EFFECT OF SOLVENT VOLUME ON SIZE OF SILICA NANOPARTICLE WHICH IS SYNTHESIZED USING MODIFIED SOL-GEL PROCESS.

By

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Acknowledgements

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Lists of Symbols and Abbreviations

Symbol		Abbreviations
DI	=	Deionised water
kDa	=	Kilo Dalton
C°	=	Celsius
L	=	Liter
mL	=	milliliter
nm	=	nanometer
PdI	=	Polydispersive index
NP	=	Nanoparticle
RPS	=	Rotation per second

Abtrak

Nano teknologi sedang merevolusikan bidang elektronik, kimia, industri bioteknologi dan biomedikal. Silika nano partikal disintisiskan melalui proses Sol-gel yang diubahsuai dibawah suhu bilik dengan mengunakan amine sebagai katalis, Tween 80 sebagai pembuat misel air dan tetraethylorthosilikat sebagai punca silica. pH air dikawal diantara 10.3 dan 10.5. Silika nanopartikal yang disinthesis mempunyai sifat larut dalam air. Saiz nanopartikal berubah apabila kita menukar isipadu pelarut, iaitu 1-Butanol di dalam kajian ini.

Perubahan adalah berperingkat menurut isipadu pelarut. Tetapi pada suatu isipadu pelarut, meningkatan berhenti dan saiz menurun secara berperingkat. Ini secara jelasnya menunjukkan pengaruh pelarut keatas formasi nanopartikal terbatas. Ini telah memberikan cara, dimana manipulasi saiz boleh dilakukan dengan bantuan pelarut juga. Pengaruh ini tidak kuat seperti air/ alkoxide nisbah dan pH air. Kajian lanjutan harus dijalankan untuk mengkaji secara jitu penagruh pelarut keatas saiz nanopartikal silica dengan mengunakan Sol-gel proses.

Abstract

Nanotechnologies are now poised to revolutionize the electronic, chemical and biotechnology industries and biomedical fields. Silica nanoparticles have been prepared in this work using modified Sol- gel method at room temperature that employs a water-soluble amine as catalyst, Tween 80 water as a micelle producer and tetraethylorthosilicate (TEOS) as the silica source. The pH value of the aqueous phase was controlled between 10.3 and 10.5. Silica nanoparticle that has been synthesized using modified Sol- gel process is water soluble in nature. The size of nanosilica particle changed when we change the volume of the solvent, which 1-butanol in this research.

The changes of the nanoparticle were gradual according to the solvent volume. But after certain amount of solvent volume, the increment is stopped and the size decreased gradually. This obviously showed that the influence of solvent in formation of Nanoparticle is limited to certain level only. This creates a new way which the manipulation of the size can be done with the help of solvent too. The influence is not as strong as water/ alkoxide ratio and pH of the solution. Further research need to be conducted to study precise influence of the solvent to the size of silica nanoparticle by modified sol-gel process.

Chapter 1

INTRODUCTION

1.1 General introduction

A major fashion in research and development has been the shift of interest towards smaller and smaller structures. This can be summarized under the general term "nanotechnology". This started in the mid-eighties and gained momentum in the nineties (Figure 1.1) (Kruis and Joshi, 2005). Dr Richard Feynman a Nobel laureate, in his lecture in1959 lecture, proposed a variety of potential nanomachines, which could be engineered to a higher level of functional efficiency than currently available manufactured devices by exploiting changes in the behavior of matter at the nanometer length scale.

In order to realize this goal, scientists and engineers have to invent strategies to synthesize specified functional nanoparticles, and then learn how to incorporate them into devices in which they might function individually or in cooperation with other nanoparticles or devices.

Applications of nanotechnology have to be brought to commercial success in numerous fields. The number of applications of nanoparticles which have been proposed is very large, ranging from information storage in information technology, materials for sensor applications, refractory materials, pharmaceutics, catalysis, novel materials, and many more. Nanoparticles are now very much in the focus of the natural sciences due to their fascinating size-dependent properties (Kruis and Joshi, 2005).

Dutrochet
Discovered
cells in tissues

Miescher
Discovered
DNA

Immunology
term
Firstly used

DNA is
discovered

1st automated
protein
sequencer
produced

1st
mAb
Produced

1st breast
cancer
gene
discovered

Dolly the
Sheep was
born

BC   1824   1830   1869   1888 1902 1906 1911 1919 1943 1953 1967 1977   1983   1995   2000

Edward Jenner
introduced
Vaccination

Protein
discovered

Waldyer
Discovered
Chromosomes

Genetics
term
Firstly used

Biotech
Term
Firstly
used

Watson&Crick
DNA structure
discovered

Genetically
engineered
hGh
produced

1st babboon
to human
bone marrow
transplantation

**Taniguchi
1stly used
Nanotechnology
in publication**

NAN report call
for
experimentation
on molecular
manufacturing

1st synthesis
of engineered
protein

1st nanobio
conference

1st report on
Nanoindustry

1959   1974   1977   1996 1998   2001 2003   2006

Drexler
introduced
Molecular
Nanotechnology

1st DNA based
nanomechanical
devices

Congressional
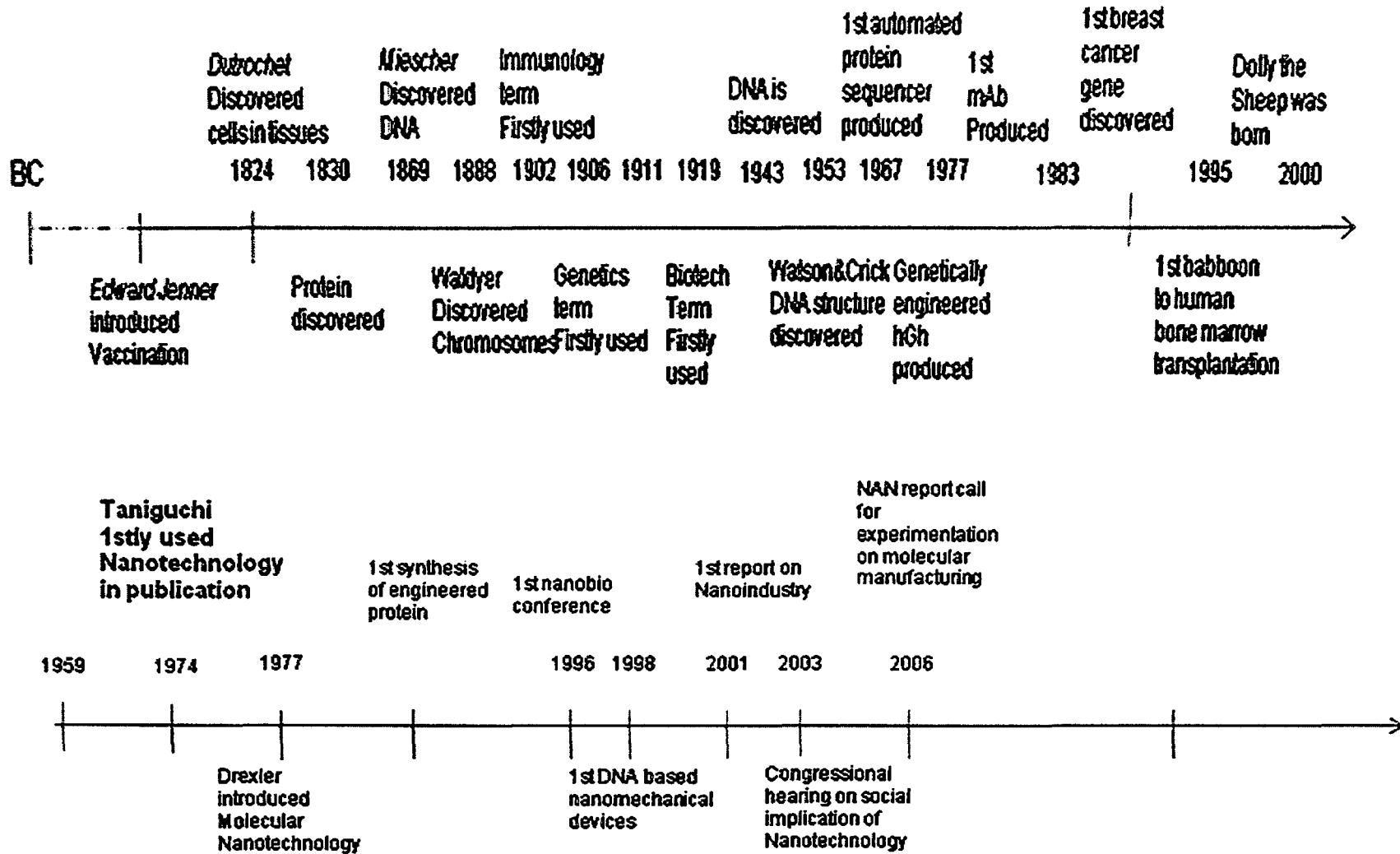hearing on social
implication of
Nanotechnology

Figure 1.1: Bionanotechnology Time Line.

Reference: Presentation "Nano Punch" By Assoc. Prof Shaharum Shamsuddin

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1.2 Bionanotechnology

Bionanotechnology or Nanobiotechnology are terms that refer to the intersection of nanotechnology and biology, are defined by science's growing ability to work at the molecular level, atom by atom, combining biological materials and the rules of physics, chemistry, and genetics to create tiny synthetic structures.

The final result of bionanotechnology is to create highly functional systems of biosensors, electronic circuits, nano-sized microchips, molecular 'switches,' and even tissue analogs for growing skin, bones, muscle, and other organs of the body (Tan *et al.*, 2004).

On the other hand, medical and biotechnological advances in the area of disease diagnosis and treatment are dependent on deep understanding of biochemical processes. Diseases can be identified based on abnormalities in the molecular level and treatments are designed precisely. Numerous methods for disease identification as well as treatments already exist. But it would be ideal to use research tools which are close to the molecular level to better understand the mechanisms that are involved in the processes of disease and disease treatment.

These tools can be nanoparticles, nanoprobes, or other nanomaterials which exist in ultra-small scale and can be designed to study biochemical process of interest (Tan *et al.*, 2004). Nanomedical approaches to drug delivery center on developing nanoscale particles or molecules to improve the bioavailability of a drug (Kashiwada, 2006).

Bioavailability is defined as the presence of drug molecules where they are needed in the body and where they will be most effective. Drug delivery focuses on maximizing bioavailability of the drug of interest both at specific places in the human body and over a period of time that needed (Patni. and Bhatia., 2008).

1.3 Nanoparticle

The term "nanoparticle" came into use long after nanoparticles were produced and investigated for the first time. In the engineering and materials science community, the terms "ultrafine particles" (UFPs) and submicron particles were used before the term "nanoparticles" (Hayashi *et al.*, 1997).

Nowadays, three main denotations for nanoparticles are being used: nanoparticle, nanocrystal and nanocluster. Nanoparticles can be defined as materials and systems whose structures and components exhibit novel and significantly improved physical, chemical and biological properties, phenomena and processes due to their nanoscale size i.e. in a range of 1-100nm (Hosokawa, 2007).

It includes particles smaller than the so called submicron particles and the longest wavelength of visible light of about 400 nm. In some applications, they are limited to particles smaller than 10–20 nm where their physical properties would drastically change (Hosokawa, 2007).

Biotechnology and biomedical field have benefited drastically from technological advancement. Nanotechnology, the processes to generate, manipulate, and set out nanomaterials, represents an area holding significant promise for health care and biotechnology for future generation (Tan *et al.*, 2004).

Nanoparticles have wide applications in the field of biology such as biolabeling, imaging, drug delivery, separation, and optical sensing (Jana *et al.*, 2007). Nanoparticles have great promise in bioanalysis and biotechnological applications because of their unique optical properties, high surface-to-volume ratio, and other size-dependent qualities.

When combined with surface modifications and material composition control, these properties provide probes for highly selective and ultrasensitive bioassay tools. The sizes of nanoparticles provide us more diverse capabilities in bioanalysis and biotechnological applications (Dabbousi *et al.*, 1998).

Figure 1.2: The scales of things: nanometers and more (http://inventors.about.com/od/sstartinventions/ss/Physics_Illustr_3.htm)

1.4 Silica Nanoparticle
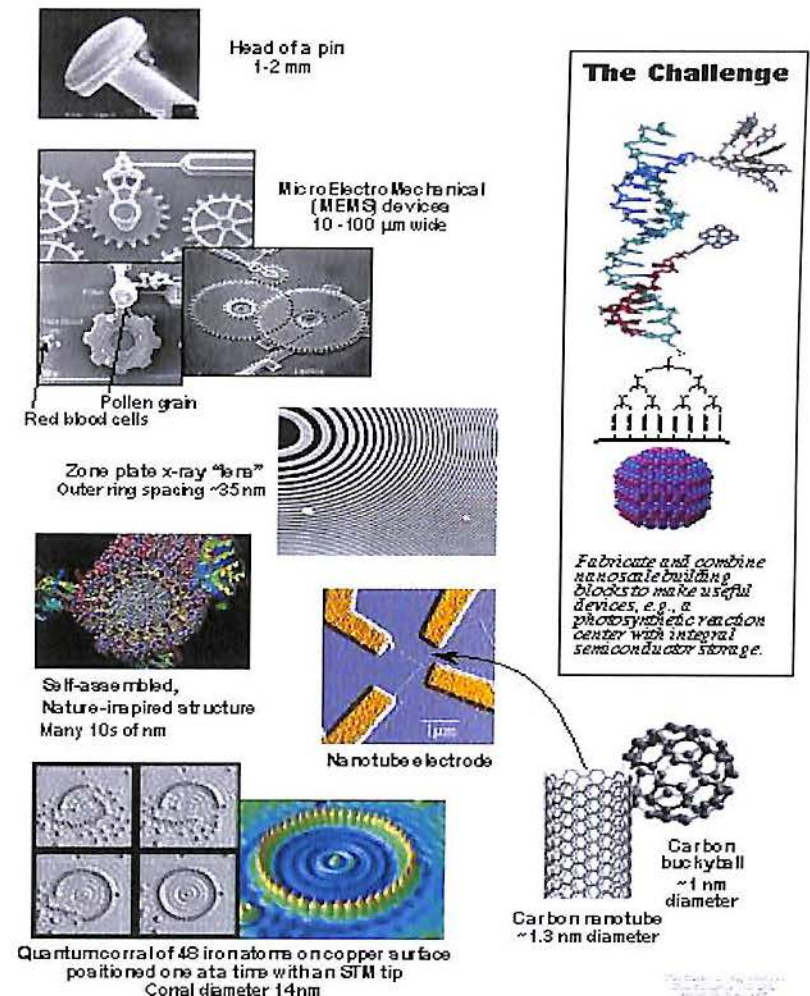
Silica is one of the most abundant materials on earth (Figure 1.3). Naturally occurring silica, such as quarts sand, rocks, and clays, are used as raw materials in the industries. In order to produce industrial silica products, such as silica gel, precipitated silica, silica sol (colloidal silica), and fumed silica, these primary raw materials are chemically treated to produce direct-silica sources, such as sodium silicate, silicon tetrachloride, and alkoxysilane (Barik *et al.*, 2008).
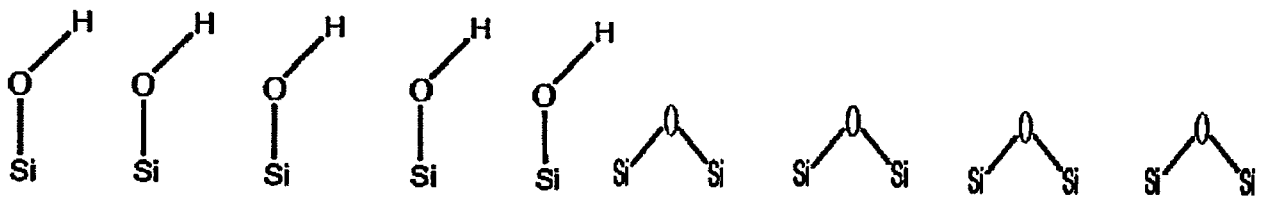
Figure 1.3: Hydrophilic (*left*) and hydrophobic (*right*) surface of silica (Barik *et al.*, 2008)

It is well known that the geometries of $(SiO_2)_n$ clusters in the small size range ($n\square>\square 12$) are chain like (Barik *et al.*, 2008). Therefore, these structures consist of some Si atoms that do not have the full fourfold(Barik *et al.*, 2008). Silica is the common name for materials composed of silicon dioxide (SiO2) and occurs in crystalline and amorphous forms. Crystalline silica exists in multiple forms. Quartz and more specifically α-quartz is a widespread and well-known material. Upon heating, α-quartz is transformed into β-quartz, trydimite and cristobalite. Porosil is the family name for porous crystalline silica. Quartz exists in natural and synthetic forms, whereas all porosils are synthetic (Napierska *et al.*, 2010) Amorphous silica can be divided into natural specimens (e.g., diatomaceous earth, opal and silica glass) and human-made products.

OBJECTIVE

- To determine relationship between solvent and the size of silica nano-particle using modified sol-gel process.

- To synthesize aqueous soluble nanosilica particles and

- To characterize aqueous soluble nanosilica particle.

Chapter 2

Literature review

2.1 Sol-gel process

"Silica" is the name given to materials with the chemical formula of silicon dioxide, SiO_2. Silica can be amorphous or crystalline, porous or non-porous (dense), anhydrous or hydroxylated (RK, 1979), regardless of their natural or synthetic nature. In a silica material, the silicon atom is in tetrahedral coordination with 4 oxygen atoms. Theoretically, an infinite variety of 3-D-ordered structures can be built from oxygen-sharing silicate tetrahedra. The number of known crystalline silica materials is limited, which leaves much room for research and development. In amorphous silica, the tetrahedra are randomly connected (Napierska *et al.*, 2010).

The sol–gel process is a simple wet chemical route for the synthesis of colloidal dispersions of inorganic and organic–inorganic hybrid materials, particularly oxides and oxide-based hybrids in various forms of powders, fibers, thin films and monoliths (Moreau *et al.*, 2001, Jin *et al.*, 2002). The sol-gel process involves the manufacture of inorganic matrices through the formation of a colloidal suspension (sol) and the gelation of the sol to form a wet gel, which after spontaneous drying, forms dry gel called xerogel. Most sol-gel techniques use water and low molecular weight alkoxysilanes such as tetraethoxysilane (TEOS) or tetramethoxysilane (TMOS), as silica precursors. (Table 2.1)
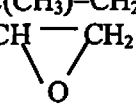
Name (acronym)	Formula
Vinyltriethoxysilane (VTS)	$(C_2H_5O)_3$ Si-CH=CH$_2$
Methacryloxypropyltriethoxysilane (MPTS)	$(C_2H_5O)_3$Si- CH$_2$CH$_2$CH$_2$-O-CO-C(CH$_3$)=CH$_2$
3-glycidyloxypropyltrimethoxysilane (GPTS)	$(C_2H_5O)_3$Si- CH$_2$CH$_2$CH$_2$-O-CH$_2$ CH $\overset{\diagdown\diagup}{O}$ CH$_2$
3-aminopropyltrimethoxysilane (APTS)	$(C_2H_5O)_3$Si- CH$_2$CH$_2$CH$_2$NH$_2$
3-mercaptppropyltriethoxysilane (McPTS)	$(C_2H_5O)_3$Si- CH$_2$CH$_2$CH$_2$SH
Chlopropyltriethoxysilane (CPTS)	$(C_2H5O)_3$Si- CH$_2$CH$_2$CH$_2$Cl

Table 2.1: Few example of Silica precursors

The alkoxide hydrolyse in the presence of water to forms silanols, with either acid or base as a catalyst. The reactions of alkoxysilanes can be summarised in terms of three steps: hydrolysis of the alkoxide, silanol-silanol condensation, and silanol-ester condensation (Figure 2.3). Hydrolysis occurs by the nucleophilic attack of the oxygen contained in water in the silicon atom. Subsequent condensation reactions take place producing siloxane bonds.

The polymerisation stages could be described as, i) polymerisation of monomers to polymers, ii) condensation of polymers to primary crystals, iii) growth or agglomeration of primary crystals to particles and iv) linking of particles into chains and to three dimensional network (Figure 2.1). Networks of the chains extend throughout the liquid medium, thickening the network into a gel. In the final stage, water and alcohol are removed from the network structure causing gradual shrinkage and even cracking of the monolithic gel.
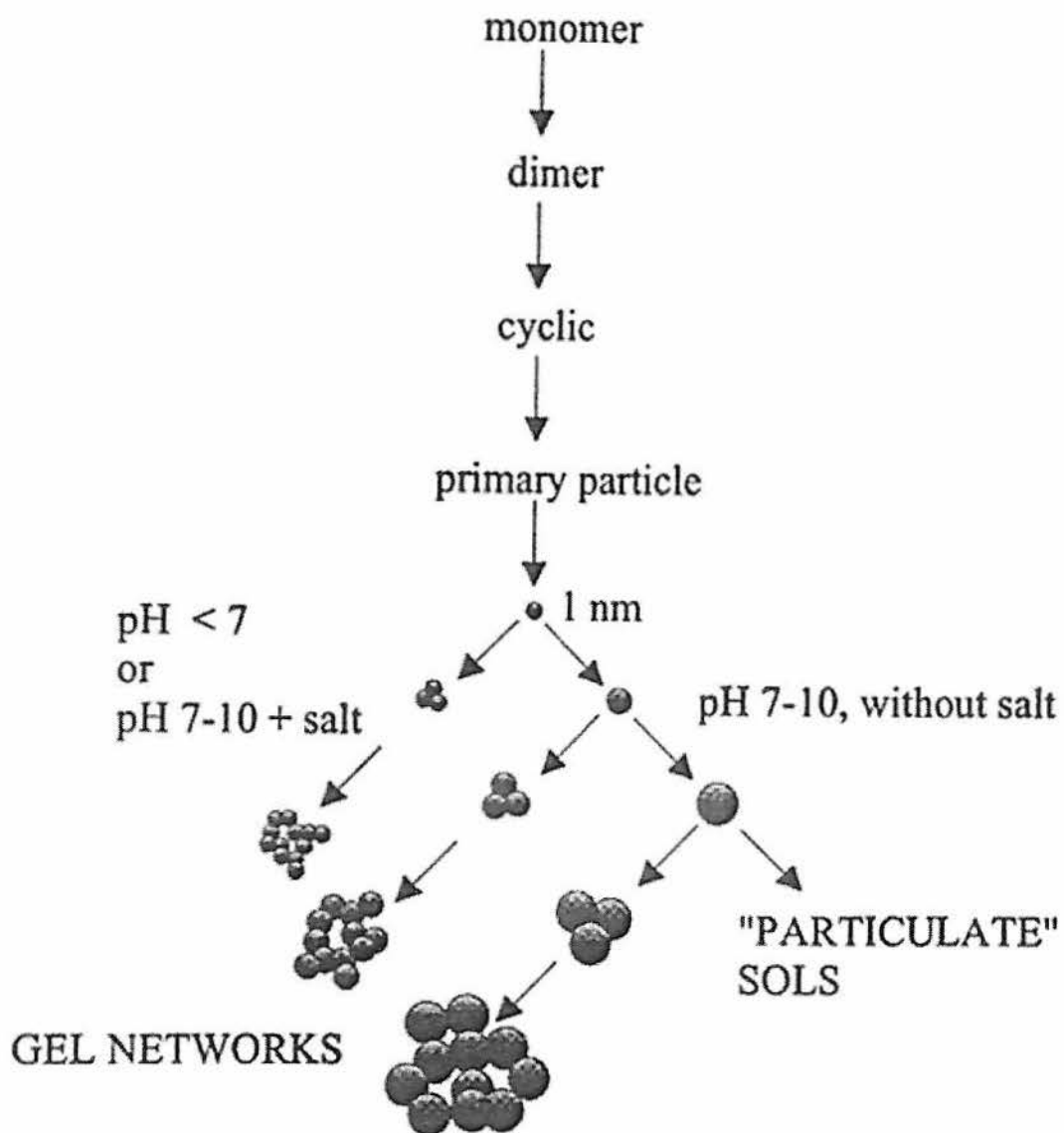
Figure 2.1: Reactions of alkoxysilanes and polymerisation behaviour of silica in various pH

2.1.1 Preparation of silica from alkoxysilanes

Most sol-gel syntheses of silica are based on the hydrolysis of tetraalkoxysilanes which is the silica precursor, Si(OR)4, according to the following equation, e.g.:

$$Si\ (OC_2H_5)_4 + 4\ H_2O \xrightarrow{\text{catalyst}} SiO_2 + C_2H_5OH$$
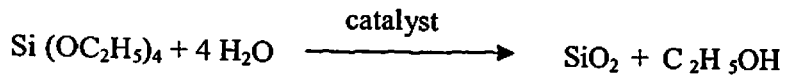
Gelation of silicon alkoxide solutions takes place as a result of the hydrolysis of the silicone alkoxides Si(OR)$_4$ and subsequent polycondensation (*dehydration*) leading to the formation of polymers and particles with siloxane bonds. The reaction can be expressed by the following formulae :

$$Si\ (OC_2H_5)_4 + 4\ H_2O \xrightarrow{\text{Catalyst}} Si\ (OH)_4 + 4C_2H_5OH$$

$$Si\ (OH)_4 \longrightarrow SiO_2 + 2\ H_2O$$
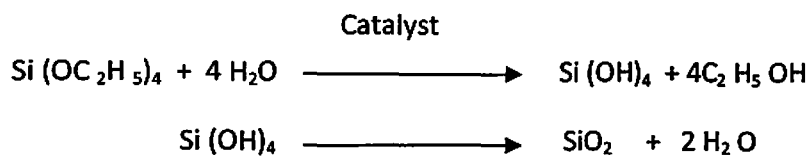
However, the mechanisms of these reactions are unknown because of the complexity of the gelation process. Theoretical reaction pathways are presented in Figure 2.2, illustrating the competition between the hydrolysis and the condensation steps of some species, the number of which increases as the reactions proceeds.

Figure 2.2: Possible reaction of tetraalkoxysilanes

Reference: (Chruściel and Ślusarski, 2003)

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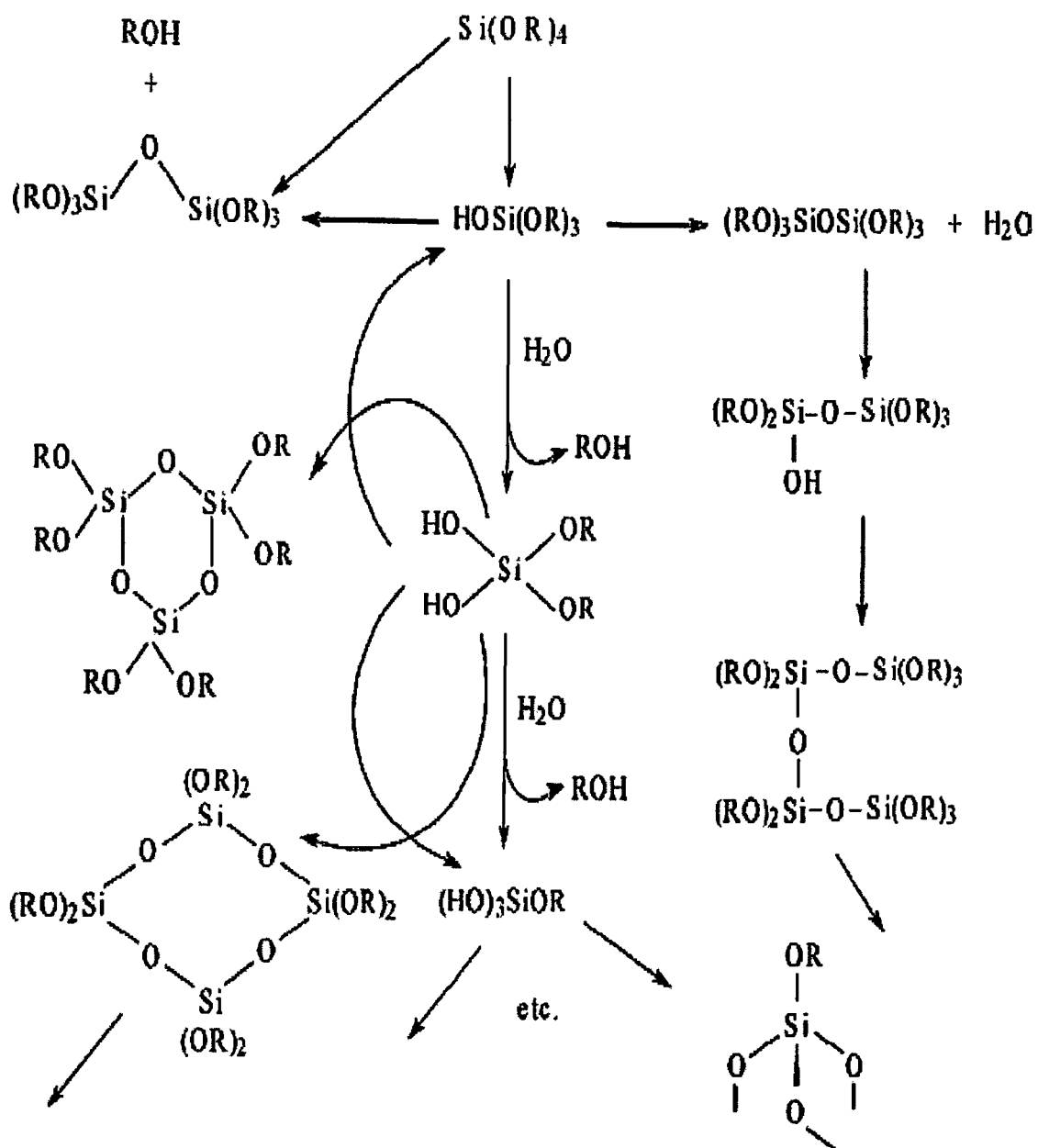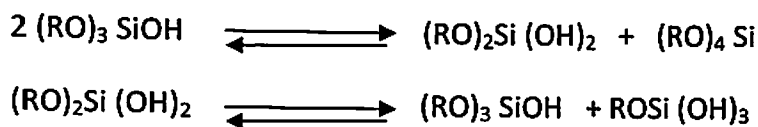Cyclic structures can be formed as well (Chruściel and Ślusarski, 2003). The condensation between this kind of intermediate polyhydroxylated species may be responsible for the gel formation. The above reactions are catalyzed by acids or bases and are accompanied by the redistribution reactions:
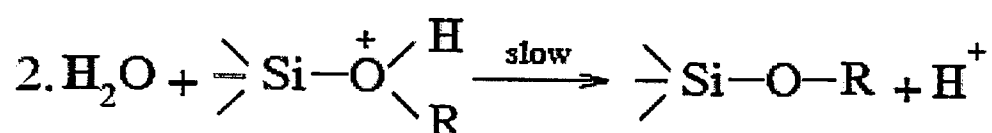
$$2\ (RO)_3\ SiOH \rightleftharpoons (RO)_2Si\ (OH)_2\ +\ (RO)_4\ Si$$

$$(RO)_2Si\ (OH)_2 \rightleftharpoons (RO)_3\ SiOH\ +\ ROSi\ (OH)_3$$

Thus, the proportions of Si–OH, Si(OH)$_2$, and Si(OH)$_3$ change during the process, which first gives a swollen gel. It is then dried, heated, and densified into the final monolithic piece of silica. The gelation may correspond to a crosslinking process taking place between macromolecular species of polysiloxanes containing free Si–OH bonds. In the presence of acids, the probability of formation of Si(OH)$_4$, which is produced by the hydrolysis of all four OR groups of an alkoxide molecule, is small and condensation reactions start before the complete hydrolysis of Si(OR)$_4$ to Si(OH)$_4$ can occur. Under basic conditions Si(OH)4 is easily formed by the preferential hydrolysis of the rest of the OR groups belonging to a partially hydrolyzed Si(OR)$_4$ molecule, and some silicon alkoxide molecules tend to remain non-hydrolized (Chruściel and Ślusarski, 2003)

The hydrolysis and condensation reactions involve a nucleophilic attack on the electrophilic silicon atom both in acid and basic catalysis, through the mechanisms depicted in Figure 2.3and 2.4 (Chruściel and Ślusarski, 2003). The catalyst and pH value of the solution, water concentration, silicon alkoxide concentration, the type of alkoxysilane and of solvent (Chruściel and Ślusarski, 2003) affect the reaction rates, the reaction mechanism

of the sol-gel transition, the gelation time, the nature of polymerized species formed in the sol, and the bulk nature of the gel.

Their structures depend on the precursor structure and deposition conditions (Brinker, 1994). It is possible to initiate the hydrolysis process of TEOS without presence solvent that is alcohol. It is done by exposing TEOS and water to ultrasounds with the help of sonicator in the presence of an acid catalyst. The gelling time of the 'sonogel' was 115–200 min for different ratios of water per 1 mole of TEOS. On the other hand, in classic method of gels, it is made by the dilution of 3 moles of alcohol per 1 mole of TEOS, the gelation may require several days (Corriu R.J.P. *et al.*, 1984)

Hydrolysis

1. $H^+ + \ \overset{\displaystyle\diagdown}{\underset{\displaystyle\diagup}{Si}} - OR \ \underset{\longleftarrow}{\overset{fast}{\longrightarrow}} \ \overset{\displaystyle\diagdown}{\underset{\displaystyle\diagup}{Si}} - \overset{+}{O} \overset{\diagup H}{\diagdown R}$

2. $H_2O + \ \overset{\displaystyle\diagdown}{\underset{\displaystyle\diagup}{Si}} - \overset{+}{O} \overset{\diagup H}{\diagdown R} \ \overset{slow}{\longrightarrow} \ \overset{\displaystyle\diagdown}{\underset{\displaystyle\diagup}{Si}} - O - R + H^+$

Condensation

1. $H^+ + \ \overset{\displaystyle\diagdown}{\underset{\displaystyle\diagup}{Si}} - OR \ \underset{\longleftarrow}{\overset{fast}{\longrightarrow}} \ \overset{\displaystyle\diagdown}{\underset{\displaystyle\diagup}{Si}} - \overset{+}{O} \overset{\diagup H}{\diagdown R}$

2. $\overset{\displaystyle\diagdown}{\underset{\displaystyle\diagup}{Si}} - OH + \ \overset{\displaystyle\diagdown}{\underset{\displaystyle\diagup}{Si}} - \overset{+}{O} \overset{\diagup H}{\diagdown R} \ \overset{slow}{\longrightarrow} \ \overset{\displaystyle\diagdown}{\underset{\displaystyle\diagup}{Si}} - O - Si \overset{\diagup}{\diagdown} + ROH$
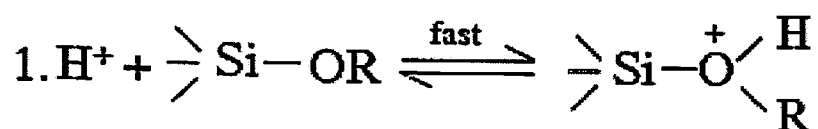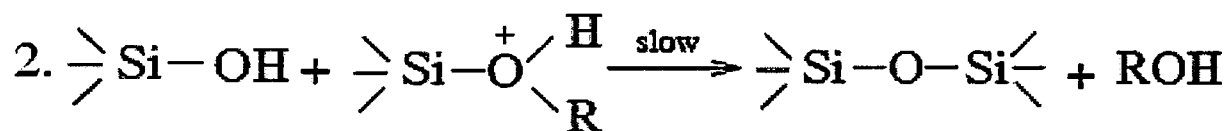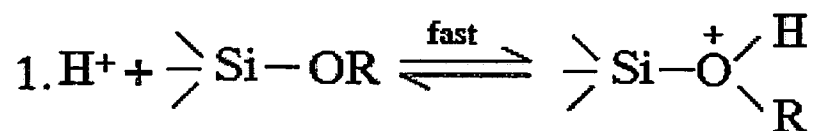
Figure 2.3 : Acid catalysis (Chruściel and Ślusarski, 2003)

Hydrolysis

$$1.\ OH^- + \underset{/}{\overset{\backslash}{\rule{0pt}{0pt}}}Si - OR \xrightarrow{\text{slow}} \underset{/}{\overset{\backslash}{\rule{0pt}{0pt}}}Si - OH + OR^-$$

$$2.\ OR^- + H_2O \xrightarrow{\text{fast}} ROH + OH^-$$

Condensation

$$1.\ \underset{/}{\overset{\backslash}{\rule{0pt}{0pt}}}Si - OH + OH^- \underset{\text{fast}}{\rightleftharpoons} \underset{/}{\overset{\backslash}{\rule{0pt}{0pt}}}Si - O^- + H_2O$$

$$2.\ \underset{/}{\overset{\backslash}{\rule{0pt}{0pt}}}Si - OH + \underset{/}{\overset{\backslash}{\rule{0pt}{0pt}}}Si - \overset{+}{O}\underset{\backslash R}{\overset{/H}{\rule{0pt}{0pt}}} \xrightarrow{\text{slow}} \underset{/}{\overset{\backslash}{\rule{0pt}{0pt}}}Si - O - Si\underset{\backslash}{\overset{/}{\rule{0pt}{0pt}}} + ROH$$
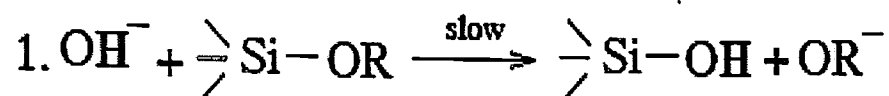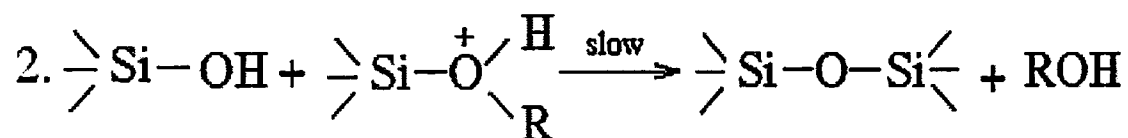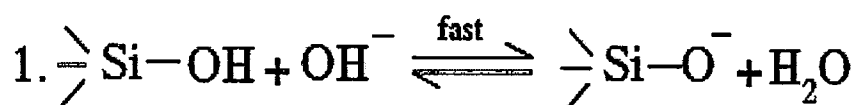
Figure 2.4 Basic catalysis (Chruściel and Ślusarski, 2003)

The combination of emulsion techniques with alkoxide hydrolysis offers a more versatile approach to preparing multicomponent oxide powders. Three general approaches can be applied. The first one involves preparation of water-in-oil emulsions, followed by the reaction of water droplets with alkoxide. In the second method, an aqueous sol is emulsified in an organic liquid and the sol droplets are then gelled. The third one involves emulsification of the alkoxide with an appropriate immiscible solvent, followed by addition of water to hydrolyze the alkoxide droplets (Chruściel and Ślusarski, 2003).

2.2 General Physico-chemical properties of nano-silica

2.2.1 Particle size and morphology.

Nanoparticles can be obtained by direct synthesis of silica sol (Cha *et al.*, 1999) or by crystallization of nano-sized crystals of quartz or porosils (Tosheva and Valtchev, 2005). The particle size is determined by the synthesis parameters which depends on the method of synthesis. Amorphous silica sol particles tend to take on the spherical shape so as to reach a minimum of interfacial surface area. The particle size of commercial silica sols prepared from sodium silicate is from 10 to 25 nm. Sols with larger primary particles can be prepared from TEOS by Stöber synthesis. For all nanomaterials, in aqueous environment, the primary nano-sized silica particles tend to form aggregates (Napierska *et al.*, 2010).

2.2.2 Porosity.

Pores are classified according to their diameter into micropores (< 2 nm), mesopores (2–50 nm) and macropores (> 50 nm). Amorphous sol particles can be microporous or nonporous (dense). The porosity of Stöber silica can be tuned by adapting the synthesis parameters: decreasing the ratio of water to TEOS promotes particle growth by aggregating smaller sub-particles, thus leading to rough particle surfaces with micropores (Napierska et al., 2010). In contrast, smooth particle surfaces are obtained with conditions of high ratio of water to TEOS.

2.2.3 Hydrophilic-hydrophobic properties.

The hydrophilicity (tendency to attract water molecules) of a silica material increases with the number of silanols, or silicon-bonded hydroxyl groups that presence in particular silica source. It also depends on the ability of forming hydrogen bonds with physical water molecules. The chemical formula of silica is represented as $SiO_2.xH_2O$. Water represents chemical water contained in silanol groups present on the surface of the silica material. The surface chemistry of silica is depicted in Figure 2.5.

Vicinal hydroxyl groups (one hydroxyl group per tetrahedron) located at mutual distances smaller than 3 nm are engaged in hydrogen bonding. Geminal hydroxyls (2 hydroxyl groups per tetrahedron) are considered to occur in minor amount of concentrations. Isolated silanols are positioned too far apart. Because of the differing chemistry of these 3 types of silanol groups, they are not all equivalent in their adsorption behavior or chemical reactivity. Vicinal hydroxyls interact strongly with water molecules and are responsible for the excellent water adsorption properties of silica.
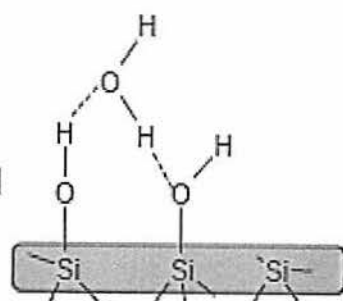
Hydrophilic surface
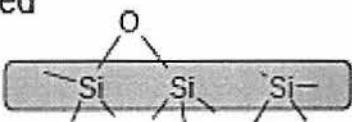
Geminal
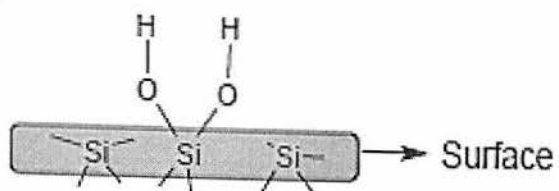
→ Surface

Vicinal,
anhydrous
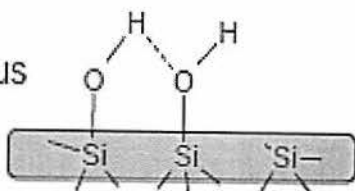
Vicinal,
hydrated

Hydrophobic surface

Siloxane,
dehydrated

Isolated
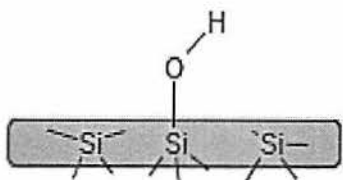

Figure 2.5: The surface chemistry of silica (Napierska *et al.*, 2010)

CHAPTER 3

Materials and Methods

Materials and Chemicals listing:

No.	Chemical	Company / Source
1	Tween 80	Sigma Aldrich
2	But-1- ol	Fisher Scientific
3	TEOS	Merck.
4	Deionised water	UKM, USM
5	Ammonia solution	Merck.

No.	Materials	Company / Source
1	Dialysis membrane	Spectrum laboratories
2	Synthesis Reactor	Favorit. Ltd
3	Syringe filter	Milipore

Table 3.1 Materials and Chemicals.

In the experiment, all the chemicals were of analytical grade and used without purification. Tetraethyl orthosilicate (TEOS, $Si(OEt)_4$) was used as a source of silica. In all the experiments were performed only using distilled water (pH= 7.00).

3.2 Methodology

3.2.1 Modified sol- gel process.

In this study, silica nanoparticles were synthesized by modified sol-gel process. We focused on the effects of the solvent volume which is the volume of 1-butanol. After 24 hours synthesis process, the silica nanoparticles had undergone 5 days of dialysis process in cold room at 5 °C. The methodology of the study has been divided into 2 phases. Firstly the micelles preparation and silica precursor is added. The second is the dialysis process of nanosilica particles.

For the first phase of water micelles preparation, 200 ml of water at pH 10 is prepared by adding NH_3 (25%) to distilled water using a digital pH meter. A total 5.5 g of Tween80 is weight and dissolved in 200ml of prepared water which is corrected to pH 10. The precursor is stirred about 15 minute for Tween 80 to dissolve completely. The pH of the suspension is measured with a digital pH meter.

The pH value of the suspension was made sure between 10.3 – 10.5. After this step, solvent is added which is 1-Butanol in our study. Table 3.2 shows different volumes of 1-Butanol that were used in this study. Solvent will form the upper layer in the beaker and we let the system stand still for 5 minutes.

Synthesis No.	Volume of Solvent (1-Butanol) ml
1	0
2	1
3	2
4	3
5	6
6	9
7	12
8	15
9	18

Table 3.2: Volume of 1-Butanol (ml) for each synthesis.

Then we let the system undergone magnetic agitation (rps = 4) for few minutes. Later the suspension was transferred in the thermostated reactor (250 ml) at 27°C with the stirring value not less than 320 rpm. Then, 2 ml of silica precursor, TEOS is added in the synthesis reactor and the solubility of the solution was checked. After the addition of the precursor, the system was let under stirring, not less than 320rpm for 24 hours.

Before the dialysis process started, 5 Liter of deionised (DI) water in a container was left overnight at 4°C in the cold room. After 24 hours of constant stirring the nano-silica suspension has undergone 5 days of dialysis. Firstly a dialysis membrane of 12 kDa was soaked with distilled water for 5 minutes. One end of the cellulose membrane was clipped and the solution was transferred into the cellulose membrane. The other end of the membrane was clipped too.

The membrane containing the suspension was transferred into a 2 liter glass beaker. The previously pre-cooled DI water was poured into the beaker. The beaker was then placed in the cold room at 4°C. The water was changed twice each day. The pH value of the dialysis water was checked constantly.