicity threshold of the drug (upper limit) [2, p. 19-22]. As a result, by maintaining the optimum therapeutic range of a drug and by targeting said drug, the overall therapeutic efficiency of the delivery system is increased. Regarding effective delivery systems, great examples include silicon dioxide -based systems.

Silicon dioxide (SiO_2), also referred to as silica, is an inorganic material widely used in different applications ranging from microelectronics to medical devices. Nevertheless, the scope of this study is on silica as a biodegradable biomaterial. More precisely, the study is focused on sol-gel processed spray-dried silica-gel microspheres applied in the field of controlled drug delivery. The mandator of the study was DelSiTech Ltd, which is a leading technology specialist in biodegradable silica-based controlled release systems. The ultimate goal was to manufacture a stable and homogenous silica-silica composite into syringes, in order to produce prefilled (“ready-to-use”) products. The composite consisted of silica-gel microparticles (as such) dispersed in a silica-hydrogel. The microparticles were manufactured prior to the study by spray-drying sol-gel derived silica sols. The silica hydrogels were also derived by a sol-gel method. Once the silica microparticle-silica hydrogel composites were prepared, their viscosity and viscoelastic properties were characterized with a rheometer. Rheological measurements are presented in Chapter 2.2, whereas the key features of sol-gel processing are described in Chapter 2.3.

The viscosity of a silica sol, which is essentially a suspension of silica nanoparticles and nanoparticle aggregates, rises as a function of time. This increase in viscosity is governed by the formation of an increasing number of silica nanoparticles within the sol. These particles and particle-chains are formed by a condensation process. Consequently, as more nanoparticles are formed they aggregate into large clusters and chains. In the end, a solid continuous three-dimensional structure is formed trapping in discrete liquid phases – the

sol has transformed into a gel. As a result, the silica microparticles had to be mixed with the silica sol before it had been allowed to form a gel. This ensured that the microparticles could be homogenously dispersed within the sol. Furthermore, after mixing the microparticles with the silica sol, the gelation rate of the mixture was seen to dramatically increase. This indicated that the microparticles may act as nucleating agents within the sol matrix hastening the gelation. [3.]

The gelation rate of the composite had to be determined, in order to control the filling process of the syringes. This rate is dependent upon several variables, but the ones investigated here were the following: temperature, evaporation and the age of the silica sol used in the manufacturing process. The results are accordingly discussed in Chapter 4. Additionally, the shelf-life of the composite was investigated by oscillatory measurements. During these shelf-life studies it was paramount to determine the first point-in-time when the composite had stabilized inside the syringe and could therefore withstand the injection process. This stabilization period also dictated how long the syringes were to be stored in a way to prevent particle sedimentation from occurring (i.e. kept in rotational motion). However, once the gelation process was complete and the microparticles were locked in place, the syringes could be stored in static conditions.

Finally, the injectability profile of the composite was studied by injecting the composite through various needle gauges (23G, 27G and 30G). In this way, the suitable needle size to be used in administering the composite to a patient was determined. Generally, a thinner needle correlates with a less painful procedure for a patient. Moreover, a thinner needle allows for a wider range of locations to which the therapeutic agent can be parenterally administered. For these reasons, the concluding aim was to manufacture a composite which could be successfully injected through the thinnest (30G) needle used in this study.

2. THEORETICAL SECTION

2.1 Rheology and rheometry

Rheology describes the deformation of solids, liquids and gasses when they are subjected to external stresses. On the other hand, rheometry determines and quantifies the rheological properties of materials (i.e. rheometry refers to the experimental techniques of rheology) [4, p. 11][5, p. 112]. Respectively, the instruments used to measure rheological properties are known as rheometers. They can either apply deformation to the material investigated measuring the subsequent force produced, or they can apply a force mode to the material measuring the deformation generated [5, p. 112-113]. Thus, viscoelastic properties of materials (e.g. solids, semi-solids, fluids) can be determined with rheometers. Separated from this definition are instruments referred to as viscometers, which are limited in their use. These devices can only be used to measure the viscous flow behavior of fluids [4, p. 13]. Although basic rheological phenomena are discussed in the upcoming chapters, it is advisable that a reader unfamiliar with the basics of rheology would refer to these excellent publications [4][5][6].

2.1.1 Shear stress and shear rate

In order to measure viscosity and understand the basics of rheology one must first define the parameters which are involved in the flow of solids, liquids and gasses. These parameters are shear stress and shear rate. Shear stress (τ) indicates a force (F) applied tangentially to an area. When sufficient shear stress is applied to a liquid, the liquid will begin to flow. The internal resistance (i.e. viscosity) of the liquid will dictate the velocity of the flow that can be maintained with a given force. [4, p. 15-16.] On the other hand, shear rate ($\dot{\gamma}$) simply defines the rate at which the shearing force is applied. For example, in a parallel-plate rheometer design (see Chapter 2.2.1) τ causes the liquid between two parallel-plates to flow as the upper plate is rotated while the lower plate is stationary. The liquid then flows with a maximum flow speed at the upper plate. When approaching the stationary plate, the flow speed of the fluid gradually drops. Thus, the fluid flows with a minimum speed ($v_{min} = 0$) at the boundary of the bottom stationary plate. This drop in speed is used to calculate the applied shear rate during the measurement [4, p. 15-16].

Now we are able to define viscosity (η) as the ratio of shear stress and shear rate ($\eta = \tau / \dot{\gamma}$). In general, viscosity expresses a fluids resistance to deformation under stress (e.g. shear stress). Furthermore when the viscosity of a liquid is constant for any values of $\dot{\gamma}$ it may be referred to as a Newtonian liquid. However in practice, this is rarely the case. A majority of liquids are Non-Newtonian liquids, meaning their viscosity is dependent on shear rate. [6, p. 62.]

Non-Newtonian behavior is commonly sub-categorized into different behavioral models. These classifications are not investigated in this study with the exception of pseudoplastic (i.e. shear-thinning) liquids. The term shear-thinning is used to describe non-Newtonian fluids which viscosity decreases as a function of shear rate. [4, p. 21-24.] The characteristic flow and viscosity curve charts for pseudoplastic liquids are shown in Figure 2.1.

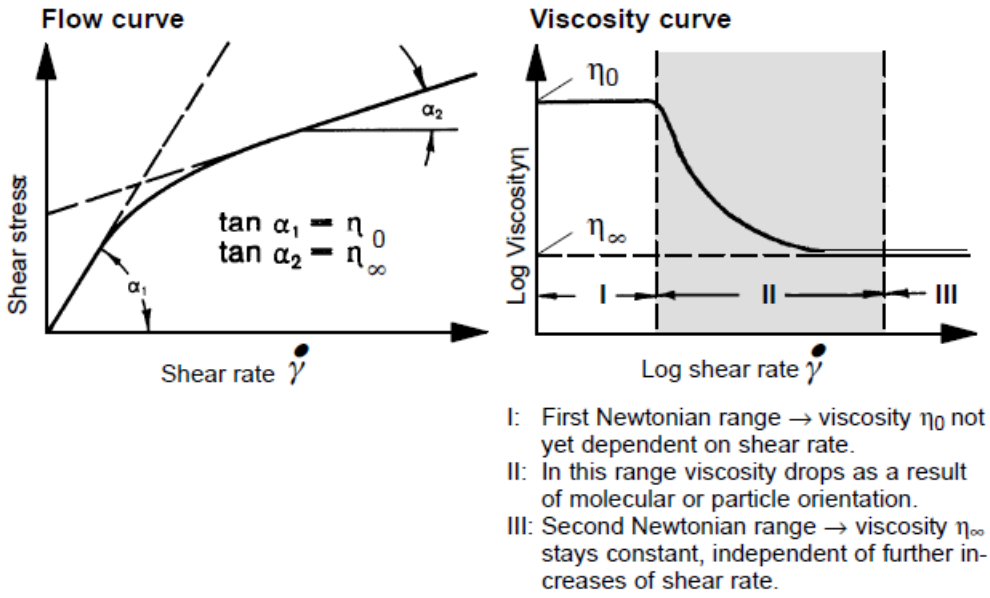

Figure 2.1. *The shear rate dependence of pseudoplastic liquids. The flow curve plots shear stress as a function of shear rate. The viscosity curve on the other hand plots viscosity as function of shear rate. [4, p. 24.]*

In conclusion, shear stress causes a rate of strain in liquids but a strain in solids. Meaning that liquids flow and solids undergo elastic deformation. Nevertheless in reality all flow and deformation of real bodies (solids, liquids, and gasses) contain some elements of both flow and elasticity [7, p. 507]. This is the concept behind viscoelasticity which is up for discussion next.

2.1.2 Viscoelasticity

When describing the rheological behavior of a majority of actual materials the most relevant term to be used would be viscoelasticity. Even liquids considered as Newtonian liquids (e.g. water) present viscoelastic effects (elastic potential) under special measurement conditions, such as very high frequencies [6, p. 245]. This aspect is also true for Non-Newtonian fluids. For instance, the transformation of a sol-gel derived silica hydrogel from a “runny” suspension into a “thick” gel can only be observed at low frequencies (i.e. slow deformation) with minor deformation. On the other hand, even solid objects (e.g. glass windows) can appear to flow, but require years of observation.

In essence, viscoelasticity is a combination of properties characteristic for liquids (dissipative viscous loss) and solids (storage of elastic energy). Thus, the general definition of a viscoelastic material holds two main components: the potential to store elastic energy and the intensity to dissipate energy. [6, p. 245-248.] Correspondingly, these components are referred to as the Young's storage modulus (E′) and the Young's loss modulus (E′′), whereas their shear counter parts are known as the shear storage modulus (G′) and the shear loss modulus (G′′) [7, p. 362-363].

Then the shear moduli values are used in calculating a variable referred to as the loss tangent (tan δ), which is the ratio of G′′ to G′ (tan δ = G′′/G′) [7, p. 363]. The loss tangent can be used to determine whether a material behaves more like a solid, G′> G′′ (tan δ < 1), or more like a liquid, G′ < G′′ (tan δ > 1). Thus the loss tangent can also be used to evaluate a materials gel-point. [6 p. 257.] For instance, silica gels derived via sol-gel processing (see Chapter 2.3.3) elicit a gel-point. In order to better understand what this gel-point is exactly, the terms sol and gel should be defined first.

The term sol referrers to a colloidal suspension of small particles dispersed in liquid. For example, a silica sol is homogenous mixture with at least one continuous liquid phase (water, ethanol, and residuals of silica precursors) and at least one dispersed solid phase (colloidal and/or aggregated silica particles) within the liquid phase. In contrast, a silica gel is a homogenous mixture of at least one continuous solid phase (silica as such and/or partially or fully hydrolyzed) and at least one dispersed liquid phase (water, ethanol and residuals of silica precursors) trapped within the solid phase. This means that a gel has dominant elastic properties over its liquid properties, whereas a sol has dominant liquid properties. Consequently, a gel-point is the point in time when a sol turns into a viscoelastic gel with dominant elastic properties. This point in time, if it exists, can be observed by rheological measurements under small angle oscillatory shear. [8, p. 4-5.]

2.2 Rheometers and measurements

Rheometers are instruments used to measure viscoelastic properties of solids, semi-solids and fluids. Furthermore, rheometers are important devices in the rheological characterization of non-Newtonian liquids and can be roughly classified into two different types: rotational and extensional rheometers. The difference being, that rotational rheometers either apply controlled shear stress or strain, and extensional rheometers either apply controlled extensional stress or strain. [4, p. 36-81][9, p. 75-106.] The Theoretical section of this study will focus on rotational rheometers owning a parallel-plate measuring geometry. Various measuring geometries (sensor systems) for rotational rheometers are defined and discussed in the next chapter.

2.2.1 Rotational rheometers

Rotational rheometers are generally categorized in the following way: controlled stress (CS-rheometers) and controlled rate (CR-rheometers) rheometers. However some modern rheometers are able to operate in both test modes. Basically, CS-rheometers input a controlled shear stress and determine the resulting shear rate, whereas CR-rheometers do the exact opposite. The similarity between these two types of rheometers is that both devices are usually supplied with the same array of sensor systems: the coaxial cylinder, cone-and-plate and parallel-plate systems. In addition to the two rheometer categories (CS- and CR-rheometers) there are two subcategories for these rotational rheometers: Searle- and Couette-measuring system. [4, p. 36.] These systems are illustrated and their differences explained in Figure 2.2.

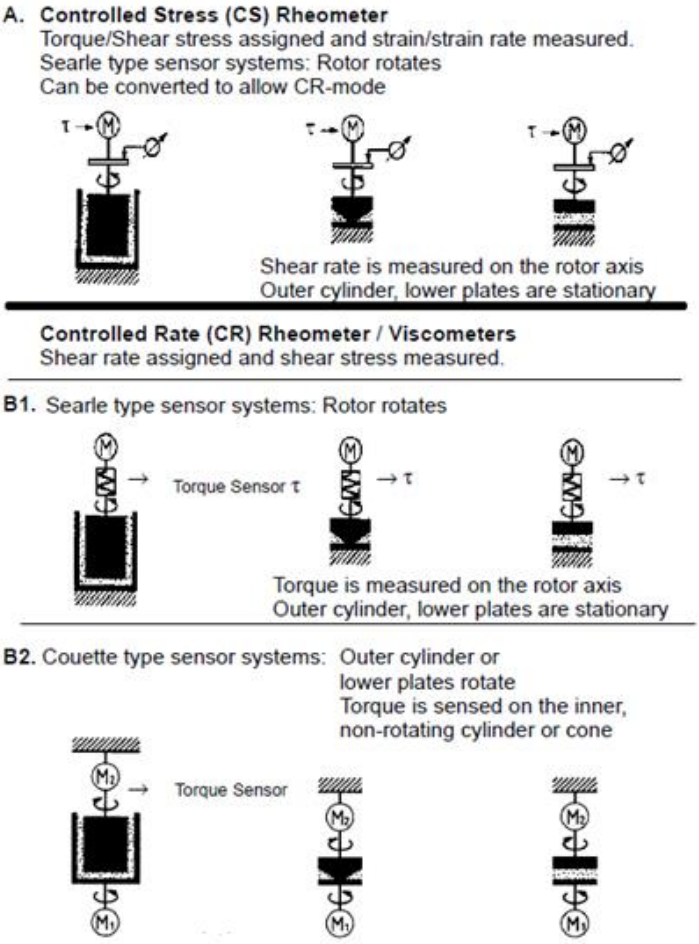

Figure 2.2 *Different types of rotational rheometers. The letter "M", in Figure 2.2, stands for a motor which rotates and drives the systems and for which specific torque values can be set. The spring (B1) acts as a torque detector between the drive motor and shaft. Picture edited from source. [4, p. 37.]*

In general rotational rheometers measure the torque needed to rotate an object in a liquid (e.g. cylinder) or on the liquid surface (e.g. plate), at a certain speed. In a Searle system

the shear rate (case A) or the torque (case B1) is measured on the rotor axis with the drive motor "M". In contrast, in a Couette system the outer drive motor "M_1" rotates at a predefined speed, which causes the liquid in the annular gap to flow. This in turn generates torque on the inner object (e.g. cylinder, cone, plate). Consequently, the generated torque would prompt the inner object to also rotate, but instead a counteracting torque is supplied by a secondary motor "M_2" – keeping the inner object from rotating. Thus, the Couette system measures the counteracting torque required to keep the inner object still. Meaning that the drive motor operates at the outer object (i.e. cup) while the viscosity related torque is measured on the shaft of the inner object (i.e. bob). In conclusion, a Searle system design has the drive motor and the torque detector on the same rotor axis, but a Couette system has these two elements separately. This is the distinctive difference between the Searle and Couette system designs. [4, p. 37-40.]

2.2.2 Viscosity measurements

Instead of leading CR or CS mode experiments with a rotational rheometer, one can have the choice of ramping or stepwise controlling the changing parameter (shear stress or rate). For example, in a CS/CR ramping -procedure either a preset shear stress or shear rate is gradually increased or decreased and the resulting shear stress/shear rate is measured. Thus, there are four different methods to form a flow or viscosity curve (see Figure 2.1) for a sample. [10, p. 54-60.] From the plotted curves, the samples Newtonian or non-Newtonian flow characteristics can be determined.

2.2.3 Oscillatory measurements

Oscillation testing is intended to be a non-destructive test method. By utilizing this type of testing, one is able to separate the elastic and viscous properties of a material (e.g. gels). During oscillation testing a sample material is exposed to a sinusoidal stress defined by an amplitude of stress (τ_0) and frequency of the applied deformation (ω). In other words, when executing an oscillatory test with a rotational rheometer the rotor no longer turns continuously in one direction as it did with viscosity measurements. This time the rotor is made to pivot with a small angle (φ) to the left and to the right. Subsequently, when a shear stress (τ) is applied to a sample it will undergo deformation (γ). Depending on the relation of viscous and elastic properties of the sample a phase shift δ may present between τ and γ. This means that the amplitude of deformation (γ_0) is not necessarily reached at the same time as τ_0. However, for pure elastic materials the phase shift is 0° and purely viscous materials present a phase shift of 90°. Accordingly, viscoelastic materials elicit a phase shift between 0° and 90°. [10, p. 80.] As a side note, in literature handling rheology, it is most common to model viscoelastic behavior as linear combinations of springs (elastic element) and dashpots (viscous element) in order to forecast the response of a material under various loading conditions. These spring and

dashpot models, which include the Maxwell and Kelvin-Voight model, are ruled out of inspection.

When conducting oscillation tests there are several types of different oscillation procedures (i.e. tests) one can choose to characterize their sample materials. Nevertheless, a popular and relevant method to study the viscoelastic behavior of gels is to conduct frequency sweep –procedure. During this procedure, the sample is exposed to an array of different frequencies under a controlled stress or deformation value. The sample must remain unchanged throughout this test procedure. Therefore, the applied stress or deformation value must remain in the sample materials linear viscoelastic range. This range can be determined by an oscillation stress sweep –test (i.e. amplitude sweep –procedure) in which the sample is subjected to gradually rising stress amplitudes. As the stress amplitude is increased, the sample will eventually yield. After this yield point, the sample no longer functions in the linear viscoelastic range in which the stress and strain amplitude have a linear relationship. Practically speaking the linear viscoelastic range is a region where material functions are independent of stress or strain values. [10, p. 80-94.]

2.3 Biomedical biomaterials

Defining the word “biomaterial” has proven to be somewhat problematic throughout history, since the field of biomaterials science has been constantly progressing and biomaterial applications have become more complex. Therefore, the definitions may vary to a certain degree, according to different sources.

The European Society for Biomaterials (ESB) defines biomaterials in the following way: “material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ or function of the body.” [11.] On the other hand, the International Union of Pure and Applied Chemistry (IUPAC) defines a biomaterial as: “material exploited in contact of living tissues, organisms or micro-organisms.” [12.] This word definition has been a subject of debate between the biomedical and environmental fields, because certain extensions to the definition (e.g. material of natural origin) cannot be extended to non-viable materials used in medical devices [12]. Nevertheless, biomaterials can be metals, ceramics, polymers and composites, and can additionally be classified into four groups: inert, natural, bioactive and biodegradable [13, p. 84]. Out of these four groups, only biodegradable biomaterials are discussed in detail. More specifically, sol-gel derived biodegradable polymeric silica gels. Before discussing polymeric silica, a short introduction into degradation is given first. Understanding various degradation mechanisms is of crucial importance when developing degradable biomedical applications, because the degradation process can govern a range of different events such as drug release, host response, material function and tissue regeneration [15, p. 179].

2.3.1 Biodegradation and bioerosion

The term biodegradation is often used to signify degradation occurring in a biological environment driven by a specific biological reaction/process [14, p. 179]. Degradation on the other hand refers to a chemical process occurring in polymeric materials, resulting in the cleavage of covalent bonds. Therefore, changing the materials initial chemical structure. In contrast, the gradual breakdown of bioceramics is often discussed and characterized by terms, such as: absorption and resorption. Absorption indicates how materials are gradually drawn into a system, whereas resorption characterizes how a material is gradually broken down into smaller components which are then assimilated into the governing system [11]. Evidently, when these phenomena occur in biological conditions the terms absorption and resorption may be referred to as bioabsorption and bioresorption. In general, resorption and bioresorption are used to emphasize that the components taken in by a system are taken in again. For example, as bone tissue undergoes resorption the bone is broken down to its mineral components, which are then assimilated by the tissue to form new bone (continuous circulation).

Consequently, the gradual breakdown of materials lead to physical property changes in them, such as mass loss and shape distortion. These physical changes in size, shape and/or mass are referred to as erosion, which can occur via degradation, dissolution, absorption, resorption, ablation and/or mechanical wear. Thus, as with erosion, the occurrence of bioerosion can also be governed by physical and/or chemical processes. However, the distinctive difference between erosion and bioerosion is that bioerosion occurs under biological conditions as opposed to being triggered by abiotic factors (e.g. heat, radiation, mechanical stress). [15, p. 179.]

Both erosion and bioerosion can be categorized into two separate modes: surface and bulk erosion. Surface erosion is limited to the surface of a device exposed to a reaction medium. Meaning, that the erosion process begins at the exposed surface and proceeds into the inner core of the device layer by layer. In contrast, during bulk erosion the whole device erodes homogenously throughout the entire matrix, because the reaction medium is able to penetrate into the bulk of the device. [16, p. 88-89.] Typically in bulk erosion the device will crumble into smaller pieces as cracks and crevices are formed throughout the device. This in turn, lets in exceeding amounts of reaction medium into the device leading to an increasing decomposition rate. This aspect is illustrated in Figure 2.3, which also depicts how the surface erosion pattern proceeds.

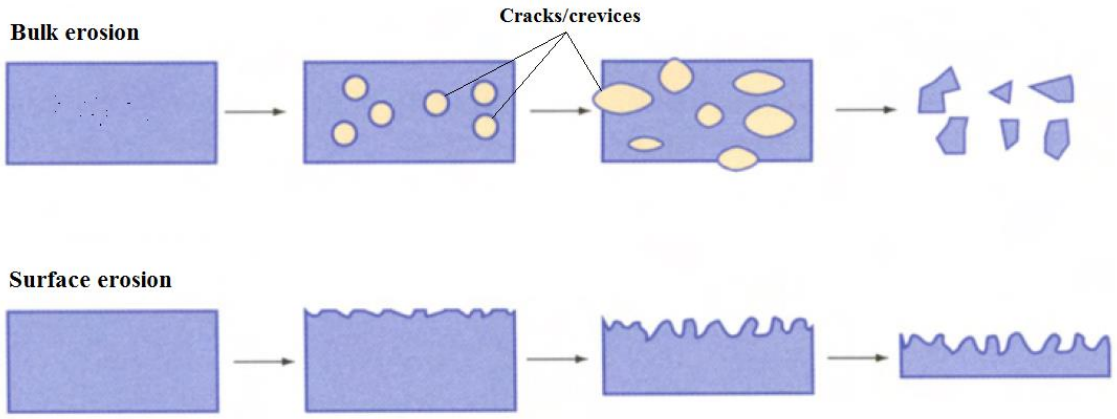

Figure 2.3. *The differences between bulk and surface erosion at arbitrary time periods. During the surface erosion process the material erodes layer by layer, whereas in bulk erosion the material erodes homogeneously as a reaction medium is taken in by the material, eventually crumbling it. Edited from source. [16, p. 89.]*

When making a distinction between degradation, biodegradation, absorption, bioabsorption, erosion and bioerosion it is adamant to understand that the prefix “bio” is not well established and can lead to some confusion in terms of terminology [15, p. 180]. Thus, biodegradation and bioabsorption may be used to simply emphasize that the structure of a material degrades in a biological entity or system. Disregarding whether or not the degradation process is meditated by biological, chemical and/or physical factors. Consequently, bioerosion can be understood as a process meditated by dissolution, degradation, ablation and/or mechanical wear in physiological and biological conditions. Bioerosion will eventually result in physical property changes of a device and in the loss of its function. [17]. Defining biodegradation and bioerosion in this manner (only a biological surrounding is needed) is crucial when speaking about silica, because silica degrades via chemical rather than biological processes in a biological environment. For example, when silica is introduced into the human body it will dissolve into the water phase of the extra cellular matrix (ECM) and is ultimately excreted out as silicic acid. Regarding silica microparticles, their bioerosion and biodegradation is discussed further in the Drug delivery technology chapter, because these mechanisms control the rate of drug released from within the microparticles.

2.3.2 Sol-gel processing of silica gels

Silica gels can be classified as polymer-silica nanocomposites (i.e. polymeric silica) which belong to a class of materials referred to as organic/inorganic nanocomposites. Unlike conventional composites, which have macroscale domain sizes, polymeric silica nanocomposites are generally nanoscopic. [18, p. 1.] Although, different methods of producing these polymeric silica nanocomposites exist, only the sol-gel approach is discussed here.

Sol-gel derived silica gels are prepared from monomeric tetrafunctional alkoxide precursors. The most common precursors used in silica gel synthesis are tetraethoxysilane ($Si(OC_2H_5)_4$) abbreviated as TEOS and tetramethoxysilane ($Si(OCH_3)_4$) abbreviated as TMOS. These monomeric precursors in an aqueous solution are commonly hydrolyzed by employing a mineral acid (e.g. HCl) or a base (e.g. NaOH) as a catalyst. Since water and alkoxysilanes are immiscible, a reciprocal solvent (e.g. ethanol) is often used to homogenize the mixture. [19, p. 108-112.] However, solvents or homogenizing agents are not necessarily required when preparing certain materials. For example, when preparing silica gels from TEOS, a solvent-free method can be utilized. This is possible due to the fact that alcohol is produced, as a by-product, when TEOS is hydrolyzed. The produced alcohol in itself is sufficient enough to homogenize the initially biphasic system allowing the system to polymerize [20].

The polymerization process of aqueous silica is driven by hydrolysis, which replaces alkoxide groups with hydroxyl groups (OH), and condensation reactions, which produce siloxane bonds (Si-O-Si). Congruently, the by-products of these reactions are alcohol and water. The hydrolysis and condensation reactions are subsequent processes, but under most conditions condensation begins before the hydrolysis is complete. [19, p. 108-112.]

The polymerization process can be described to progress in three stages. The first stage being the polymerization of the monomer to form particles, which then grow in size. In the end, as the particle grow and aggregate, they link into chains which extend throughout the liquid medium (i.e. thickening it to a gel) [21]. Furthermore the growth and aggregation behavior of the particles are greatly affected by pH and/or by the presence of flocculating salts. This dependence is illustrated in Figure 2.4.

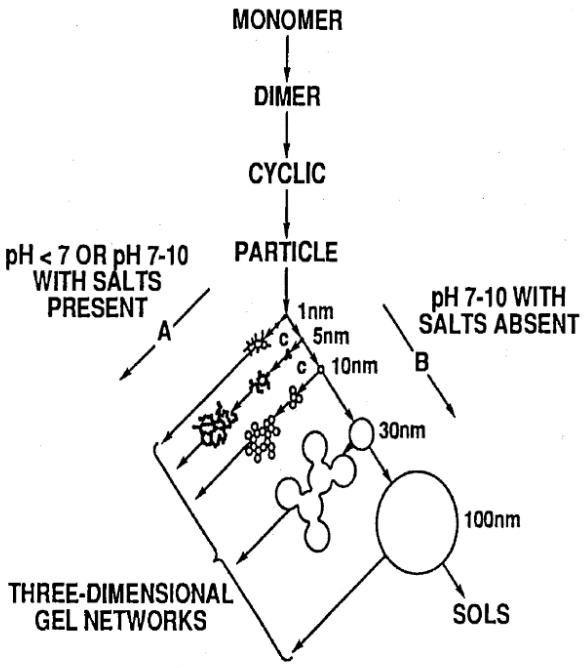

Figure 2.4. *In acidic solutions or in the presence of salts (A) particles aggregate to three-dimensional networks and form gels. In basic solutions (B) particles grow in size simultaneously decreasing in number [21, p. 174]. Considering this study, the sol-gel reactions were led via route A. Choosing route B over A would have most likely resulted in an unstable final product or the desired three-dimensional gel structure might have not formed in the first place.*

The pH-dependence of the polymerization process is of great significance in the successful manufacturing of silica gels. According to Ilier [21, p. 213-222], the polymerization process can be divided into three pH domains: the first domain being < pH 4, the second domain pH 4-7 and the final domain > pH 7. The first domain is characterized by the point of zero charge (PZC), where the surface energy is zero, and the isoelectric point (IEP), where the electrical mobility of the silica particles is zero. Both of these points are found in the range of pH 1-3. Therefore pH 4 performs as a boundary point. The other boundary point is at pH 7. Above this pH silica particles are ionized, which mainly leads to particle growth without sufficient aggregation or gelation from occurring within the sol. [19, p. 103][22, p. 775.] These domains are depicted in Figure 2.5, in which sol stability is plotted as a function of pH.

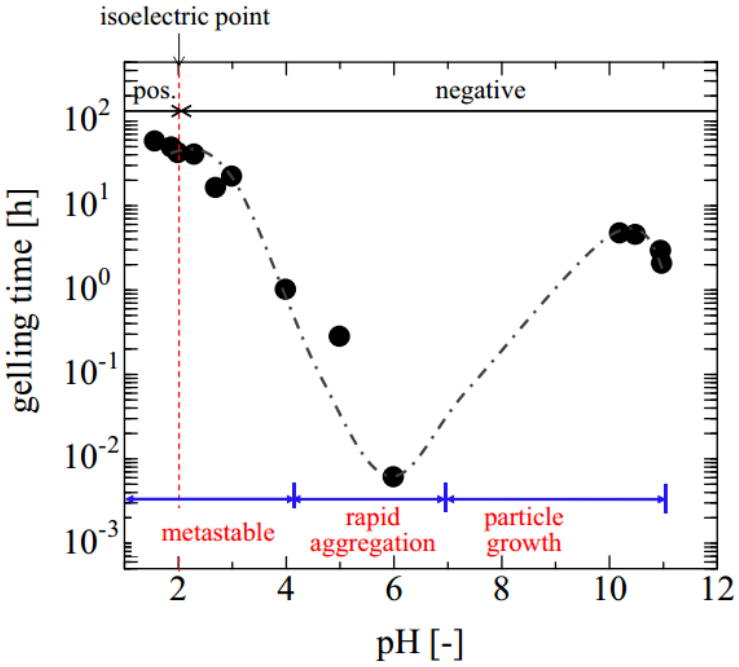

Figure 2.5. *The pH-dependence of colloidal silica-water systems. Three different pH domains can be distinguished: metastable, rapid aggregation and particle growth without aggregation or gelation [22, p. 775].*

The metastable domain around pH 2, illustrated in Figure 2.5, clearly depicts a peak value in sol stability and an increase in gelation time. In this domain hydrolysis reactions are dominant over condensation reactions. This is due to the fact that under acidic conditions an alkoxide group is protonated decreasing the electron density of silicon. This results in a more electrophilic silicon, which in turn makes it more susceptible to hydrolysis. In addition to hydrolyzing monomers, the reaction produces alcohol as a by-product, which serves as a homogenizing agent. [19, p. 131.]

Acid-catalyzed hydrolysis is reported to be more effective compared to a base-catalyzed technique. Moreover an increased R value, which indicates the mole amount of water to TEOS, has been shown to promote hydrolysis resulting in a more complete hydrolysis of monomers. Although high values of R cause immiscibility in the biphasic mixture, alcohol produced by the hydrolysis reaction and the partial hydrolysis of the TEOS precursor lead to homogenization. [19, p. 127.] An R value of 400 is considered to be an extremely high value, since sols at this point are at the brink of not being able to form a cross-linked system through-out the aqueous medium (i.e. unable to form a gel). One reason behind this inability is that, besides promoting hydrolysis, high values of R also promote siloxane bond hydrolysis. This reaction is the reverse reaction of the polymer condensation and is promoted by large R values [19, p. 127].

Condensation reactions on the other hand are promoted and accelerated near neutral pH (see Figure 2.5). The gel time (t_{gel}) of sol-gel systems is often used to measure overall all

condensation kinetics, so that the average condensation rate is expressed as inversely proportional to the gel time (average condensation rate ≈ 1 / t_{gel}) [19, p. 140]. Consequently, the minimum condensation rate can be observed near the isoelectric point and maximum rate near pH 6 (see Figure 2.5). It is evident that by manipulating the pH of silica sols one can control the rate of hydrolysis and condensation reactions occurring in the system by accelerating or de-accelerating the other.

2.3.3 Applications of silica gels

The use of sol-gel derived silica materials have already been incorporated in various commercial applications in many different fields of technology and research such as construction, tissue engineering, drug delivery and coating technologies. This widespread attention in many areas is a result of the fact that porous silica matrices are easily tailored into several different morphologies. Ranging from spherical nano- and microparticles to monolithic devices with various shapes and sizes. Additionally sol-gel derived silica owns a unique set of physio-chemical properties which have led it be incorporated in the manufacturing of construction materials.

Silica nano- and microparticles have been used to mechanically reinforce cement and to enhance thermal, abrasion and surface hardness properties of composite coatings. [23, p. 31-34.] In contrast different biomedical fields such as controlled drug delivery and tissue engineering has benefited from the fact that amorphous sol-gel processed silica is both compatible and degradable in living tissue [17, p. 382]. For example, silica nano- and microparticles have been incorporated into commercial bioactive glasses in order promote their bioactivity. Silica has enhanced the adhesion properties of these biomedical ceramic implants and stimulated cell proliferation as well as migration of cells into the implant. This has helped in preventing marginal gap formation at the implant-tissue interface [24][25]. Nevertheless, the most interesting field, regarding this study, is the field of controlled drug delivery. Specifically, the application of sol-gel derived amorphous silica microparticles, which provide the possibility to encapsulate different biologically active agents [17][26].

2.4 Drug delivery technology

The main objective of the interdisciplinary field of drug delivery is the accurate administration of bioactive agents, commonly referred to as drugs, to achieve a sought after clinical response. Achieving this objective is known to be somewhat problematic, but recent advances have led to the development of sophisticated drug delivery technologies, such as controlled release, sustained release and targeted delivery systems.

Modern drug delivery technologies can offer the advantages of reducing drug dose frequencies, provide a more constant drug effect over time, reduce drug side effects, maintain therapeutically effective drug concentrations in plasma and offer the capability

of being selectively active in specific areas in the body (e.g. cancer tissue). [27][28.] The terms targeted, controlled release and sustained release systems are defined as follows. Sustained release systems prolong the duration of drug effects by slowing down the release of the drug. On the contrary, a controlled release dosage form does not merely delay the release rate, but delivers the drug at a specific rate for a preset time. Moreover, targeted delivery systems are often understood to be a part of controlled release systems, because they often provide a site-specific drug delivery. In addition to attaining temporal control. [27.] This focus of this study is on controlled release systems, more precisely on the dissolution controlled systems (see Chapter 2.4.2). Furthermore, the use of spray-dried silica microparticles in drug delivery is discussed. Ruling other drug delivery systems out of inspection.

2.4.1 Routes of drug administration

Every drug molecule, upon administration, warrants a delivery system to carry the drug to the site of action in a patient. Therefore, various routes of drug administration and dosage form types exist. Conventional dosage forms, up until the 1970s, essentially comprised of injectable solutions, oral formulations and topical creams and ointments. Since then many other dosage forms have been invented for many different routes of administration [29, p. 1-3]. These routes can be categorized into invasive and non-invasive routes. The distinctive difference being that non-invasive routes utilize the natural orifices of a patient (e.g. rectum). However, invasive methods rely on entering the body by punctures or incisions. Thus, non-invasive methods include oral, topical (e.g. skin), transmucosal (nasal, buccal, vaginal, ocular and rectal) and inhalation routes, while invasive methods include parenteral routes, such as subcutaneous, intravenous, intramuscular and intraocular [27].

When designing a parenteral route for a delivery system, many different parameters must be taken into account. Nonetheless, one major factor exists: needle gauge from which the product can be injected through. The size of the needle often determines the final site in which the product can be appropriately injected to. For example intraocular administration methods tend to require very small needle sizes (e.g. 30G), whereas subcutaneous injections are commonly performed with larger needles (e.g. 23-26G). Although fine needles reduce the pain of injection thus increasing patient comfort, they also require an increased force upon the injection of the drug. [30][31.] While patient convenience and compliance plays a vital role in developing a successful drug delivery technology, maximum patient comfort cannot always be achieved, since pain is a subjective experience and certain drug characteristics (e.g. large molecule size) prevent the use of fine needles, such as 27G-30G.

2.4.2 Controlled drug release systems and kinetics

In principal, controlled drug release systems deliver a preset drug dose, either systematically or locally, at a predetermined rate for a predetermined time period [32, p. 56]. Drug release can be understood as the process in which a drug molecule migrates from its initial position inside the drug delivery device to the outer surface of said device and then to the release medium (e.g. blood). This unseeingly difficult process is affected by a multitude of different intricate factors and their reciprocal interactions. These factors include, but are not limited to, device geometry, the release environment and solubility of the drug compound. [33, p. 2.] Despite of this complexity, the mechanism by which drug release is controlled can be categorized into five different groups: diffusion-, dissolution-, osmosis-, mechanical-, and bioresponsive controlled release mechanisms [32]. As with any classification method, grouping the different controlled drug release mechanism can be quite arbitrary. In this case even more so, since other release mechanisms can be considered to be the subsequent result of another mechanism. For example, swelling is sometimes classified as its own main category, but it can occur as the succeeding result of another reaction (e.g. a bioresponsive ligand-substrate interaction) [32, p. 60]. Also, swelling is mainly characteristic to just polymer-based drug delivery devices [33, p. 2]. Thus, Table 2.1 is merely one representation of how controlled drug release mechanisms can be classified.

The focus of this chapter is on the dissolution release mechanism and other mechanisms are ruled out of inspection. The reason being, that the whole study focuses on amorphous silica microparticles, which are dissolution-controlled matrix devices in which minimal drug diffusion occurs [26]. Provided that the active agent is successfully encapsulated upon manufacturing the particles.

Table 2.1. *The table represents one possible way of categorizing different controlled release rate drug delivery systems. Edited from source. [32.]*

Release mechanism	Subcategory
1) Diffusion-controlled	a) Reservoir devices
	b) Matrix devices
2) Dissolution-controlled	a) Reservoir devices
	b) Matrix devices
3) Mechanical-controlled	a) Zero-order controlled drug release
	b) Intermittent drug release
4) Bioresponsive-controlled	a) Biodegradation
	b) Biotransformation
5) Osmosis-controlled	

Dissolution-controlled devices can be divided in into two groups: reservoir and matrix devices. Reservoir devices retain the drug behind a membrane, whereas in matrix devices the drug is dispersed within a continuous matrix. Their common denominator is that both devices (i.e. dissolution-controlled devices) must be water soluble and/or degradable in water. [32, p. 58.] The structural differences of these two devices are schematically illustrated in Figure 2.6.

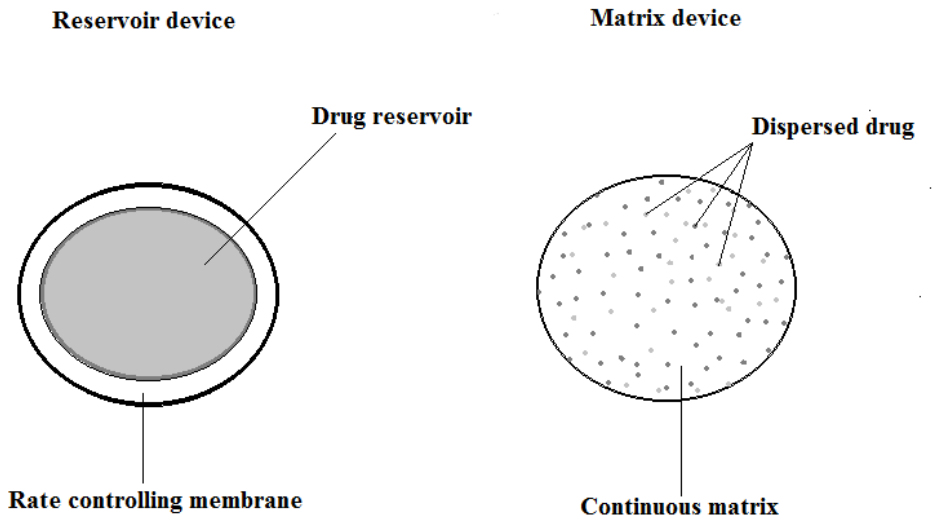

Figure 2.6. *Schematic illustration of a diffusion-controlled reservoir (left-hand side) and a diffusion-controlled matrix (right-hand side) systems. Edited from source. [32, p. 57.]*

The membrane, commonly a polymer, of a reservoir device is designed to dissolve after a certain time period thereby releasing the drug. The drug can also be released through the membrane by the drug first dissolving into it. However, in a matrix device the drug is released solely by the gradual dissolution or degradation of the device matrix. For a reservoir device the drug release can be controlled by modifying the thickness and/or dissolution rate of the membrane surrounding the drug core. More intricate reservoir devices incorporate different membrane materials and/or varying coating thicknesses in order to hasten and/or delay the drug release rate at certain time periods. Similarly, for matrix devices the release rate can be adjusted by the dissolution rate of the matrix. Another way to adjust the release rate is to design a non-linear drug concentration profile in the matrix, which is necessary for monolithic devices where the drug released decreases as the matrix decreases in size. [32, p. 59.]

Often, the drug release mechanisms overlap one another. Meaning that at a given time more than one mechanism may be involved in delivering the drug and different mechanism may be dominant during the different stages of the drug delivery process [2, p. 19]. An array of different tests can be conducted in order to determine which mechanisms overlap or are dominant, but one underlining factor must be determined and characterized: a drug release profile. This profile is commonly presented either as the released drug concentration in plasma or the cumulative release-% of a drug as a function of time [28]. Generally, these release profiles are modeled by various mathematical models (e.g. Fick's law, zero-order and first-order release,) with the purpose of simplifying complex release mechanisms and to comprehend the release process of a specific system. Therefore, a mathematical model concentrates on one or two dominant driving forces and are often inadequate to describe intricate multicomponent material

systems (e.g. stimuli-responsive systems). [33, p. 2.] Figure 2.7 depicts two different release profiles (zero-order and immediate) and illustrates how a therapeutically effective controlled release system would ideally deliver a drug compared to an immediate release drug delivery device (e.g. conventional orally administered drug capsules).

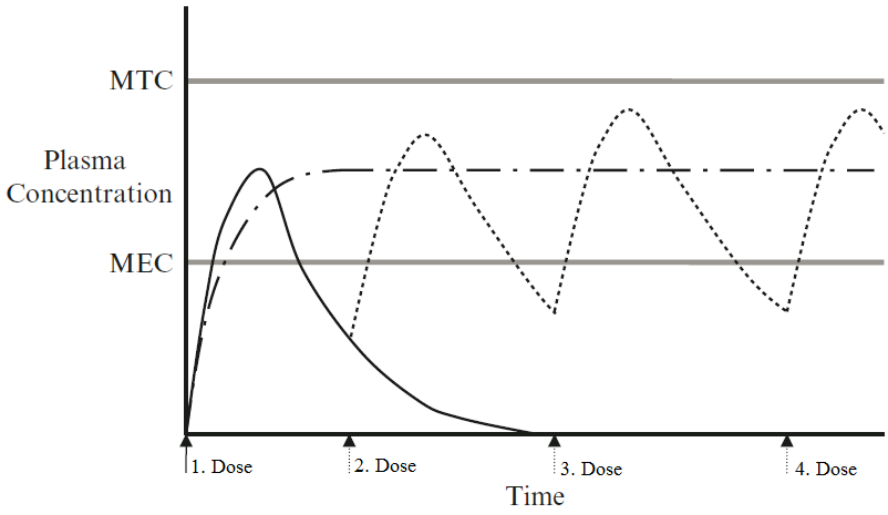

Figure 2.7. *Two different drug release profiles represented as drug concentration in plasma and plotted as a function of time. The two profiles are defined as: zero-order release (dot-dash curve) and immediate release (solid curve followed by a dotted curve). MTC stands for minimum toxic concentration and MEC for minimum effective concentration. The ideal therapeutic effect is achieved below the MTC and above the MEC, referred to as the therapeutic window. Multiple doses (dotted arrows) at regular intervals are required with immediate release drugs, resulting in a rapid rise and fall in drug concentrations whereas a zero-order release system will lead to constant concentration in plasma, after an initial rise, with a single dose (solid arrow). Edited from source. [2, p. 21.]*

In most cases, when designing a controlled release device, a zero-order release profile is desired. The reason being, that drug concentration fluctuations in bodily fluids can be minimized (see Figure 2.7). In other words, with zero-order release systems the delivery rate does not vary with time. Ideally the drug is released at a constant rate, subsequently maintaining an effective drug concentration in the body for a prolonged period of time. [29, p. 29-30.] This type of release is advantageous in treating many disease types, which makes it a desirable and sought after design.

2.4.3 Silica microparticles and controlled drug delivery

Extensive advances have been made in the past decades to enable more effective administration of drugs. Therefore a range different organic and inorganic drug delivery

systems have surfaced, some of which are illustrated in Figure 2.8. Still, the focus of this chapter is on silica particles.

Drug delivery system	Structure	Chemical properties
Liposomes		Consists of hydrophobic tail and hydrophilic head group Forms closed vesicles with an aqueous core Internal aqueous domain between the lipid bilayers Encapsulation of drugs occurs either in the aqueous space or intercalated into the bilayer
Dendrimers		Hyper branched and globular macromolecules Well defined core, backbone and multivalent periphery By hydrophobic and electrostatic interactions, incorporate biomolecules Convergent – endo-receptor Divergent – exo-receptor
Carbon nanotubes		Rolling up a single layer of grapheme sheet – single walled Rolling up many layers to form concentric cylinders – multi-walled
Gold nanoparticles		Gold nanoparticle serves as core Photosensitive
Iron oxide nanoparticles		Superparamagnetic particles Need trigger to release biomolecules, for example, laser irradiation
Titanium dioxide nanoparticles		Self-ordered Nano-tubular structure Photodynamic therapy
Silica nanoparticles		Mesoporous structure Honeycomb-like structure Active surface

Figure 2.8. *Different types of drug delivery systems and a short description of some their chemical properties. Edited from source. [34, p. 3.]*

Organic systems such as micelles, liposomes and polymeric nanoparticles tend to be limited in their use due to owning poor thermal and/or chemical stability. In addition, these materials are often rapidly eliminated by the immune system. Although, every system has its own advantages and disadvantages, silica particles can offer a biocompatible and stable alternative to organic controlled drug delivery -systems. Combining a sol-gel polymerization method (see Chapter 2.3.2) with either spray-drying or emulsion chemistry, biologically active agents can be encapsulated within silica particles with ease. [35-39.] Moreover, the sol-gel polymerization method provides the possibility of encapsulating a large variety of different biologicals into silica, such as small molecules, proteins, viruses and even entire cells [17, p. 382]. This is possible, because the sol-gel method is an ambient-temperature polymerization technique – essential when handling biologically active agents [35, p. 1959-1960].

After the sol-gel processing, spray-drying is a favorable method to produce spherical silica microparticles. Spray-drying also allows the processing of heat sensitive biologicals and offers the possibility to straightforwardly adjust various particle properties such as: size, porosity and size distribution. Although spray-drying faces certain challenges, such as low yield and surface segregation, it offers the advantage of being a single-step process. The process can even be readily scaled up and together with the sol-gel method it is an economically beneficial processing method. [26][35][40][41.]

Silica particles have also attracted ample attention as a novel drug-delivery system, because of their certain intrinsic properties: inherent hydrophilicity, non-toxicity (excluding crystalline silica), biodegradability and biocompatibility in living organisms [17, p. 382][35, p. 1960].

Amorphous silica microparticles intended for drug administration, undergo bulk erosion (defined in Chapter 2.3.1) meditated by the dissolution and biodegradation of silica under physiological conditions. Studies have shown that spray-dried silica microparticles are dense enough to prohibit any significant diffusion from occurring even with considerably small molecules (e.g. propranolol). However, trace amounts of drug diffusion is almost always witnessed in the form of an initial drug burst. For example, the drug burst (see Figure 2.9) witnessed with the majority of spray-dried silica microparticles can be explained by the presence of non-encapsulated and partially encapsulated drug molecules. These non-encapsulated drug molecules tend to rapidly dissolve in to the reaction medium. On the other hand, the partially encapsulated molecules tend to swiftly diffuse from the microparticle surface or from the vicinity of said surface. [17][26, p. 223.] In an ideal scenario, every drug molecule is encapsulated in a way that this initial burst effect would be non-existent (i.e. every drug molecule is encapsulated into the vicinity of the inner core of a microparticle).

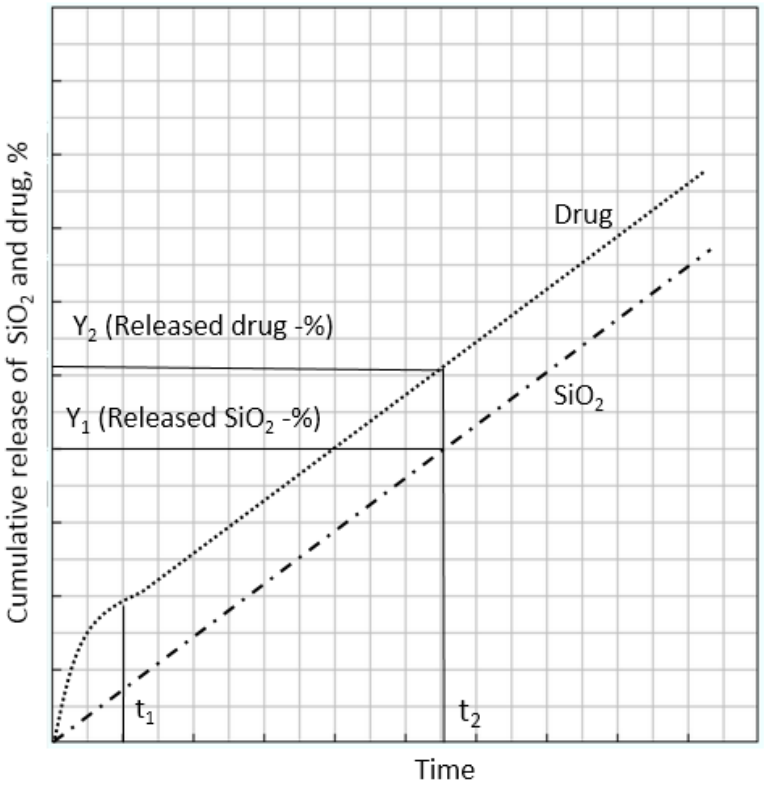

Figure 2.9. *The effect of an initial drug burst ($t \leq t_1$) on the release kinetics of zero-order drug delivery systems. After the burst effect ($t \geq t_1$), the drug release rate changes into a constant release mode.*

Disregarding the initial drug burst effect, the drug release from within the microparticles is controlled by the biodegradation and dissolution of silica. Meaning that by modulating these two factors one can dictate, to some extent, the release profile of the silica-based drug delivery system. Studies have shown that the dissolution of silica can be adjusted relatively effortlessly with the following parameters: water-to-alkoxide ratio, amount of solvent, catalyst concentrations and various process parameters (e.g. aging and drying). In contrast, the degradation process and kinetics of silica microparticles are governed by parameters, such as porosity, chemical structure and the type of the drug molecule. [42][43.]

Despite the fact that the dissolution and biodegradation of the microparticles result in bulk erosion, a zero-order release profile is observed for porous silica microparticle systems [44]. This is interesting, because typically a surface eroding drug delivery system exhibits zero-order kinetics, whereas a bulk eroding systems tend to show a variable release rate. Both of these release profiles are graphically represented as a function of time in Figure 2.10.

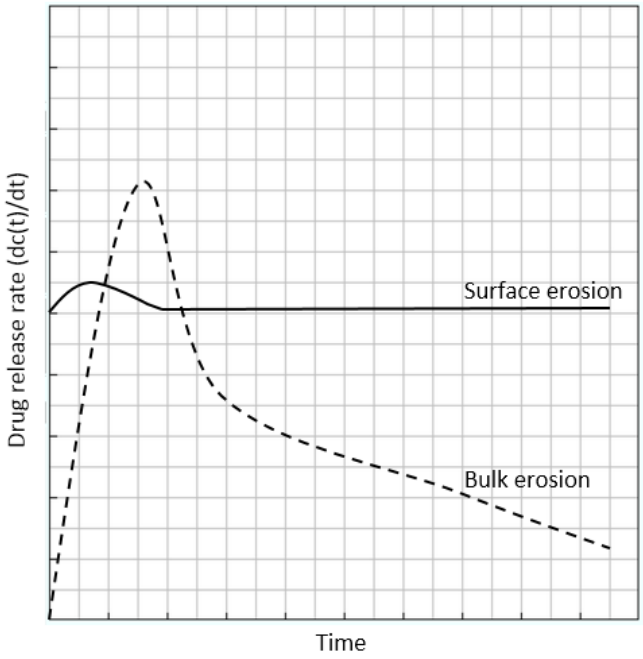

Figure 2.10. *Typical release rates corresponding with surface (solid line) and bulk erosion (dash line). For bulk erosion the release rate depends on the total amount of material and as the material is depleted the release rate decreases. The resulting profile is known as variable release rate. In contrast, for surface eroding materials the release rate is directly proportional to the systems outer surface area.*

The reason why silica-based porous microparticle systems display zero-order kinetics, despite the fact that they undergo bulk erosion, is because the solubility of silica in aqueous solutions is substantially low. For this reason, in some scientific communities

there remains a misconception that silica is insoluble in aqueous conditions. [45, p. 12.] Consequently, as the porous silica microparticle is penetrated by an aqueous solution, the solution inside the microscopic pores quickly reach saturated silica levels. As a result, the dissolution rate of silica inside the concise pores is slow. Successively retarding the drug release. In a best case scenario, this results in a zero-order drug release profile, which is a desired and highly sought-after characteristic for many drug delivery systems. [44.]

3. EXPERIMENTAL SECTION

3.1 Materials

The materials used in this study were a standard array of different laboratory equipment (e.g. flasks and beakers), various reagents used in the sol-gel process and spray dried silica gel microparticles used as a carrier for pharmaceutical agents. Regarding this study, no drug molecules were encapsulated within the microparticles. In other words, all of the microparticles used were placebos. Furthermore, the microparticles had been manufactured before the study began. The silica gels were however synthesized during the study. All of the silica gels prepared can be referred to as hydrogels, since the dispersion medium was methodically water [46, p. 696].

3.1.1 Sol-gel derived silica gels

The prepared silica sols consisted of a 98% reagent grade tetraethoxysilane (TEOS), deionized water, 0.1 M hydrochloric acid (HCl) and 0.1 M sodium hydroxide (NaOH). The TEOS was supplied by Sigma-Aldrich® (USA). The deionized water was produced with a MILLI-Q Academic (USA) water purification system. Both the HCl and NaOH were supplied by Merck Millipore (USA) (reagents were Titripur® grade). The sol-gel process and the molar ratios of these reagents are discussed further on in the Methods chapter.

3.1.2 Silica microparticles

All of the microparticles were manufactured by a Contract Manufacturing Organization in Europe. The microparticle size distribution (PSD) for the batch (14SIT078 BR1A) of microparticles used were: X_{10} = 2.70 µm, X_{50} = 9.98 µm and X_{90} = 18.68 µm. The PSD was measured by laser diffractometry by using the Sympatec/Helos laser diffraction apparatus (Sympatec GmbH, Germany).

In this study the process parameters applied in the spray-drying process and the process itself will not be discussed in great detail. For a more thorough investigation of the process it is suggested that the reader would review these excellent publications [26][47][48][49].

3.1.3 Syringes and needles

The silica-silica composite was filled into two different types of syringes and injected through three different needle gauges. A clear majority of composites were prepared at DelSiTech (Finland) and were filled into 1 ml plastic luer syringes supplied by BD (USA).

A smaller portion of composites investigated in this study were manufactured by a Contract Manufacturing Organization (CMO) in Europe. The CMO filled the composites, they had manufactured, into 1 ml glass Gx RTF® luer-lock syringes supplied by Gerresheimer (Germany). Both of the syringe models could be fitted with separately supplied needles.

The syringes were ultimately fitted with three different needles gauges to study the effect of needle size on the injectability of the composite. The needle gauges used were the following: 23G (0.6 x 30 mm), 27G (0.4 x 20 mm) and 30G (0.3 x 13 mm). The first numeral inside the brackets signify the outside diameter of the needle and the latter denotes the length of the needle. The 23G and 27G needles (Neolus™) were manufactured by Terumo (Japan), whereas the 30G needles (Microlance™) were manufactured by BD (USA)

3.2 Methods

The preparation of the composite basically consisted of two different stages. First the silica sol was prepared by a sol-gel method. Then the silica sol was pipetted into a plastic container holding previously weighed silica microparticles. Then the mixture was stirred for 10 minutes and filled into plastic syringes. The whole process however is described in greater detail in the upcoming chapters.

3.2.1 Sol-gel method

The formulation of the silica sol was kept constant during the whole study. The sol was prepared by first measuring TEOS, deionized water and HCl (0.1 M) at a mole ratio of 1:400:0.01. In order to ensure a homogenous product, 200 ml of the sol was prepared each time. It is noteworthy that in calculating the amount of water needed, one must take into account the amount of water that exists in HCl (0.1 M).

After measuring the reagents, the solution was vigorously stirred (1100 rpm) in a sealed laboratory bottle with a magnet stirrer for 25 minutes at room temperature. This was done in order to achieve two things: firstly to speed up the hydrolysis process and to ensure that the initial solution had time to be entirely hydrolyzed into a sol. After the stirring, the pH of the sol (initially pH < 2) was raised to pH 6.2 (±0.1) with 0.1 M NaOH. This raise allowed for the condensation reactions to accelerate, lowering the gel-time of the sol accordingly. The purpose of this routine is explained in Chapter 2.3.3.

3.2.2 Composite preparation and prepared samples

Once the pH of the silica sol was set to a value of 6.2, the sol was considered to begin aging. Thus, each reported aging time was calculated from this point onwards. The composite was manufactured by mixing together spray-dried silica microparticles and a

silica sol, before the sol had thickened into a gel. Some experiments were however conducted in which microparticles were mixed into a silica gel. This method resulted in a heterogeneous final product owning poor injectability characteristics and the results were ruled out of this study.

The microparticles were weighed into a 50 ml lidded plastic container. After the microparticle weighing, the silica sol (~ pH 6.2) was pipetted into the plastic container holding the microparticles. Utilizing a large pipette tip (5 ml tip) the mixture was stirred by hand for approximately 5 to 10 minutes.

The concentration of the composite, reported as a mass concentration (g/ml), ranged from 0.5 to 1 g/ml through-out the whole experiment. The exception being the process and manufacturing optimization study, in which only 1 g/ml concentrations were systematically studied. In addition to the microparticle concentration, the total amount of microparticles influenced the mixing process. Making it difficult to mix extremely low or high amounts of composite. Thus, to ensure a facile stirring process roughly 3 ml of composite was prepared each time into a single 50 ml plastic container.

As the composite was mixed and when it appeared to be homogenous it was deemed ready. The next step was to either fill syringes with the composite or leave the composite to age in the plastic container. Respectively, the filled syringes were stored to be later studied by rheological measurements or by conducting an injection test array. The syringes were stored horizontally, exposed to ambient light at room temperature. In contrast, a portion of the syringes manufactured by the CMO were horizontally stored at elevated temperatures (40 °C).

The composites left to age in the plastic container, were used to study the aging profile (i.e. gelation time) of the composite. The gelation time was investigated under three different processing conditions: temperature, evaporation, age of the initial silica sol.

The gelation time was known to be reliant on temperature, hence the gelation was studied in room temperature (23 °C) and in refrigerator temperatures (4 °C). Furthermore, in order to evaluate the effects of moisture evaporation, the silica-silica composites were set to age in both open and closed systems. This meant that the plastic containers were either left lidless (open system) or sealed with a lid (closed system). Regarding the open systems, each time 200 ml of silica sol was prepared and allowed to age in a 500 ml decanter either in room or refrigerator temperatures. Lastly, the age of the initial silica sol was also taken into account. Hence, samples were prepared with unaged sol (referred to as fresh sols) and aged sols (aged for 1-3 hours). The ultimate aim of these studies were to hinder the gelation rate of the composite, so that the syringes could be filled within a wider time frame.

The successful filling of the syringes entailed that the composite remained as a suspension for the entire duration of the filling process. Once the composite had gelled, the syringes

were next to impossible to be filled. Figure 3.1 depicts a process chart of the whole composite preparation process and the studies conducted for the final product. In conclusion, the amount of replicate samples and measurements used to study different characteristics of the composite are tabularized into Table 3.1.

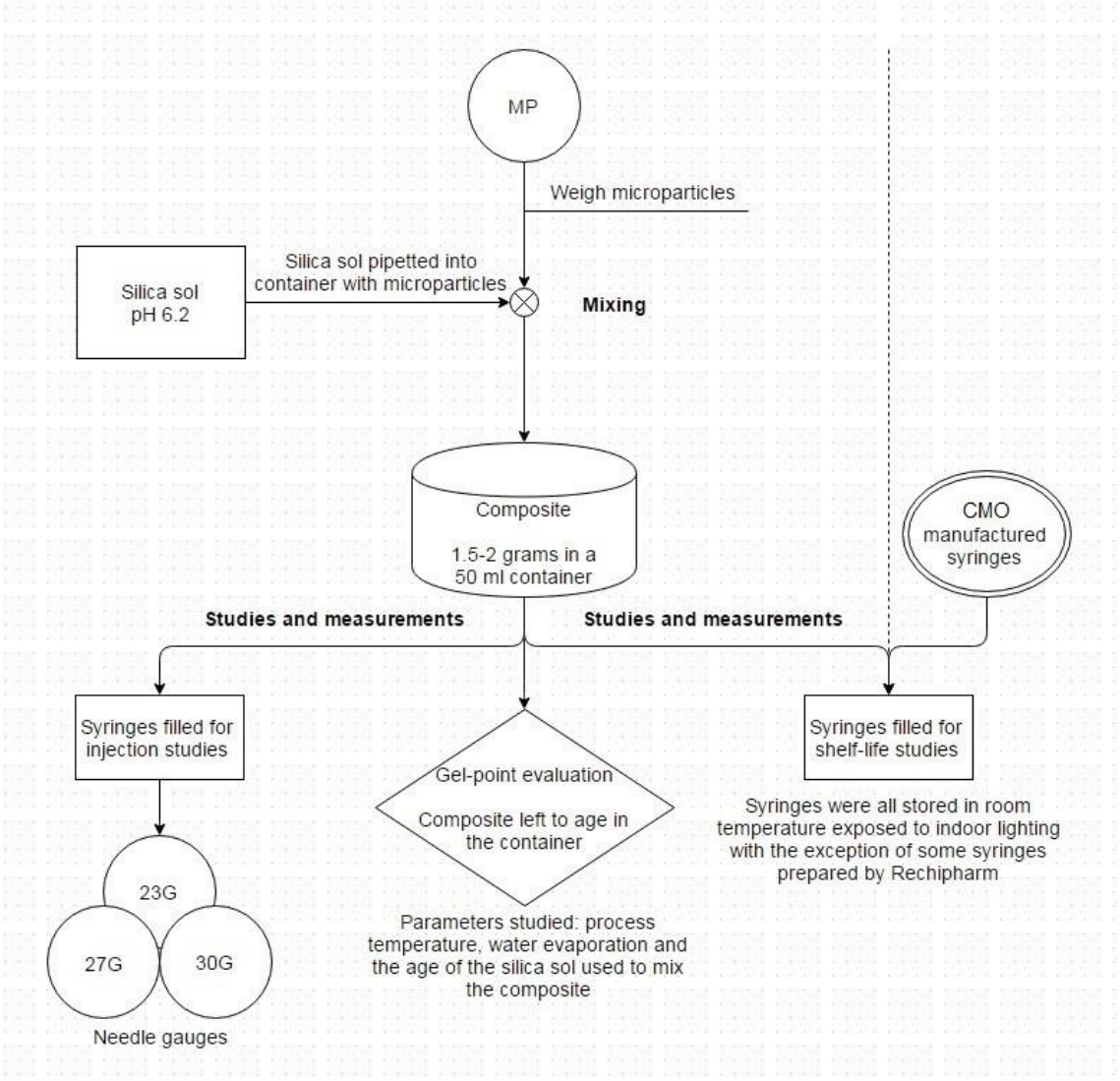

Figure 3.1. *Flow chart of the composite preparation process and the three different sets of studies conducted afterwards. The injection studies aided in defining the appropriate needle gauges that could be used to inject the final product. The gel-point evaluation study made it possible to optimize the manufacturing process of the composite as well as the filling process of the syringes. Lastly, the shelf-life study determined the point-in-time when the composite had stabilized inside the syringe – ready to be conventionally stored and injected out.*

Table 3.1. *The amount of replicate samples prepared and studied. The brackets referrer to the test method used to characterize each property. The measurements included oscillatory and viscosity measurements with a rheometer. The injectability study was conducted by injecting samples through different needle gauges (25G, 27G and 30G).*

Formulation		**Amount of replicate samples measured** (three replicate measurements per sample)		
C_{mp} (g/ml)	**Sol R-value**	**Gel-point (oscillatory)**	**Shelf-life (oscillatory)**	**Shear-thinning (viscosity)**
0.5	**400**	Not measured	3	3
0.75	**400**	Not measured	3	3
1.0	**400**	3	3	3
Formulation		**Amount of replicate samples injected** (injectability study)		
C_{mp} (g/ml)	**Sol R-value**	**Needle gauge: 23G**	**Needle gauge: 27G**	**Needle gauge: 30G**
0.5	**400**	Not injected	1	1
0.6	**400**	Not injected	1	1
0.7	**400**	Not injected	1	1
0.75	**400**	Not injected	1	2
0.8	**400**	Not injected	1	1
0.9	**400**	Not injected	1	1
1.0	**400**	12	18	6
C_{mp} = microparticle concentration of the composite sample R-value = H_2O:TEOS for the used silica sol				

3.2.3 Rheological measurements

A single rotational rheometer (ThermoHaake RS 300, Germany) equipped with a parallel-plate with a HPP20 TC measuring geometry (D = 20 mm). This system was used to measure all materials. A CS/CR rotatory ramp -program with a gap of 0.3 mm and shear rate ranging from 0.1000 1/s to 1000 1/s at 25 °C was used to measure viscosity. Oscillatory testing was conducted with a frequency sweep with frequency range of 0.01-10 Hz at 25 °C with a 0.4mm gap. Furthermore the testing was conducted under controlled deformation where the deformation was set to 0.002 which was previously determined with an amplitude sweep –program.

4. RESULTS AND DISCUSSION

4.1 Manufacturing process optimization

During the manufacturing of the silica-silica composite it is paramount to evaluate, understand and measure certain process parameters, which are known to either hinder or assist the process [19][21]. The parameters investigated in this study were moisture evaporation, process temperature and the age of the sol used in the mixing of the composite. These were chosen for under inspection, because they could be controlled with ease and were known to either increase or decrease the gelation time of the composite. The objective was to determine the time frame in which the syringes had to have been filled before the composite turned from a low viscosity suspension into a thick gel with dominant elastic properties. Thus, after a certain point in time the homogenous filling of the syringes would be jeopardized and eventually made next to impossible. Consequently, oscillatory measurements were conducted from which a loss tangent (tan δ) was calculated for each set of measurements. The loss tangent, explained in Chapter 2.3.3, was then used to determine the gel-point and evaluate the strength of the prepared composites [50][51].

In order to plot data obtained from the oscillation measurements as a function of time, the frequency value (range in this study was from 0.01 Hz to 10 Hz) must be set constant. The lowest frequency value was chosen so that the possible gel-point could be observed, since at higher frequencies viscoelastic materials have a tendency to act stiffer in their linear viscoelastic range (see Figure 4.1) [51].

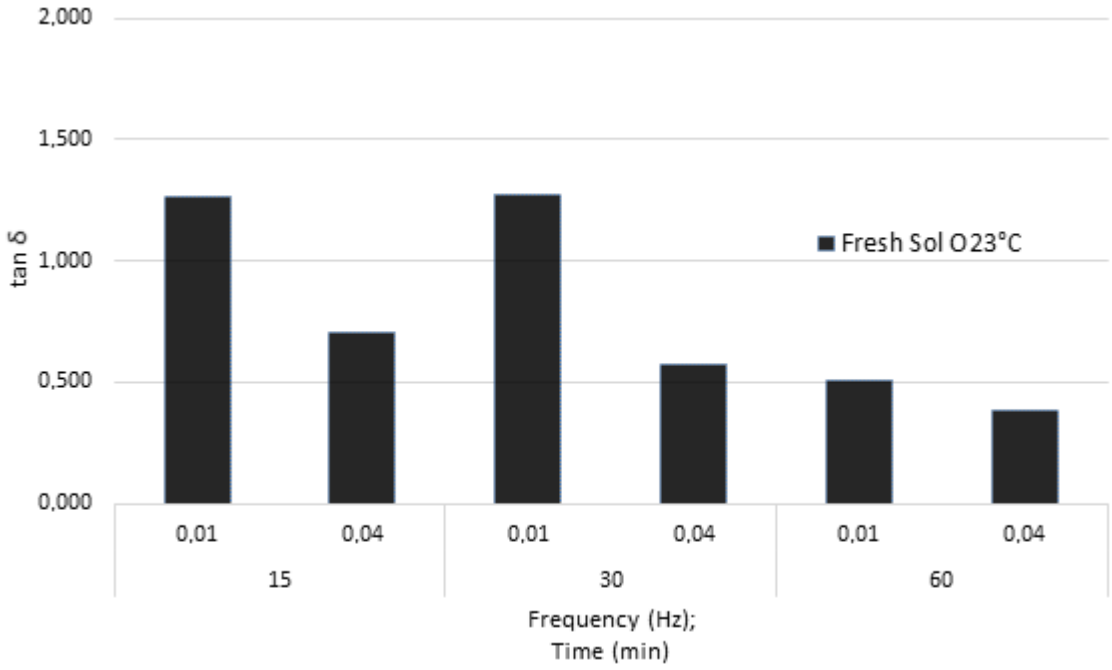

Figure 4.1. *The composite transitions from a suspension (tan δ > 1) into a gel (tan δ < 1) after 30 minutes can be observed at 0.01 Hz (single measurement). However, at 0.04 Hz the same sol-to-gel transition cannot be detected. "Fresh Sol" indicates that an unaged silica sol was used and the prefix "O" stands for open system. Mixing began at 0 min. The reported values represent a single measurement.*

The phenomena observed in Figure 4.1 clearly demonstrates how viscoelastic properties are dependent on measurement conditions. If the composite is not subjected to appropriately low frequencies, the sol-to-gel transition-point is undetectable. Understandably, utilizing even lower frequencies than 0.01 Hz one can gain even more accurate data about the material at rest. However, this would greatly increase the measurement time. Subsequently, if the measurement procedure takes an excessively long time to conduct, the time-dependent viscoelastic properties of the sample may change during the measurement [10]. As a result, unreliable data may be obtained. In conclusion, the measurement conditions influence the results obtained from oscillatory measurements.

4.1.1 Comparing process temperatures

The impact of both room (23°C) and refrigerator process temperatures (4-8°C) were investigated. All samples investigated in this section, plotted in Figures 4.2, 4.3, 4.4 and 4.5, were prepared using unaged silica sols (referred to as Fresh Sols) with a microparticle concentration of 1 g/ml. Figure 4.2 compares the gelation time of composites manufactured in open systems to composites prepared in closed systems both processed at room temperatures. Similarly, Figure 4.3 compares these same systems, but at

refrigerator temperatures. In conclusion, Figures 4.4 and 4.5 illustrate the gelation time differences of similar composites processed at the two different temperatures.

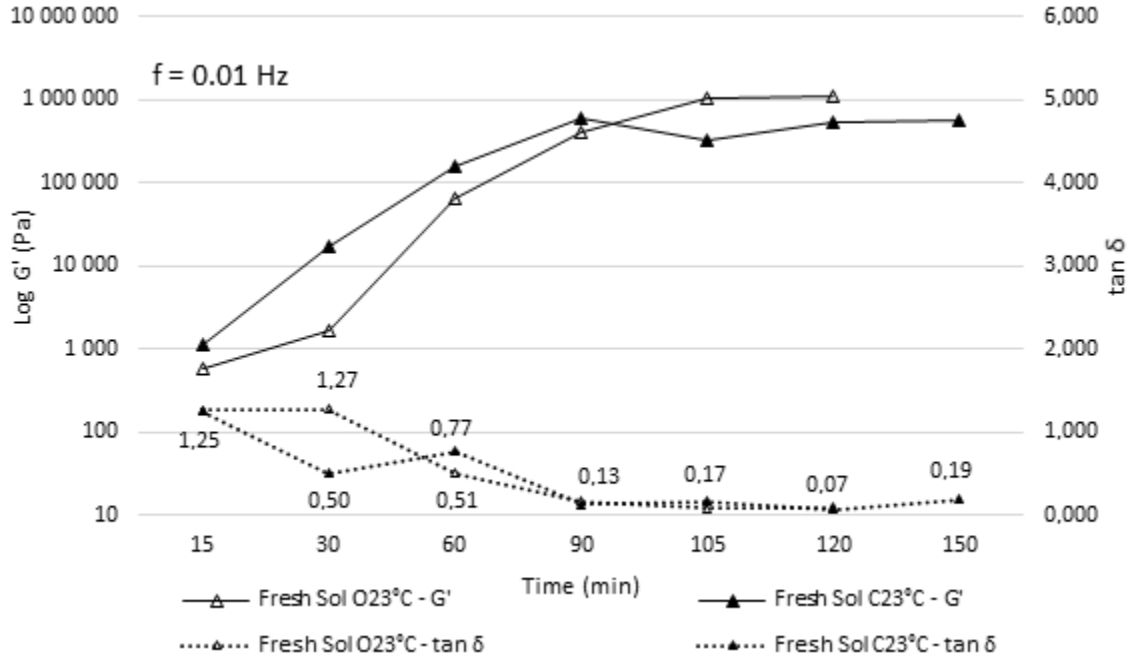

Figure 4.2. *Comparing the effects of minimizing evaporation during processing at 23 °C. "Fresh Sol" indicates that an unaged silica sol was used to prepare the composite sample. The prefix "O" stands for open system (hollow markers) and correspondingly the prefix "C" for closed system (filled markers). Tan δ describes the viscoelastic nature of the measured samples. If tan δ > 1, the sample is a suspension, and if tan δ < 1, the sample is a gel. Thus, the sol-gel transition point is at tan δ = 1. Mixing began at 0 min. Three replicate measurements were conducted from which average values were calculated (reported here).*

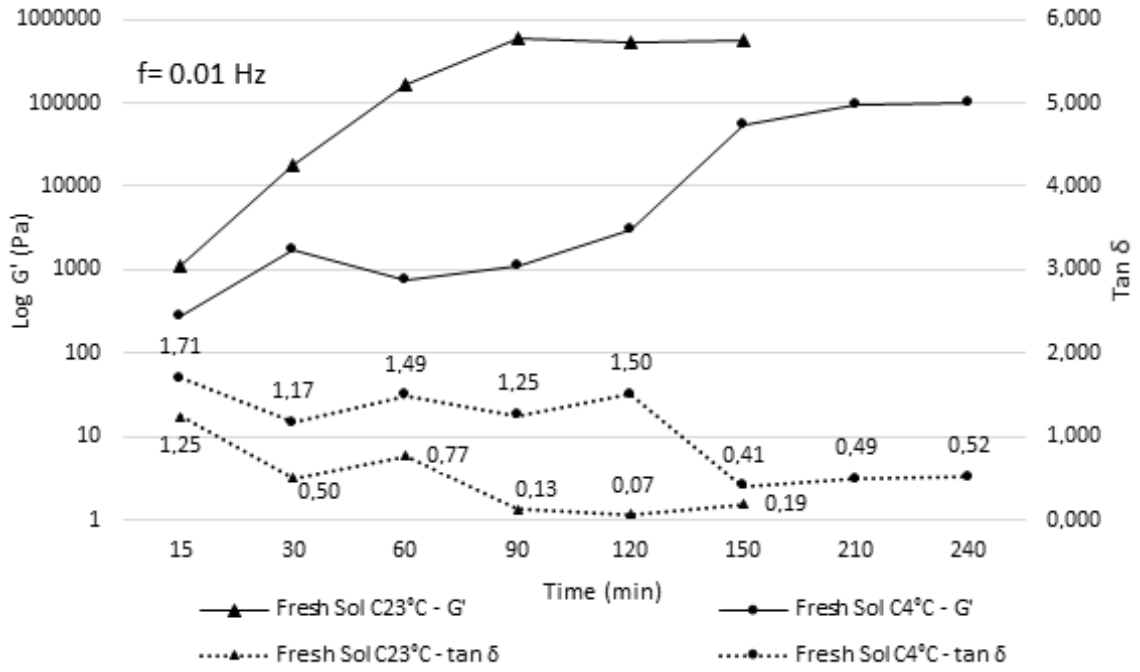

Figure 4.3. *Comparing the effects of minimizing evaporation during processing at 4-8 °C. "Fresh Sol" indicates that an unaged silica sol was used to prepare the composite sample. The prefix "O" stands for open system (hollow markers) and correspondingly the prefix "C" for closed system (filled markers). Tan δ describes the viscoelastic nature of the measured samples. If tan δ > 1, the sample is a suspension, and if tan δ < 1, the sample is a gel. Thus, the sol-gel transition point is at tan δ = 1. Mixing began at 0 min. Three replicate measurements were conducted from which average values were calculated (reported here).*

The gelation time can be understood as the first measuring point where tan δ < 1. Accordingly, the gelation of the composite in room temperature is observed to occur in less than 60 minutes regardless of minimizing evaporation (see Figure 4.2), but with lower temperatures the gelation is witnessed to occur after the 120 min mark (see Figure 4.3). The syringes could arguably still be filled near the vicinity of the gel-point, since the composite could be pipetted from the sample container on to the rheometer plate with relative ease. Ultimately, the flow characteristics of the composites drastically changed once the storage modulus of the composite had reached 100 000 Pa (at 0.01 Hz). At this point the composite was difficult to pipette, because it no longer flowed as single droplets, but as a continuous elastic fluid rapidly thickening into a rigid gel ($G' \approx$ 1 000 000 Pa).

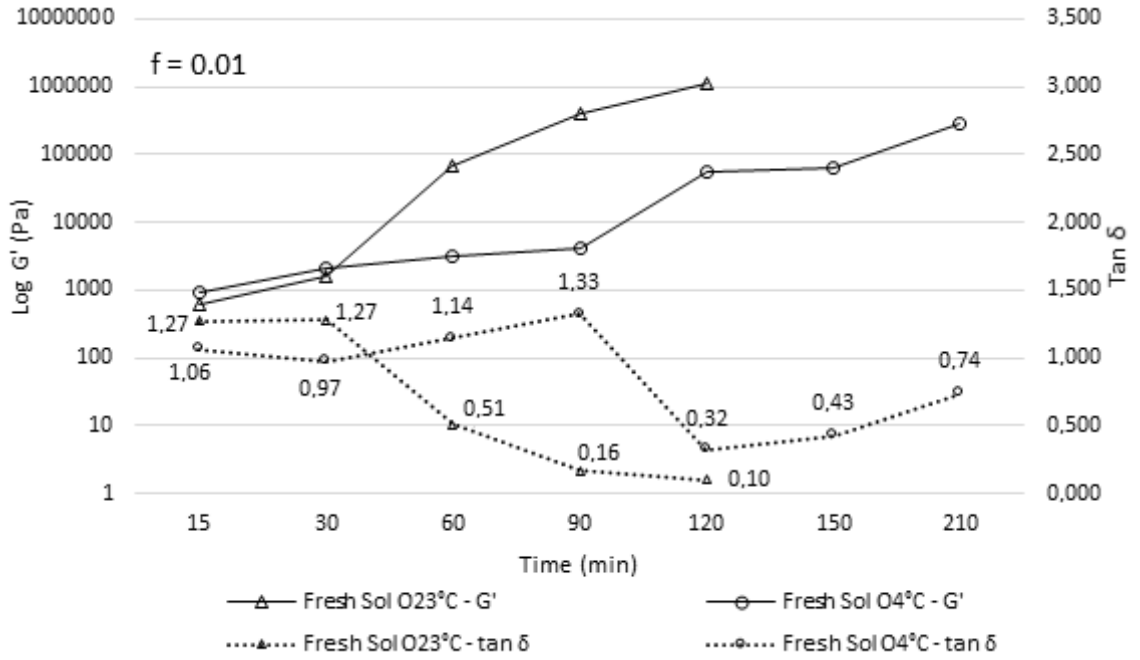

Figure 4.4. *Comparing the effects of lowering the process temperature from 23°C to 4-8°C for open systems (prefix "O"). Tan δ describes the viscoelastic nature of the measured samples. If tan δ > 1, the sample is a suspension, and if tan δ < 1, the sample is a gel. Thus, the sol-gel transition point is at tan δ = 1. Mixing began at 0 min. Three replicate measurements were conducted from which average values were calculated (reported here).*

As is evident from Figure 4.4 and 4.5 that lowering the process temperature increases the gelation time of the composite. Resulting in a wider filling time frame of syringes. Although, lower temperatures inherently tend to increase a materials viscosity [51], in this case the viscosity increase was subtle and did not pose any problems during measurements.

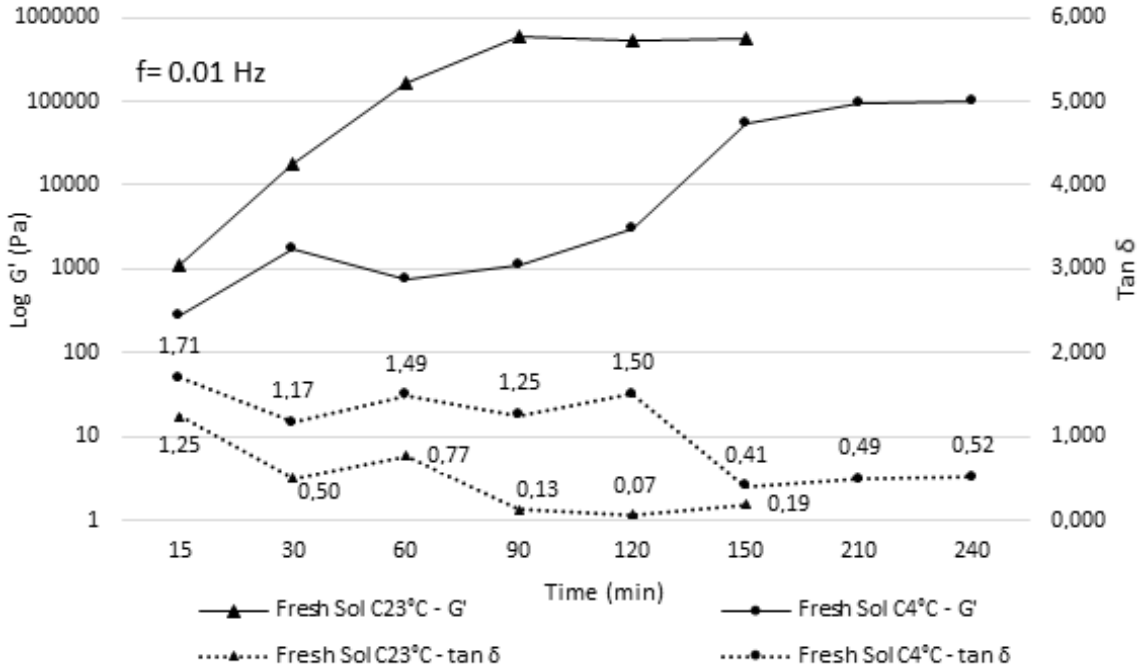

Figure 4.5. *Comparing the effects of lowering the process temperature from 23°C to 4-8°C for closed systems (prefix "C"). Tan δ describes the viscoelastic nature of the measured samples. If tan δ > 1, the sample is a suspension, and if tan δ < 1, the sample is a gel. Thus, the sol-gel transition point is at tan δ = 1. Mixing began at 0 min. Three replicate measurements were conducted from which average values were calculated (reported here).*

The process temperature seems to have a greater influence on the gelation time of the composite than controlling the amount of water evaporating during the process. However, this correlation is insubstantial, because the evaporation surfaces were relatively small, whereas the temperature difference was relatively large. Nevertheless, it is certain that optimal conditions for retarding the gelation time of the composite is achieved by keeping process temperatures in the range of 4-8°C and minimizing evaporation of moisture.

4.1.2 Comparing aged and unaged silica sols

It was postulated that manufacturing the silica composites from aged silica sols would decrease the gelation time of the composite [44]. Consequently, constricting the time frame in which the syringes could be homogenously filled. The postulation was found to be true. Figures 4.6, 4.7 and Table 4.1 compare the effects of using aged sols in the composite manufacturing process. The term "Fresh sol" indicates that sol had not been allowed to age prior to manufacturing, whereas the prefixes "1 h Sol","2 h Sol" and "3 h Sol" specify the age of the sol used. Respectively, the initial silica sols were aged for 1, 2 and 3 hours. The prefixes "O" and "C" anterior to the process temperatures stand for open and closed systems, analogous.

Table 4.1. *The values were measured at a frequency of 0.01 Hz. The circled area values indicate the time frame in which the gelation of the composite samples occurred. The gel-point being at tan δ = 1. Mixing began at 0 min. Three replicate measurements were conducted from which average values were calculated (reported here).*

	Fresh Sol O23°C		**1 h Sol O23°C**		**2 h Sol O23°C**		**3 h Sol O23°C**	
Time	**G' (Pa)**	**tan δ**	**G' (Pa)**	**tan δ**	**G' (Pa)**	**tan δ**	**G' (Pa)**	**tan δ**
15 min	592	1.268	1 214	1.272	1 771	1.468	12 258	0.660
30 min	1637	1.275	14 400	0.276	21 200	0.226	27 041	0.734
60 min	65 720	0.506	189 700	0.740	254 900	0.629	130 900	0.455
90 min	401 200	0.163	-	-	-	-	-	-

Circled area = the occurrence of the sol-to-gel transition

When preparing the composites with sols aged for 1 to 2 hours the gelation of the composite is observed to occur within the first 30 minutes of the manufacturing procedure (Table 4.1). However, when composites are prepared with fresh sols the gelation is observed to occur between 30 to 60 minutes (Table 4.1). Remarkably, no gel-point was observed for composites prepared with 3 hour old sols (Table 4.1). This indicates that the gelation occurred within the first 15 minutes. Moreover, within 90 minutes the composites prepared from aged silica sols thickened into a rigid paste-like gel, which could no longer be aptly measured. Thus, no rheological measurements could be conducted for the “1 h Sol”-, “2 h Sol”- and “3h Sol”-samples at the 90 minute mark.

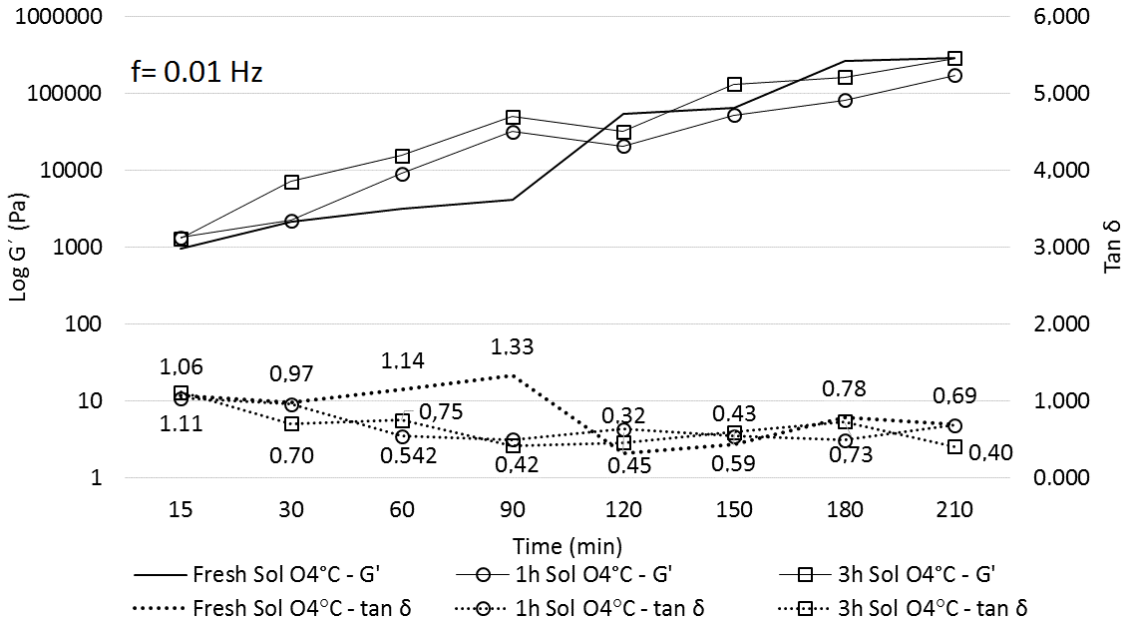

Figure 4.6. *Comparison of composites prepared from aged sols with composites prepared from fresh (i.e. unaged) sols. The composite samples were aged in refrigerator temperatures as open systems. Tan δ describes the viscoelastic nature of the measured samples. If tan δ > 1, the sample is a suspension, and if tan δ < 1, the sample is a gel. Mixing began at 0 min. Three replicate measurements were conducted from which average values were calculated (reported here).*

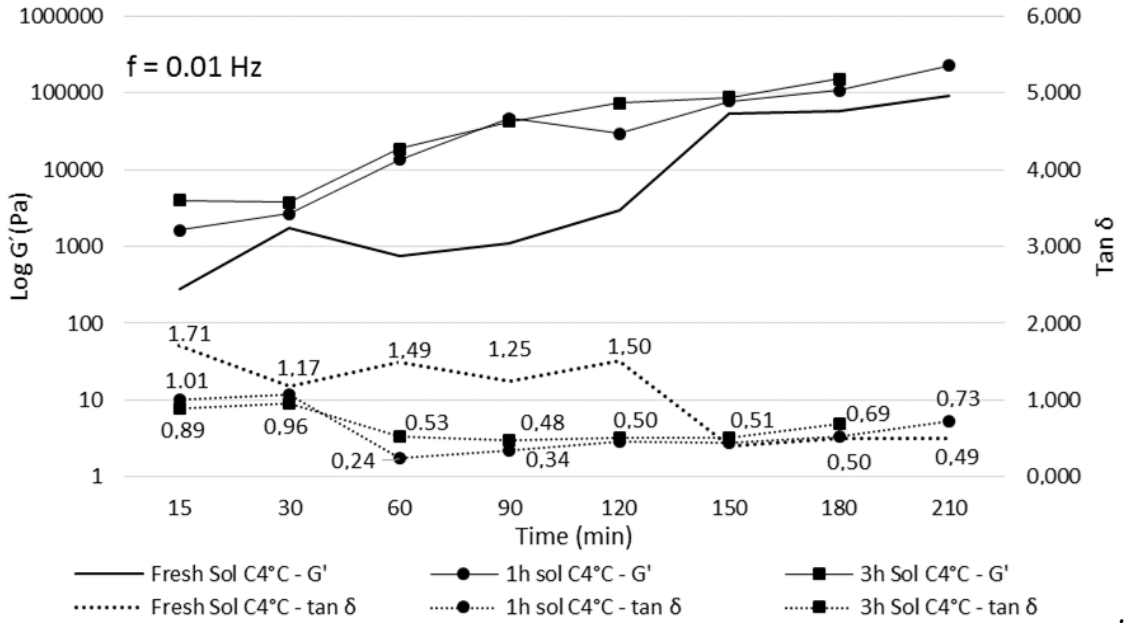

Figure 4.7. *Comparison of composites prepared from aged sols with composites prepared from fresh (i.e. unaged) sols. The composite samples where then aged in refrigerator temperatures as closed systems. Tan δ describes the viscoelastic nature of the measured samples. If tan δ > 1, the sample is a suspension, and if tan δ < 1, the sample is a gel. Mixing began at 0 min. Three replicate measurements were conducted from which average values were calculated (reported here).*

The age of the initial silica sol has a great impact on the gelation time of the composite, which can be seen in Figures 4.6 and 4.7. Regarding open process systems at refrigerator temperature, composites manufactured with aged silica sols were detected to gel approximately 60 minutes faster than composites prepared with unaged sols (Figure 4.6). This difference was even greater under closed process conditions (at 4°C), where a 90 minute difference in the gelation time was witnessed (Figure 4.7). Consequently, the homogeneity of the final product may be compromised, by simply neglecting to use unaged silica sols during manufacturing. Even if the sol is allowed to age for an hour (at 23 °C) prior to mixing the composite, the homogeneity of the final product is jeopardized.

As the silica sol ages, condensation reactions polymerize the silica to form larger particles, particle chains and agglomerates [19][21]. Ultimately, forming the initial suspension into a three-dimensional gel. This polymerization process is accelerated by the addition of the silica microparticles, which simultaneously act as nucleating agents in the condensation process and as particle-reinforcements in the gel [52, p. 14-15]. For this reason, the silica sol on its own transforms into a gel more slowly than the silica sol microparticle mixture. Successively, the composites gel transformation is dependent on the polymerization degree of the initial silica sol. Table 4.1 illustrates this phenomena well, which shows that the gelation time of the composite decreases as the silica sols gradually age.

4.2 Shelf-life study

After the silica-silica composite is processed and the syringes are successfully filled. The next logical step is to evaluate the shelf-life of the final product. This was done by conducting an injection test array through various needle sizes and oscillatory measurements to witness, if any gel structure deterioration had occurred over time. In this circumstance, the shelf-life studies included determining whether the final product would remain stable (i.e. homogenous) for two months and to study the effects of elevated storage temperatures (40 °C). Furthermore, product samples were prepared with varying microparticle concentrations (0.5, 0.75 and 1.0 g/ml) in order to evaluate how the microparticle concentration might affect the shelf-life of the product. Naturally, as the microparticle concentration is decreased the cross-linking density of the gel structure decreases. As a results, the structure of the silica-silica composite becomes more fragile [53][54]. During preliminary injection testing, it was witnessed that products with a microparticle concentration lower than 0.5 g/ml could not withstand the injection process and visible phase separation occurred.

In addition, the time-frame in which the composite became stable inside the syringe was determined. Only after the composite had stabilized inside the syringe, the structure of the composite could withstand the injection process (entails high shear stresses). Therefore, this stability experiment was conducted by injecting varyingly aged composites through 23G and 27G needles to see how much time the composite needed to

stabilize. For the first twelve hours of this follow-up, measurements were conducted in one hour intervals and then in 24 hour intervals continuously up to a month.

4.2.1 Viscoelasticity of the composites

Once the syringes had been filled, the progressive gelation of the composite within the syringe was mapped utilizing rheological measurements under small angle oscillatory shear. The objective was to determine the point-in-time when the gelation process was complete and whether the composite remained homogenous during the whole study [51]. The results for 1 g/ml samples are presented in Figure 4.8, whereas Figure 4.9 presents the results for 0.5 g/ml and 0.75 g/ml samples.

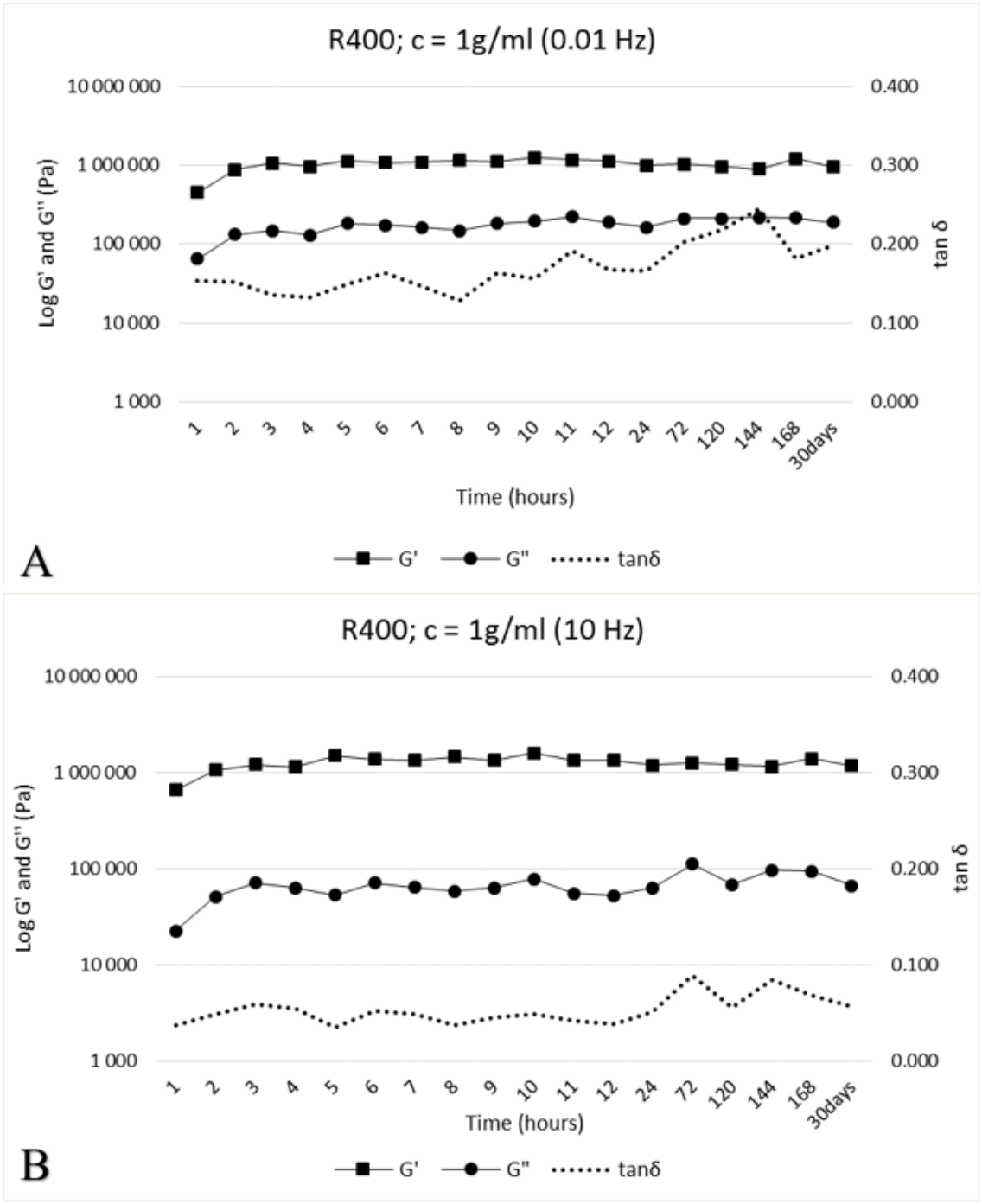

Figure 4.8. *The two graphs represent measured viscoelastic properties as a function of time at two different frequencies: 0.01 Hz (graph A) and 10 Hz (graph B). The samples had a microparticle concentration of 1 g/ml. The plotted values are averages of nine replicate measurements from three replicate samples. The x-axis designates the time which has passed from the point when the syringes were filled.*

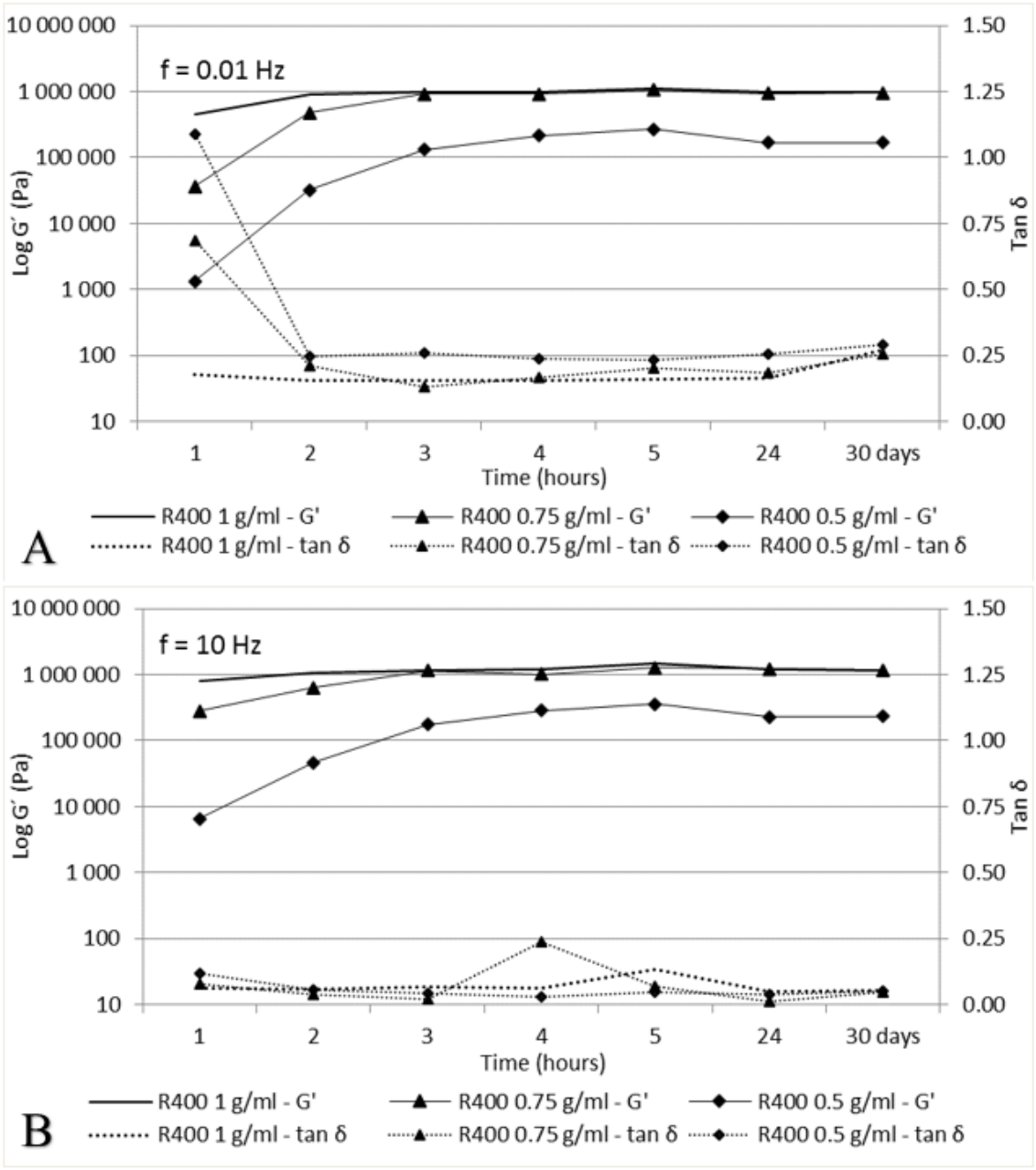

Figure 4.9. *The effects of lowering the microparticle concentration of the composites under 1 g/ml was studied under two frequencies: 0.01 Hz (graph A) and 10 Hz (graph B). The plotted values are averages of nine replicate measurements from three replicate samples. The x-axis designates the time which has passed from the point when the syringes were filled.*

As was expected, the microparticle concentration had an effect on the viscoelastic properties of the composite, because the microparticles in fact reinforce the gel-structure of the composite [52]. Therefore, as the microparticle volume concentration is decreased, the storage modulus (G') of the composite decreases (Figure 4.9).

In addition to affecting the elastic dominance of the composite, the microparticle concentration also affects the gelation time of the composite. This phenomena is apparent

from Figure 4.9, where the G' values of the samples are seen to stabilize within different time periods, according to their microparticle volume concentration. The term "stabilization" is to be understood as the point-in-time where the viscoelastic properties (G′, G′′ and tan δ) begin to plateau (seen in Figures 4.8 and 4.9). Nonetheless, lowering the microparticle concentration of the composites appear to slightly impede the stabilization of the composite. This aspect must be taken into account when manufacturing the composite syringes to prevent particle sedimentation. Thus, to ensure that the composite inside the syringes remain homogenous, after the filling, the syringes are to be subjected to a rotational motion (e.g. with a tube rotator). This motion must be kept at least till the composite has stabilized, in a way, that the microparticles are suspended in the gel and can no longer migrate. After the composite has stabilized it can be stored conventionally in static conditions without its structural integrity being jeopardized. However, understandably this integrity is lost over time. Therefore, it was important to evaluate how the composite retained its viscoelastic properties over time.

The samples were seen to retain their viscoelastic properties for at least a month, even at high frequencies, since no drastic increases or decreases in the measured moduli values are observed in neither Figure 4.8 nor Figure 4.9. For example, a sudden increase in tan δ (i.e. tan $\delta > 1$) values would have indicated that the three-dimensional structure of the composite had reverted back to its initial two-dimensional network. The weak chemical bonds between the silica macromolecules could have been dissipated by internal and/or external forces (e.g. shear stress, thermal energy) resulting in the depolymerization of the composite. In contrast, a sudden increase could indicate the onset of polymer aging (i.e. cross-linking of the macromolecules). This aging process would initially harden and increase the mechanical properties of the composite. Ultimately making the product brittle and susceptible to the formation of cracks and crevices. The aging process is caused by chemical transformation of macromolecules and is often initiated and meditated by exposure to extreme weather conditions (e.g. UV-light, ozone,) and/or oxidative chemicals (e.g. hydroperoxides) [55].Therefore, under controlled storage conditions the latter scenario is unlikely to occur in the silica-silica composite matrix.

Most likely the silica-silica composite will eventually depolymerize and regress back to a viscous fluid rather than developing dominant elastic properties. This statement is supported by the results denoted in Table 4.2. The samples studied in Table 4.2 are identical to the samples studied in Figure 4.8 in respect to their formula. However, the samples were manufactured by the CMO and the syringes used were made of glass instead of plastic. For this reason the CMO manufactured tips of the syringe were not cut off prior to the oscillatory measurements. Thus, preceding the measurements the CMO samples were subjected to shear stresses leading to the shear-thinning of the samples. As a result, the measured absolute values for G′ and G′′ are a magnitude lower for the samples in Table 4.2 compared to the samples investigated in Figure 4.8.

Table 4.2. *The CMO manufactured syringes. Sample R4001 was the reference sample, R4002A was stored for 1 month in room temperature and R4003A for 2 months. Consequently R4002B was stored for 1 month and R4003B for 2 months with the exception of being stored in elevated temperatures (40 °C). The values of the variables were measured at a frequency of 0.01 Hz. The reported values are average values of three replicate measurements.*

Sample	Storage time	G' (Pa)	G'' (Pa)	tan δ
R4001	Unaged	189 867	83 757	0.445
R4002A	1 month	181 167	81 323	0.450
R4003A	2 months	68 830	35 280	0.513
R4002B	1 month (40°C)	34	30	0.777
R4003B	2 months (40°C)	48	50	1.259

The final silica-silica composite product cannot withstand temperatures above ambient temperatures for an extended period of time during storage, and should therefore be stored in a temperature controlled environment. This is evident when comparing the viscoelastic properties of the samples tabulated in Table 4.2. Furthermore, the tabularized data supports the visual observations made during the oscillatory measurements: the samples stored in 40°C were observed to be flowing fluids as supposed to resembling solid-like gels. This factor could pose a significant problem when transporting and respectively storing the product in countries with a tropical climate. In addition, to deteriorating in elevated temperatures, the silica-silica composites seem to lose some of their elastic strength within the first 2 months after being stored at room temperature. This aspect can be seen in Table 4.1, but is further highlighted in Table 4.2. The represented data (Table 4.2) compares the measured viscoelastic properties of R4002A- and R4003A-samples in the entire frequency range used to study the materials.

Table 4.3. *The CMO manufactured syringes. Both samples were stored in room temperature. Sample R4002A was stored for a month, whereas sample R4003A was stored for 2 months before they were measured under small angle oscillatory shear. The reported values are average values of three replicate measurements.*

	Sample R4002A			Sample R4003A		
Hz	**G' (Pa)**	**G'' (Pa)**	**tan δ**	**G' (Pa)**	**G'' (Pa)**	**tan δ**
0,01	181 167	81 323	0.450	68 830	35 280	0.513
0,04	289 933	66 563	0.225	114 500	20 790	0.182
0,07	308 100	77 353	0.250	123 333	25 013	0.203
0,1	327 333	53 367	0.158	120 300	22 390	0.186
0,4	353 633	29 473	0.085	139 200	22 913	0.164
0,7	358 133	19 391	0.054	146 600	15 747	0.106
1	356 700	12 074	0.036	149 433	12 199	0.081
4	361 533	25 220	0.072	154 833	14 177	0.091
7	335 033	37 400	0.106	157 933	11 882	0.075
10	367 033	15 940	0.048	152 667	12 880	0.084

It is apparent that the measured G´ values at the two month time-period are approximately half of what they are for the samples stored for a period of one month, regardless of the subjected frequency (see Table 4.3). However, no distinctive visual observations could be made between the two samples as they seemed to have similar viscoelastic properties. Meaning, that the samples should be, at some point, studied by injecting them through thin needles in order to evaluate, if a difference in terms of injectability exist between them. The injection studies would also aid in determining whether the two month sample is actually unusable. This distinction cannot be made simply by stating that the G´ values plummet within 2 months after being manufactured. In conclusion, the final silica-silica composite product should be stored and kept in ambient temperatures to ensure an extended shelf-life.

4.2.2 Injectability of the composites

The composites aging process was investigated by conducting injection studies through two needle gauges (23G and 27G). In order to support and further focus the observations

made from the small angle oscillatory shear studies. The results have been tabularized into Tables 4.4 and 4.5.

Table 4.4. *Composites with microparticle concentrations of 1 g/ml were injected through thin needles (23G and 27G). The storage time indicates the age of the composite syringe before it was injected (calculated from when the syringe was filled).*

Storage time	Syringe #	23G	27G	Observations
1 h	1.	—	1000 µl*	Not gelled, phase separation
2 h	1.	—	1000 µl*	Not gelled, phase separation
3 h	1.	—	1000 µl*	Not gelled, phase separation
4 h	1.	800 µl**	200 µl*	Weak Gel, little separation
5 h	1.	600 µl**	400 µl*	Weak Gel, little separation
6 h	1.	500 µl**	500 µl*	Gel, close to final product
7 h	1.	900 µl**	100 µl*	Stable Gel
	2.	300 µl**	600 µl*	Stable Gel
8 h	1.	1000 µl*	—	Stable Gel
	2.	—	1000 µl*	Stable Gel

* The amount of composite successfully injected through the needle (out of 1000 µl).
** The composite was first injected through a 27G needle and then the rest was injected through a 23G needle

Table 4.5. *Composites with microparticle concentrations of 1 g/ml were injected through thin needles (23G and 27G). The storage time indicates the age of the composite syringe before it was injected (calculated from when the syringe was filled).*

Storage time	Syringe #	23G	27G	Observations
24 h	**1.**	1000 µl*	—	
	2.	—	1000 µl*	
	3.	—	700 µl*	
	4.	—	700 µl*	
48 h	**1.**	1000 µl*	1000 µl*	§ All composites injected were stable gels meaning no phase separation was observed.
	2.	—	1000 µl*	
96 h	**1.**	1000 µl*	—	§ Only small amounts of fluid is leeched out from the composite after it has been injected unto thin tissue paper.
	2.			
120 h	**1.**	1000 µl*	—	
	2.	—	900 µl*	
144 h	**1.**	1000 µl*	—	§ The final product resembles a smooth and matte paste.
	2.	—	1000 µl*	
	3.	—	1000 µl*	
168 h	**1.**	1000 µl*	—	
	2.	—	1000 µl*	

* The amount of composite successfully injected through the needle (out of 1000 µl).

According to the injectability data in Table 4.4 the silica-silica composite (c = 1 g/ml) should not be injected within the first seven hours of being filled, because the composite cannot withstand the forces applied to it upon the injection process. This observation designates that even though the samples G´ values are seen to stabilize within three hours (see Figure 4.8) the formation of the three-dimensional gel structure of the composite is not completely finished by this time. It is most likely, that the subtle progression of the elastic properties of the composite cannot be witnessed by the rheological setup used in this study. Nonetheless, the key information is that at least for the first eight hours of being filled, the syringes should be subjected to a rotational motion in order to prevent microparticle sedimentation. After this procedure the syringes can be stored conventionally. This statement is further supported by comparing the injectability data of Tables 4.4 and 4.5: the injectability through a 27G needle improves after the first eight hours of being filled.

The final objective was to determine whether it was possible to successfully inject the silica-silica composite through a 30G needle. This was hypothesized to be extremely difficult due to the size of the silica microparticles. After preliminary injection tests, no composites with a particle concentration of 1 g/ml were successfully injected through a 30G needle. In this context, a successful injection entailed that the whole sample volume could be injected through the 30G needle. The whole volume in this case being 1000 µl. After the failed injections, it was apparent that the particle concentration should be lowered below 1 g/ml. Consequently another array of composite syringes, with concentrations ranging from 0.5 to 0.9 g/ml, were prepared to be injected through very fine needles. The obtained data is tabularized in the Table 4.6.

Table 4.6. *Composites with different microparticle concentrations were injected through 27G and 30G needles. The composite syringes were injected after 7 days had passed from the day they were filled. Visual observations of the injected composites are also described in order to further characterize the composites.*

Microparticle concentration	Needle gauge	Amount injected (out 1000 µl)	Observations
0.5 g/ml	27 G	1000 µl	Phase separation occurred – extremely weak gel
	30 G	200 µl	
0.6 g/ml	27 G	1000 µl	Phase separation occurred – weak gel
	30 G	600 µl	
0.7 g/ml	27 G	1000 µl	Successful injections, although tiny visible clusters present
	30 G	1000 µl	
0.75 g/ml*	30 G	1000 µl	Resembles "1 g/ml"- samples: stabile gel
	30 G	1000 µl	
0.8 g/ml	27 G	200 µl	Resembles "1 g/ml"- samples: stabile gel
	30 G	200 µl	
0.9 g/ml	27 G	1000 µl	Highly resembles "1 g/ml"- samples
	30 G	100 µl	

* The samples were stored for a period of 30 days before they were injected whereas the other samples (with different concentrations) were stored for 7 days.

Lowering the microparticle concentrations can pose problems regarding the stability of the final product. In other words, as the microparticle concentration is decreased, the composite mechanical properties weaken. As a result, the inherent ability of the composite to withstand shear stresses and strains decreases. Thus, composites with a lower microparticle concentration are more shear-thinning than composites with a higher concentration (Figure 4.10).

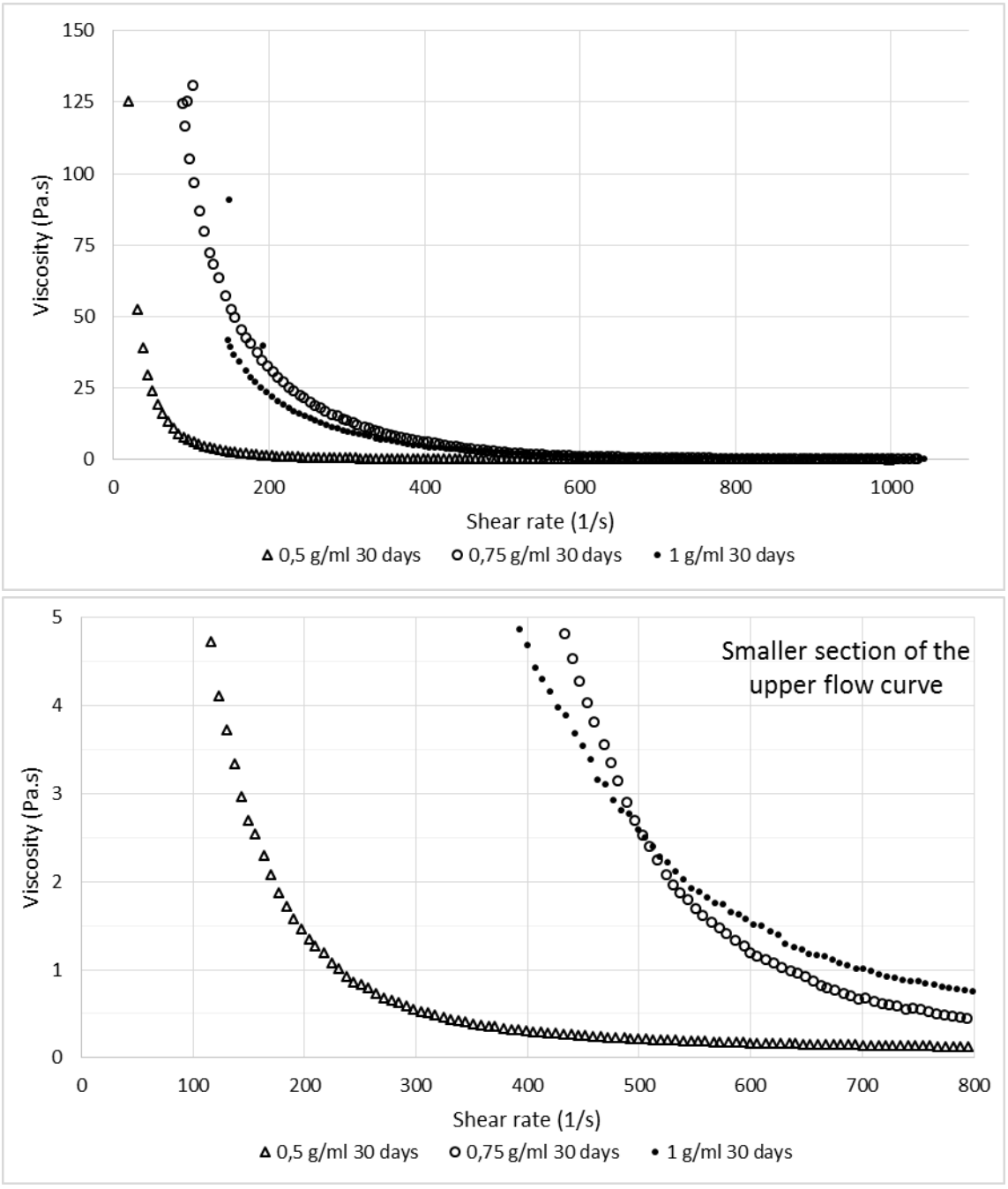

Figure 4.10. *Shear thinning becomes progressively larger as the volume concentration of the solid silica microparticles decreases. The legend shows each sample's storage time and microparticle concentration.*

In spired by the data gathered in Table 4.6 and in Figure 4.9, composites with a microparticle concentrations of 0.75 g/ml were prepared. After being stored for a period of one month, the 0.75 g/ml composites were injected through thin needles (see Table 4.6). As a result, two out two of the prepared samples were successfully injected through 30G needles. In conclusion, if the soul objective is to manufacture a silica-silica composite that could be injected through a 30G needle, composites owning a microparticle concentration between 0.7-0.75 g/ml would be the most prominent to investigate further.

5. CONCLUSIONS

Suspending silica gel microparticles in a sol-gel derived silica hydrogel offers a promising alternative to parenterally administered organic drug delivery systems. The benefits of encapsulating therapeutic agents within sol-gel derived silica microparticles have been discussed in several other reports [17][18][23][24][34][45].

The objective of this study was to manufacture syringes with an injectable silica-silica composite and to study its processing methods, injectability and shelf-life. The composite in itself is a shear-thinning material and the viscosity of the composite increases, to a certain degree, as a function of time. This meant that once the composite had been mixed, the syringes were to be filled before the composite turned into a thick gel. This ensured that the syringes could be homogeneously filled and that the composite would gradually gel inside the syringes – suspending the silica microparticles in place.

The shear-thinning characteristic of the composite allows the composite gel to be injected through even particularly thin needles (e.g. needles suitable for intraocular administration). Naturally, this shear-thinning property progressively decreases as the volume concentration of solid particles within the composite is increased or as the R-value of the silica hydrogel is decreased. It is to be noted that in this study the R-value for the silica hydrogel was a constant R400 and the effects of lowering the R-value was not investigated. Another noteworthy aspect is that by decreasing the microparticle concentration the structure of the composite becomes weaker in terms of mechanical strength. The reason is that the microparticles act as reinforcing agents stabilizing the hydrogel matrix. As a result, weak composites cannot withstand the injection process (i.e. the composite is too shear-thinning).

Before any injection studies were conducted, it was necessary to closely examine the manufacturing process of the composite in order to ensure the production of a viable final product. In this context, a final product is defined as a 1 ml syringe filled with a composite owning a 1 g/ml silica microparticle concentration. Accordingly, the gelation process of the composite was characterized under small angle oscillatory shear to create a process timeline. The results indicated that by lowering the process temperature to 4-8°C from room temperatures (23°C), the gelation process was considerably hindered. This resulted in a broader time frame in which the syringes could be homogeneously filled. In addition to temperature effects, evaporation and the age of the silica sol (with a pH of 6.2) used in the manufacturing process were also studied. Since the sample sizes and the evaporation surfaces were relatively small, evaporation did not seem to greatly influence the gelation process. In contrast, using unaged silica sols to mix the composite had a greater impact than striving to minimize the amount of evaporation taking place during the process. The optimal processing conditions defined by a broad filling-time frame were achieved by

utilizing fresh sols (sol age < 1 hour), closed process systems (i.e. closed containers, flasks, etc.) and employing refrigerator temperatures (4-8°C). Under these conditions the syringes could be filled within the first 210 minutes. This time was calculated from the point when the two separate components of the composite were mixed together. In contrast, composites prepared in room temperature had to have been filled within the first 60 minutes. The point-in-time when the syringes could no longer be filled was determined by a visual observation: the composite was witnessed to flow ineptly as a single elastic fluid and not as a droplet forming liquid. The same point may also be characterized by a G´ value of 100 000 Pa measured at a frequency of 0.01 Hz.

Once the syringes were successfully filled, the composite stabilized inside the syringes within the first 8 hours. Meaning that, after this point-in-time the product could be injected through a fine needle in a way that its physical structure was not compromised in the process. Therefore, as soon as the syringes were filled they should be subjected to a rotational motion to avoid any particle sedimentation from occurring. Once the microparticles can no longer migrate inside the composite matrix, the product can be safely stored conventionally in static conditions.

Upon storage, the composite was observed to preserve its viscoelastic properties for a duration of 30 days as was the case with the syringes manufactured by the CMO. Regarding the injectability studies, composites owning a 1 g/ml microparticle concentration were systematically injected through 23G needle in a way that the whole sample volume (1 ml) was successfully injected through. As the needle size was decreased to 27G and 30G, the amount of successful injections also decreased. Approximately two thirds of the samples injected through a 27G needle were successfully injected out. In contrast, with the 30G needles, every injection attempt was unsuccessful. However, by decreasing the microparticle concentration from 1 g/ml to 0.75 g/ml the composites injectability through 30G needles significantly improved without jeopardizing the composites viscoelastic characteristics. These are promising preliminary results, which should be investigated more thoroughly in the future.

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