SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

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PROPERTIES OF ORGANOSILICA ENCAPSULATED DYE FOR BIOIMAGING APPLICATION

By

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(Materials Engineering)

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DECLARATION

I declare that I have led, finished the exploration work and composed the paper entitled "**Properties of organosilica encapsulated dye for bioimaging application**". I additionally declare that it has not been beforehand submitted for the honor of any degree or confirmation or other comparative title of this for some other examining body or University.

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Date :

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TABLE OF CONTENTS

Page

CHAPTER 1 INTRODUCTION

CHAPTER 2 LITERATURE REVIEW

CHAPTER 3 METHODOLOGY

CHAPTER 4 RESULTS AND DISCUSSION

CHAPTER 5 CONCLUSION

LIST OF FIGURES

Page

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	**Compound**
≡Si-O-Si≡	Siloxane
APMS	3-aminopropyltrimethoxysilane
APTES	3-aminopropyltriethoxysilane
SiNPs	silica nanoparticles
Ba^{2+}	Barium ion
$BaCl_2$	Barium chloride
AOT	bis(2-ethylhexyl) sulfosuccinate
CTAB	hexadecyltrimethylammonium bromide
Vtes	vinyltriethoxysilane
C_{14}MIMBr	1-tetradecyl-3- methylimidazolium bromide
Ca^{2+}	Calcium ion
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
CMC	Critical micelles concentration
CMPS	colloidal mesoporous silica
DDS	Drug delivery system
DLS	Dynamic light scattering
H_2O	Water
HAS	Human serum protein
HCl	Hydrochloric acid
IEP	Isoeletric point
II-14-3	trimethylene-di(tetradecacyloxyethyl-dimethylammoniumbromide)
INH	Isoniazid
Mg^{2+}	Magnesium ion
$MgCl_2$	Magnesium chloride
MHSNS	Mesoporous hollow silica nanoparticles

MPTMS	3-mercaptopropyl trimethoxysilane
MSNs	Mesoporous silica nanoparticles
Na^+	Sodium ion
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NH_3	Ammonia
NH_4OH	Ammonium
NIR	Near infra red
NP5	polyoxyethylene (5) nonylphenylether
NPs	Nanoparticles
O/W	Oil in Water
OD	Optical density
OH^-	Hydroxyl ion
ORMOSIL	Organically modified silica
PBS	Phosphate buffer solution/saline
PDI	polydispersity index
PEO	Polyethylene oxide
PS	Polystyrene
PT	Propane-triol
PTMS	Phenyltrimethoxysilane
ROH	Alcohol
ROS	Reactive oxygen species
RT	Room temperature
Ru(bpy)	Ruthenium-tris(2,2′-bipyridyl) dichloride
SD	Standard deviation
SEM	Scanning electron microscope
$=Si(OH)_2$	Silanediols or geminal silanols
$Si(OH)_4$	Silicic acid
$Si(OR)_4$	Silica precursor
$Si{\equiv}OH$	Silanol group
Si-Cl	Silica chlorination

SiINH	Silica nanoparticles colloids entrapped Isoniazid
SiRIF	Silica nanoparticles colloids entrapped Rifampicin
SiRN	Silica nanoparticle colloids entrapped Nile Red
TEM	Transmission electron microscope
TEOS	Tetraethyl orthosilicates
TMOS	Tetramethyl orthosilicates
TPOS	Tetrapropyl orthosilicates
TRIS	tris- (hydroxymethyl) aminomethane
TSTMP	trisodium trimetaphosphate
Tween 80	Polysorbate 80
UV-Vis	UV visible spectrophotometer
VTES	Vinyltriethoxysilane
VTMS	Vinyltrimethoxysilane
W/O	Water in Oil
XRD	X-ray diffractometer

LIST OF SYMBOLS

nm	Nanometer
µm	micrometer
mm	milimeter
l	liter
ml	mililiter
µl	microliter
g	gram
µg	microgram
λ	Wavelength
°C	Degree Celcius
°	Degree
θ	theta
d	diameter
M	Molarity
v/v	Volume per volume
wt	weight
%	percent
h	hour
min	minute
rpm	Revolutions per minute
ζ	potential
±	Plus minus
r_m	Radius

SIFAT-SIFAT ORGANOSILICA YANG DIPERLUKAN UNTUK APLIKASI BIOIMAGING

ABSTRAK

Dalam kajian ini koloid nanopartikel silika (SiNPs) telah disintesis dengan menggunakan kaedah pembentukan misel. Isipadu pelarut 2-butanol diubah dari 2, 4 dan 6 ml. Saiz zarah diperoleh dari TEM meningkat dari 30.46, 34.354 dan 42.47 nm dengan peningkatan isipadu 2-butanol dari 2, 4 dan 6 ml. Kesan penambahan D-glukosa dan trisodium trimetaphosphate (TSTMP) sebagai campuran excipien pada keberkesanan penapisan telah dikaji. Tambahan excipien ini sepatutnya menyediakan salutan biologi tambahan pada permukaan silika yang meningkatkan kestabilan dan keserasianbio untuk analisis *in vitro*. Keberkesanan penapisan selepas penambahan TSTMP menunjukkan isipadu 2-butanol yang lebih besar juga memberikan keberkesanan penapisan yang lebih rendah berbanding dengan isipadu yang lebih kecil (98.03% - 89.82%). Dalam kajian ini, perwarna merah Nile digunakan sebagai model perwarna untuk menghasilkan silika yang dirumuskan oleh pewarna merah Nile (SiNR). Kestabilan SiNR pelbagai saiz dalam larutan natrium klorida (NaCl) dan serum tetikus dianalisis. Peningkatan kepekatan NaCl meningkat menyebabkan kestabilan menurun. Hasil yang sama diperolehi untuk kestabilan SiNR di dalam serum tetikus, di mana kestabilan SiNR menurun dengan meningkatkan kepekatan serum tetikus. Kestabilan optimum diperolehi dari SiNR yang dihasilkan menggunakan 4 ml 2-butanol. Dalam medium biologi, kecekapan kestabilan SiNR dalam NaCl dengan kepekatan 0.1, 0.5 dan 1.0 M adalah 57.7%, 56.05 dan 55.6% dan kestabilan dalam serum tetikus dengan kepekatan 5, 10 dan 25% adalah 58.9, 51.7 dan 34.3%. Fotostabiliti pelbagai saiz SiNR diuji di bawah penyinaran lampu halogen 100 watt selama

1 jam untuk memerhatikan fotodegradasi SiNR.Kecekapan penyinaran isipadu 4 ml 2-butanol lebih rendah (27.25%) berbanding isipadu 2 dan 6 ml 2-butanol.

PROPERTIES OF ORGANOSILICA ENCAPSULATED DYE FOR BIOIMAGING APPLICATION

ABSTRACT

In this work silica nanoparticles (SiNPs) colloids were synthesized by using micelle formation approach. The volume of 2-butanol co-solvent was varied from 2, 4 and 6 ml. Particle sizes were observed from TEM increased from 30.46, 34.354, and 42.47 nm with increasing amount of 2-butanol from 2, 4 and 6 ml respectively. The effect of D-glucose and trisodium trimetaphosphate (TSTMP) as excipient mixture on filtration efficacy was studied. This excipient addition is supposed to provide additional biological coating on the silica surface that enhances stability and biocompatibility for *in vitro* analysis. Filtration efficacy after the addition of TSTMP showed higher volume of 2-butanol gave lower filtration efficacy compared to smaller volume (98.03% - 89.82%). In this study, Nile red dye was used as a dye model to produce silica encapsulated Nile red dye (SiNR). The stability of SiNR of various sizes in sodium chloride (NaCl) solution and mouse serum volume rates were analysed. As the concentration of NaCl increased, the stability efficiency decreased. Similar result was obtained for stability of SiNR in mouse serum, where by the stability of SiNR decreased with increasing the concentration of mouse serum.The optimum stability of SiRN was obtained was volume of 4 ml of 2-butanol.In biological medium, the stability efficiency of SiNR in NaCl with concentration of 0.1, 0.5 and 1.0 M are 57.7%, 56.05 and 55.6% respectively and stability of SiNR in mouse serum concentration of 5, 10 and 25% are 58.9, 51.7 and 34.3% respectively. Decolorization

efficiency of SiNR produced using 4 ml of 2-butanol is lower (27.25%) compared to SiRN produced using 2 and 6 ml of 2-butanol.

CHAPTER 1

INTRODUCTION

1.1 Research background

Nanotechnology can be characterized as materials and system where the components and structures exhibit novel and altogether enhanced physical, substance and organic properties, wonders and procedures due to their nanoscale estimate i.e. in a range of 1-100nm. Nanotechnologies have turned out to be one of the promising exploration areas, which may bring a critical advance into material and device improvement (Provenzale et al., 2009).

Lately, silica nanoparticles (SiNPs) are one of the perfect materials for marking in bioanalysis and biotechnological applications. Silica nanopartilces (SiNPs) have demonstrated their shell is biocompatible and non-poisonous (Zeng et al., 2002), shields fluoroscent dye molecules from the surrounding condition and encapsulated the dyes in its system (Wang et al., 1998), giving photostability and signal enhancement, separately (Vansant et al., 1995). In addition, simple surface change of silica advances conjugation of biomolecules, for example, proteins, peptides, antibodies, oligonucleotides, and so forth (Bringley et al., 2008). There have two common techniques have been synthesis of silica-based nanoparticles: the reverse microemulsion (Santra et al., 2001) and the Stöber strategy (Sharma et al., 2006). These strategies offer certain advantages with a few limitation. For example, in the reverse microemulsion technique, excellent consistency of the particles at the nanometer scale can be gotten and their surfaces are easily modified with functional group including thiol, amine, and carboxylate amid response (Kickelbick et al., 2003). Then again, dye molecules can be discharged from silica matrix as a result of the absence of the covalent connection of a fluorophore to the silica lattice, leading decreased brightness (Lindberg et al., 1995). What's more, this strategy requires huge measure of surfactants and natural solvents. On the other hand, the method presented by Stöber in 1968 (Arriagada et al., 1994) is generally the decision for the planning of pure silica particles and hydrophobic natural encapsulated dye particles (Arriagada et al., 1999). This technique is relatively basic, yet restricted by the consistency of particles, especially those in the sub-50 nm go. Consequently, filtration and further separation technique are normally connected to get particles with uniform size distribution. Studies pointing of controlling particle size have been focussed on how nanoparticle synthesis is affected by the relative measures of reagents utilized (Van Blaaderen et al., 1992). This paper will concentrate on the part of synthesis, size dependent properties, stability of Silica Nanoparticles (SiNPs) and photostability of SiNPs under irradiation of halogen lamp.

1.2 Problem statement

Fluorescent imaging is a generally utilized device in biological research. The increasing expanding interest for checking different biological procedures at the same time calls for fluorescent probes that can meet numerous necessities, including, however not restricted to high stability and sensitivity in complex conditions, great solubility and an appropriate emission spectrum. The one of a kind attributes, great biocompatibility, non-toxicity, high hydrophily, optically transparent, size-tunable and simplicity to covalently connect to diverse biomolecules, have enabled SiNPs to build fluorescent probes for successful imaging inside living cell and at the small animal level. Due to the robust and bright discharging light, fluorescent SiNPs have risen as potential fluorescent labels. Although enormous advances have been made utilizing SiNPs in labeling, detachment, bioimaging, disease diagnosis and treatment, a few limitation hinder their further advancement. Right off the bat, the leakage of dye from the fluorescent SiNPs over time, which could essentially shrink the fluorescence intensity of the particle and increase the background signal, require encourage examination. Be that as it may, organic fluorescent dye have many drawbacks, particularly quick photobleaching. The photobleaching of the dye is an after effect of the numerous molecules in cellular matrix capable of including photochemical responses. One solution is the numerous molecules in cellular matrix capable for inducing photochemical responses. One solution is to encapsulated the fluorescent dyes, which shields them from chemical reaction, and along these lines, the chemical stability and biocompatibility of the fluorescent dye can be incredibly upgraded. Numerous sorts of encapsulated approaches have been proposed, including utilizing lipid micelles, polymer matrix or silica network (Guo et al. 2014). Silica network turned into a more attractive decision because of those attributes, for example, biocompatibility, non-toxicity, water-solubility, size control and simple adjustment.Thirdly, it is vital to standardize the technique of the synthesis and functionalization to guarantee that SiNPs have a great dispensability in aqueous solution, controllable size and uniform multifunction. In addition, to accomplish ultrasensitive examination, numerous fluorescent dye molecules should be co-typifying inside one single SiNPS (Zhang et al. 2016).

This principle subject of nanomaterials is devoted to the utilization of nanoparticulate materials in biological imaging. The point highlights the rapidly extending utilizations and interest in nanomaterials for bioapplications; specifically, bioimaging. A few highlights of the distributions in the topic are discussed. In their review article, Zhang et al. (2016) exhibit current achievements in the advancement of new dye loaded fluorescent silica nanoparticles (SiNPs) for non-invasice fluorescence bioimaging which that both in vitro and in vivo. SiNPs are easy to functionalise and show great biocompatibility, low poisonous quality, high hydrophilicity and phenomenal optical transparency. From these properties, SiNPs are extremely appropriate for the fabrication of fluorescent probes utilized as a part of the compelling imaging of living cells and little creatures. It likewise talked about in various difficulties that limit applications of these nanomaterials, for example, leakage of dye from the fluorescent SiNPs, rapid photobelaching for traditional organic fluorescent dye and their dispersibility in fluid media.

In this work, the challenge could be overcome by synthesis the silica NPs colloids utilizing the micelles development approach. In this technique, a lesser measure of surfactant was utilized without extra functionalizer. Additionally, the achievement rate of silica NPs encapsulated dye is promising with finish evacuation of surfactant was completed by means of dialysis handle. This technique offers a simple procedure of silica nanoparticle doped whereby the dye molecules are specifically entrapped inside the micelle structure before development of colloidal silica NPs. Silica NPs colloids encapsulated dye (SiNR) with uniform particles size distribution can be controlled by differing the synthesis parameters, for example, stirring rate, amount of surfactant, butanol volume, and color focus. What's more, stability of SiNR in salt arrangement and organic media (NaCl and mouse serum) is additionally inspected to evaluate the performance of SiNR as a carrier. Also, dye discharge properties under irradiation are systematically considered by varying the photostability under irradiation by using halogen lamp for 60 minutes.

1.3 Objectives

The main objectives of this research are:

1. To synthesis and characterize silica NPs colloids encapsulated fluorescent dye (SiNR).
2. To study stability of silica NPs colloids encapsulated fluorescent dye with dye in biological media by using sodium chloride (NaCl) and Mouse serum.
3. To study the photostability effect under irradiation of silica NPs colloidsencapsulated fluorescent dye of halagon lamp.

1.4 Thesis scope

This thesis comprises of five sections. Part 1 examines on the Introduction, problems statement and objectives of the research. Literature review of related works is discussed in Chapter 2. While in Chapter 3, experimental details and characterization used are explainedin details. In Chapter 4, the results and discussions of this research are presented. Finally, conclusion and recommendations for future work are stated in Chapter 5.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Nanotechnology is utilization and control of matter at a very small scale. Particles and molecules work differently at this size, and give variety of surprising and fascinating properties. Nanotechnology have given chances to the advancement of materials, including those for medical applications, where conventional procedures may reach their limits. Nanotechnology should not be seen as a single technique that only influences particular area. Despite the fact that regularly referred to as the 'tiny science', nanotechnology does not just mean small products and structures. Nanoscale highlights are frequently incorporated into bulk materials and large surfaces. Nanotechnology speaks to the design, creation and application of materials at atomic, sub-atomic and macromolecular scales, so as to deliver new nanosized materials (Van Blaaderen, & Vrij, 1992).

Based of the properties with high effectiveness, affectability and selectivity, nanoparticles (NPs, for example, natural fluorescent dye have been generally utilized as a part of bioimaging applications (Van Blaaderen, & Vrij, 1993). Be that as it may, organic fluorescent dye have many drawbacks, particularly quick photobleaching. The photobleaching of the dye is an after effect of the numerous molecules in cellular matrix capable of including photochemical responses. One of solution is the numerous molecules in cellular matrix capable for inducing photochemical responses. To overcome this limitationis to encapsulated the fluorescent dyes, which shields them from chemical reaction, and along these lines, the chemical stability and biocompatibility of the fluorescent dye can be incredibly upgraded. Numerous sorts of encapsulated approaches have been proposed, including utilizing lipid micelles, polymer matrix or silica network (Guo et al., 2014). Silica network is an attractive decision because of those attributes, for example, biocompatibility, non-toxicity, water-solubility, size control and simple adjustment.

2.2 Silica nanoparticles (SiNPs)

Silica-based NPs (SiNPs) are as recently used as a part of numerous areas of bioanalysis. Compared to polymer-based NPs, which have been utilized regularly in bioanalysis and labeling, SiNPshave demonstrated less aggregation and it simply have little dye leakage (Yang et al., 2013). By utilizing reasonable synthetic conditions, a major number of dye molecules either organic or inorganic can be fused inside a single silica molecule (there can be a huge number of dye molecules) (Figure 2.1). Dye doped nanoparticles (NPs) create a highly amplified optical signal compared with single dye molecule. On the off chance that connected properly in bioanalysis, SiNPs can improve analytical sensitivity (Figure 2.2). Besides, as the dyeis caught inside the silica matrix, which gives a powerful barrier keeping the dye from the surrounding environment, both photobleaching and photodegradation can be minimized (Bae et al., 2013).

The excellent photostability makes these NPs suitable for applications where high intensity or delayed excitations are required. For instance, intracellular optical imaging experiences extremely photobleaching. With SiNPs, this issue can be minimized and avoided. The flexible silica chemistry gives versatile routes to surface change. Different types of functional groups can be effectively presented onto the NPs for conjugation with biomolecules. Furthermore, the silica surface makes these NPs chemically inert and physically stable (Santraet al., 2005). These properties make silica NPs magnificent labelling reagents for bioanalysis and bioimaging (Tan et al., 2004).

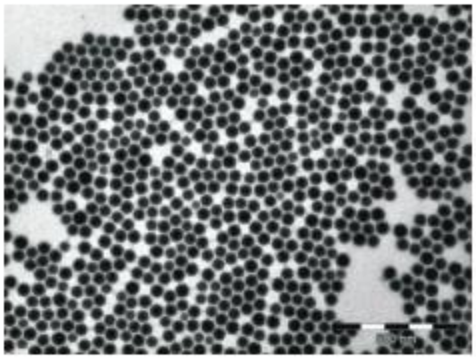


Figure 2.1: Transmission electron microscopy (TEM) image of Rubpy dye-doped silica NPs. Scale bar = 500 nm (Tan et al., 2004)

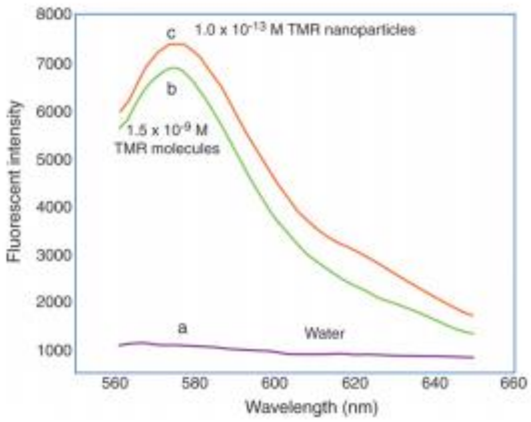


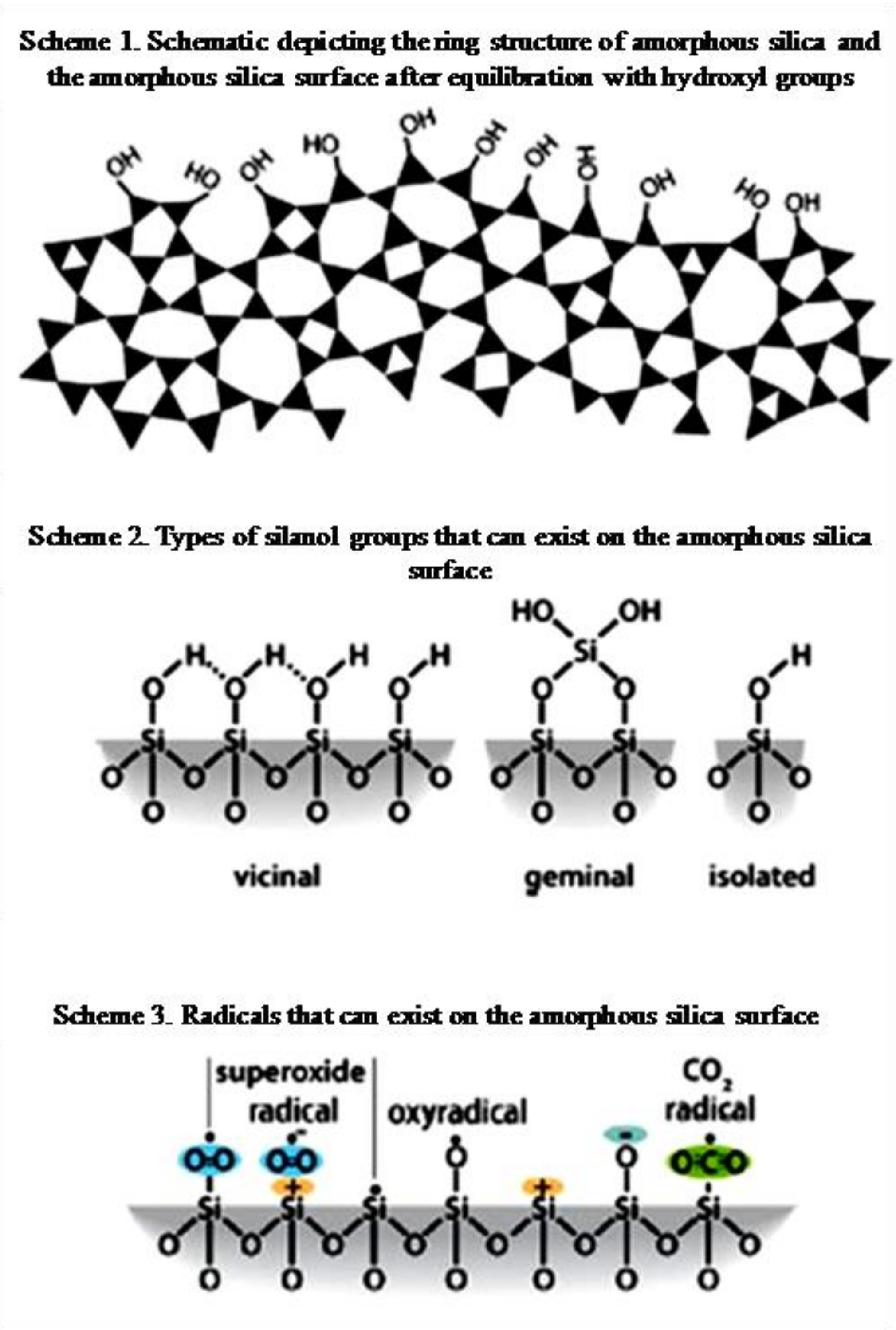
Figure 2.2 : Fluorescent signal amplification using dye-doped NPs: (a) pure water (control); (b) tetramethylrhodamine (TMR) dye, 1.5 x 10-9 M; (c) TMR-doped NPs, 1 x 10-13 M (Smith et al., 2006).

2.2.3 Properties of SiNPs

The properties of SiNPssuch as water-soluble, non-toxic, odourless particles which can undoubtedly be synthesized easily at low cost (Metin, Bonnecaze, &Nguyen, 2012). SiNPsare described by their low bulk density, which around 0.04 – 0.1 g/cm^3 , and a high specific surface area, commonly 200 – 300 m^2/g (Napierska et al., 2010).

There are a few approaches to produce amorphous silica. Fumed or pyrolitic silica can be produced by pyrolysis process which utilizes high temperature (1200-1400 °C). Also, colloidal, precipitates and mesoporous silica can be produced by utilizing hydrolysis and condensation reaction aqueous solution or hydrothermal process (Zhang et al., 2012). Not at all like crystalline silica, amorphous silica has short-run order and its structure is strongly dependent to kinetic and environmental impact as a result of a weak energy site. Schematic diagram in Figure 2.3 shows how amorphous silica structure is built from the siloxane framework architecture, which comprises of combination of closed siloxane rings (Figure 2.3, Scheme 1), alongside the pattern, concentration, and extent of hydrogen bonding of silanol gatherings (=Si−OH) that terminate the siloxane rings at the silica surface (Figure 2.3, Scheme 2). Silica could contain generally high concentration of surface-related radicals (Figure 2.3, Scheme 3) as it has high surface zone (Zhang et al., 2012).

Water adsorbs specially on the remaining silanols which can be separated into three group: isolated silanols, geminal silanols and vicinal silanols on the siloxanes. The most active silanol group is geminal silanols however their concentration on the surface is the lowest (10–12 %). Isolated silanols concentration is the most astounding (60–65 %), while vicinal silanols on the siloxanes (Si-O-Si) make the rest (Buszewski et al., 2012). Physical and chemical properties of various sorts of amorphous silica like pyrogenic, accelerated and colloidal silicaare listed in Table 2.1 (Fruijtier et al., 2012).



Scheme 1. Schematic depicting the ring structure of amorphous silica and the amorphous silica surface after equilibration with hydroxyl groups

Scheme 2. Types of silanol groups that can exist on the amorphous silica surface

Scheme 3. Radicals that can exist on the amorphous silica surface

Figure 2.3: Schematic diagrams of amorphous silica structures and reaction on its surface (Zhang et al., 2012).

Table 2.1: Physical and chemical properties of different amorphous silica (Fruijtier et al., 2012)

Property [units]	Pyrogenic	Precipitated	Colloidal
Space group	Not measurable for amorphous materials		
SiO_2 content [wt%]	≥ 99.8	> 95%; gel:>95% (dry)	≥ 99.5
Loss of drying [%]	< 2.5	5-7; gel: 2-6	>> 2.5
Melting point [°C]	1600-1725	1600-1725	1600-1725
Density (g/cm^3 at 20 °C)	2.2	2.2	2.2
Water solubility (saturation) [mg/L] at 37 °C and pH 7.1-7.4	144-151: (15-68 at 20 °C and pH 5.5-6.6)	141; gel: 127-141	Colloidal dispersion in water
Specific surface area (SSA)	50-400	30-500; gel: 250-1000	50-380 (spherical) ~640 (porous)
Zeta potential	Negative	Negative	Usually negative, can be positive with certain stabilizer
Behavior towards water	Hydrophilic	Hydrophilic	Hydrophilic
Porosity	Microporous	Meso- or macroporous	-

2.3 Synthesis of SiNPs

2.3.1 Introduction

SiNPs can be synthesized as highly monodisperse, exceedingly circular nanoparticles. The basic advances in silica nanochemistry depend on the hydrolysis and condensation of tetraethylorthosilane (TEOS) (Figure 2.4), or the sol-gel approach (Bourgeat-Lami, & Lang, 1998).

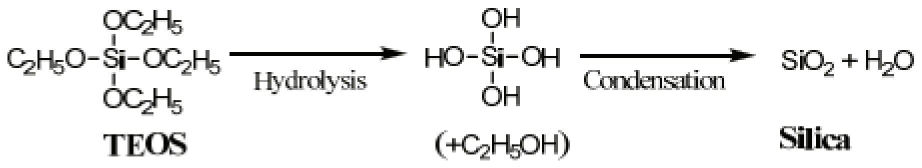


Figure 2.4: Hydrolysis and condensation of TEOS (Bourgeat-Lami, & Lang, 1998).

The hydrolysis of TEOS can be catalyzed by both base and acid. Water in the system starts the hydrolysis and forms monosilicic acid. As Iler express that the general hypothesis of polymerization of silica in aqueous system (Figure 2.5) (Fripiatet al., 1963). At the point when the solution of monomer, $Si(OH)_4$, is formed at a concentration higher than the solvency of the solid amorphous silica without solid phase on which the solvent silica may be deposited, at that point the monomer is polymerizes by condensation. Therefore, at pH = 7–10, the silica nanoparticles are negatively charged and repulsed each other. Consequently, the particles may proceed with development without aggregation. Above pH 10, the silica starts to dissolve as silicate. Nonetheless, at low pH, the silica particles bear next to very little ionic charge. The particles can aggregate and collide into chains and form shape gel networks. This additionally happens when saltis introduced at high pH, because salts can neutralize the silica (Fripiat et al., 1963).

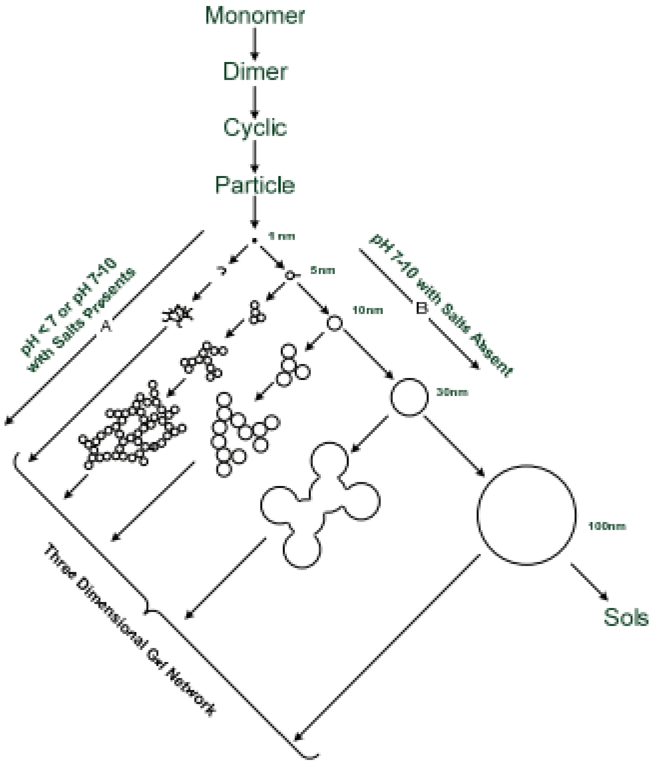


Figure 2.5: Polymerization of silica. In basic solution (B) particles in sols grow in size with decrease in number; in acid solution or in presence of flocculating salts (A), particles aggregate into three-dimensional networks and form gels (Fripiat et al., 1963).

2.3.2 Stöber Method

This name of this technique takes from a name Werner Stöber, who found the controlled synthesis of colloidal silica in 1968 (W. Stöber et al., 1968) in light of a past work by Kolbe , and later further created by van Blaaderen et al., (1993). This method includes the ammonia catalyzed hydrolysis and condensation of tetraethyl orthosilicate (Teos) in an ethanol/water mixture.

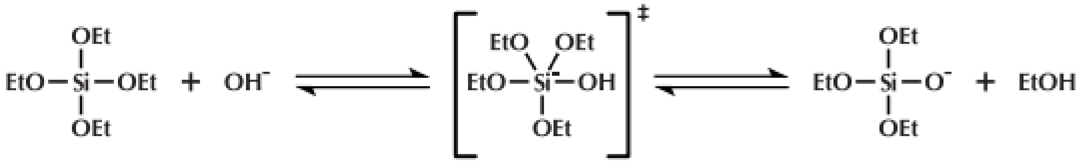


Figure 2.6: Reaction mechanism for the base catalyzed hydrolysis of TEOS (van Blaaderen et al., 1993).

The hydrolysis step (Figure 2.7) is comprehended to advance through a bimolecular nucleophilic substitution (Sn^2) on the silicon atom, which is characterized by a negatively charged, pentacoordinate transition state (Cushing et al., 2004). The OH^- as nucleophile is included in this progression, subsequently by increasing the concentration of NH_3 as well as H_2O, it can prompts an expansion in the reaction rate. In addition, since the hydrolysis decreases the electron density on the silicon particle, OH is more electron and pulling back than OEt – while the negative charge of the transition state is more balanced out for each subsequent step; consequently, the rate-deciding step for monomers is the hydrolysis of the principal ethoxy aggregate (Cushing et al., 2004)

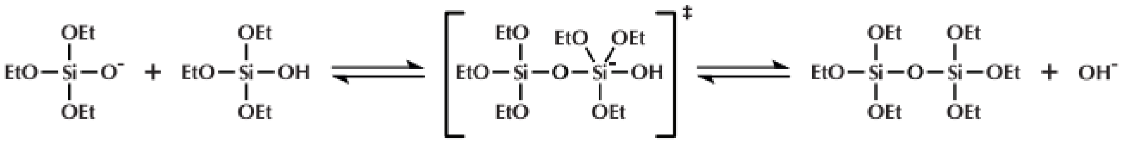


Figure 2.7: Reaction mechanism for the base catalyzed hydrolysis of teos (Cushing et al., 2004).

The condensation step (Figure 2.8) is similar with hydrolysis process. Condensation step continues through a Sn^2 sort nucleophilic substitution at the silicon atom, which can brings about a negatively charged, pentacoordinate transition rate; the nucleophile is a deprotonated silanol group.The reaction rate is affected by the concentration of NH_3 as well as H_2O, and it can decrease the electron density on the silicon particle – OSi is more electron pulling back than OH.

The method includes more consolidated species with more vulnerable both to hydrolysis and to condensation, in view of the acidity of the silanol groups are expanded too. The rate of condensation is significantly quicker than the rate of hydrolysis. The most likely reaction in solution is between a monomer and a higher polymerized species (condensation between the extensive siloxane moieties is obstructed by steric impacts), which prompts the development of a spheroidal molecule.

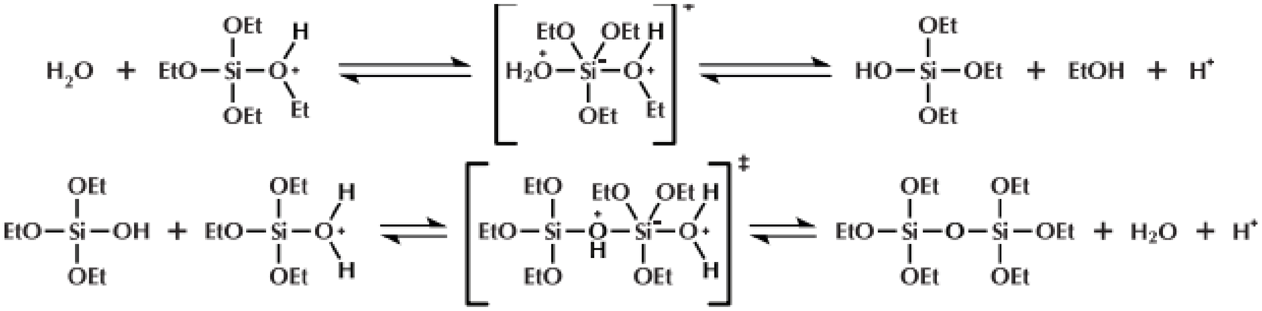


Figure 2.8: Reaction mechanism for the acid catalyzed hydrolysis (above) and condensation (below) of TEOS (van Blaaderen et al., 1992).

Indeed, these two reactions may happen in acidic solution too (Figure 2.9). With acid catalysis, the previously mentioned impacts are turned around: the transition state is presently positively charged and in this manner is less stabilized for every hydrolysis or condensation step, whose rate is decreased as the polymerization procedure continues.

Hydrolysis turns into the quicker procedure. Hence, rather than spheroidal agglomerates, chains and branches are specially formed, which cross-link in the long run happen at later stages. It is cleae the morphology of the final structure is firmly subject to the catalysis utilized (Gallagher et al., 1989).

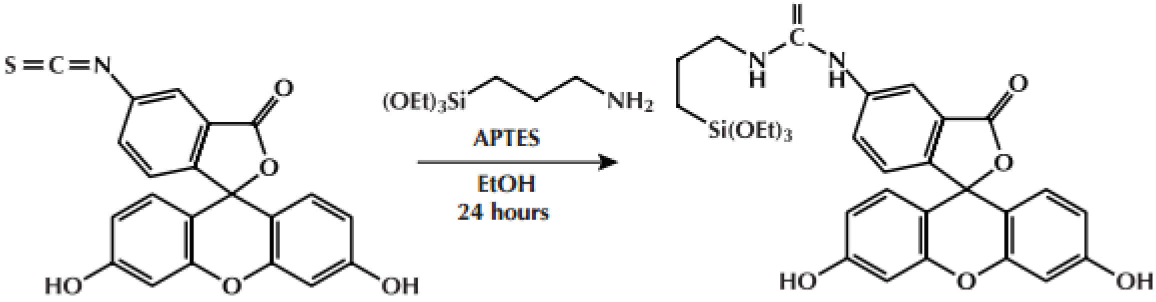


Figure 2.9: Exemplifying synthesis of a trialkoxysilane functionalized fluorophore: coupling of fluorescein isothiocyanate with Aptes as in reference (van Blaaderen et al., 1992).

The preparation of the primary dye doped silica nanoparticles was adapted from van Blaaderen's concept of altering the structure of fluorophores, by coupling (3-aminopropyl)triethoxysilane (Aptes) with fluorescein isothiocyanate (Figure 2.9) (van Blaaderen et al., 1992) and rhodamine B isothiocyanate The after effect of triethoxysilane derivatized particles is then ready to co-consolidate with TEOS amid the nanoparticle development, yielding system in which the organic dyes are covalently grafted to the silica matrix (Figure 2.10). This is a crucial change, due to separation from minimizing leaching issues, permits to achieve high doping levels (Verhaegh et al., 1994).

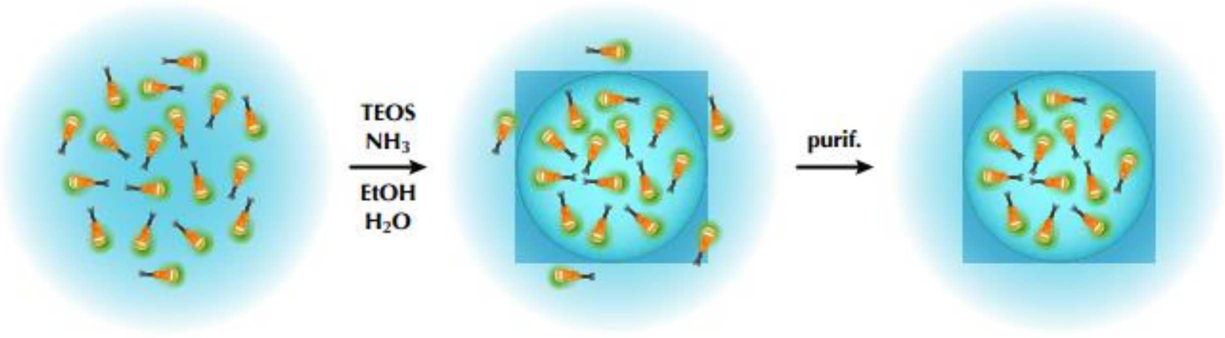


Figure 2.10: Schematic representation of the Stöber synthesis of fluorescein isothiocyanate doped silica nanoparticles (Blaaderen et al., 1992).

It should to be reminded that the inclusion inside the nanoparticles prompts a change of the photophysical properties of the fluorophores, for example, quantum yield or photostability (Larson et al., 2005). This isolation diminishes the interaction of the dyes with external interfering agent as the dissolvable or sub-atomic oxygen, that is regularly in charge of luminescence quenching – and additionally their excited state conformational mobility (Rampazzo et al., 2007). These components hinder non-radiative decay pathways and could counteract photoreactions that comes from the irreversible degradation of the fluorophores. Besides, due to the water compatibility of silica, numerous hydrophobic fluorophores can be effectively moved in water – and in this manner their properties exploited – as a result of their inclusion inside the nanoparticle.

The Stöber technique has been studied for the impact of different synthetic the parameters, e.g. the concentration, temperature, or nature of the solvent, reactants, on the size of the prepared particles. Subsequently, it is realized that the particles size change by changing these variables. Reaction temperature is an essential parameter when tuning the size of the particles. The diameter of the particles decreases with increasing reaction temperature (Bogush et al., 1991). When the reaction of temperature increases, hydrolysis and condensation reaction rates also increase, which increases the nucleation rate. Furthermore, it has been reported for that linear development and formation of cyclic structures of silica particles happen all the while. However, the latter can happen more rapidly at elevated temperatures (Wang et al., 2011).

The select of dissolvable, for example, a low molar-mass alcohol, is additionally an essential parameter to consider while preparing silica particles. For instance the polarity and diffusivity of TEOS as solvent. The diffusivity of TEOS in the solvent, is identified with its viscosity decrease with expanding sub-atomic weight of the solvent. Moreover, the polarity of the solvent affect the final size of the particles. Alcohol solvents have diverse relative dielectric constants (methanol: 32.6, ethanol: 24.3, propanol: 20.1), which influences the particle size in the development procedure (Iler et al., 1997). A solvent with a lower dielectric consistent can prompt a circumstance where the repulsive force between particles decreases compared to van der Waals attractive force. In this manner, the development of the particles is favored. Furthermore, the lower the dielectric constant of the solvent, the bigger the particles is formed. The particles arranged in methanol are smaller than the one arranged in ethanol or propanol (Hartlen et al., 2008).

The concentration of ammonia being utilized as catalyst as a part of the Stöber strategy is additionally an important factor in the final size of the particles. As a catalyst, ammonia advances the hydrolysis and condensation reaction of TEOS through increasing concentration of hydroxide particles (OH-) (Kremer et al., 1996). Increasing initial concentration of ammoniaforms many nuclei that later produces smaller particles. However, another imperative variable must be taken over while considering the impact of ammonia concentration. The ionic strength of the suspension increases with the ammonia concentration, which decreases double layer thickness of the particles (Harris et al., 1990). The static repulsive force between the particles is believed to be overwhelmed by the van der Waals attractive force, prompting the arrangement of bigger particles. The balance between both contribution, for example, the ionic strength caused by the ammonia concentration and its catalyst affect hydrolysis of TEOS is firmly dependant on the water substance of the suspension. As revealed by Bogush et al. (1991) the sizeof the particles increases with the concentration of water and ammonia until the point that it achieves maximum, after which the particles become smaller. The impact of ammonia concentration relying upon a low or high water content has been reported in the literature (Abbas, et al., 2008).

2.3.3 Microemulsion

2.3.3.1 Basic Concepts of Microemulsion

Microemulsions are clear, isotropic liquid mixture of water, oil, surfactant and in some cases co-surfactant which was first revealed in 1943 by Hoar and Schulman. Dissimilar with traditional macroemulsion, a microemulsion is thermodynamically steady (Akartuna et al., 2009). In the present model of microemulsion, surfactant molecules form spherical aggregates in the continuous stage. In the event that water is in the centre of the aggregates, it is called water-in-oil (w/o) microemulsion, or reverse micelle and is characterized by the molar ratio of water to surfactant

$$(S),\ w0 : w_0 = [H2O]/[S] \qquad (2\text{-}1)$$

Then the relationship between w_0 and the micellar radius $r_m$can be established:

$$r_m = \frac{3Vs}{\sum s} + \frac{3VwW0}{\sum s} \qquad (2.2)$$

where, Σ_S is the molar interfacial area at the surfactant-oil boundary; V_S , Vw is the molar volume of surfactant and water, respectively (Akartuna et al., 2009).

The linear relationship among r_m and w_0 has been tentatively confirmed in different sodium bis(2-ethylhexyl) sulfosuccinate (AOT) and hexadecyltrimethylammonium bromide (CTAB) reverse micellar system over a basic w_0 (Stump et al., 1999). In this way, the extent of the water droplets can be controlled by modifying w_0. The constant Brownian movement of switch micelles affect the exchange of materials in the water droplets and inevitable balance distribution of all substance (Essawyet al., 2010). On the basic of the above model of reverse micelles, the advantage of reverse micelles as nanoreactors are self-evident. The reaction can be kept in a constrained space of which that the size can be decisively controlled. The products have uniform and controllable size and shape.

Same with Stöber's approach the particles synthesized by microemulsion system are formed by the hydrolysis and polymerization of silane precursor within the sight of ammonia. In the microemulsion approach, the size of micelles is fundamentally in the range of 5-100 nm, dependent on a few parameters, for example, surfactant type and concentration (Najjar et al., 2012).The microemulsion system has a few phases region which is the water in oil (W/O), oil in water (O/W), combination of both O/W/O or W/O/W, reverse micelles, micelles and bicontinuous structure as appeared in Figure 2.11 (Malik et al., 2010). The differences of every technique are illustrated in Figure 2.11.

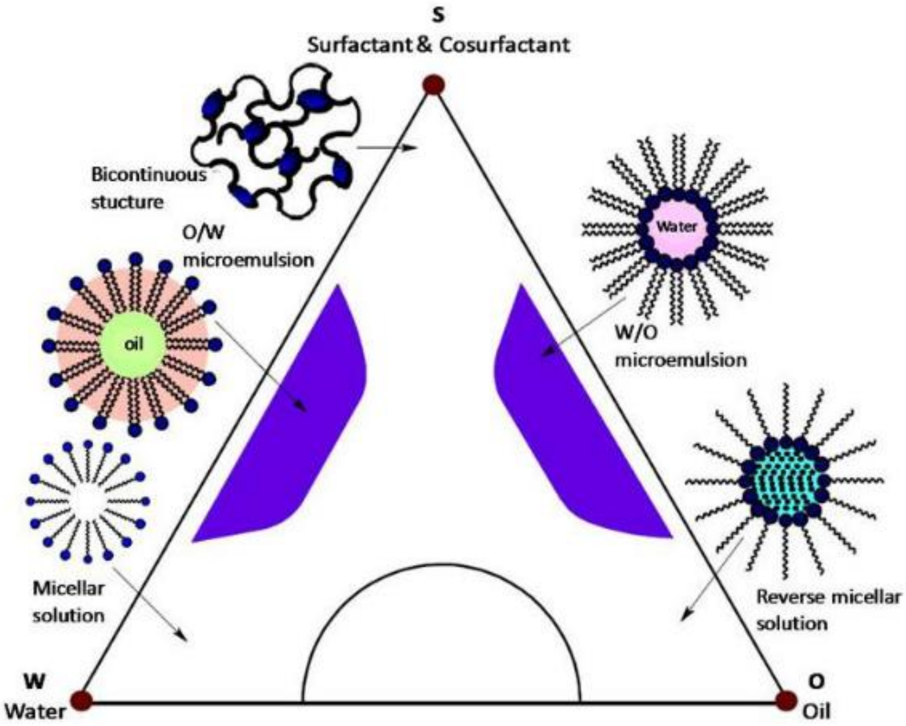


Figure 2.11: Hypothetical phase regions of microemulsion systems (Malik et al., 2010).

2.3.3.2 Water in Oil microemulsion (W/O) or reverse micelle

This technique was found by Arriagada et al., (1995) and laterenhanced by Tan and associates Zhao et al., (2004). The important reaction of this procedure depends on the medium where hydrolysis and condensation of TEOS occur, i.e. in the center of a reverse microemulsion. This impossible to miss system begins when a surfactant, alongside a controlled amount of water, is dispersed in a hydrocarbon, under specific conditions.

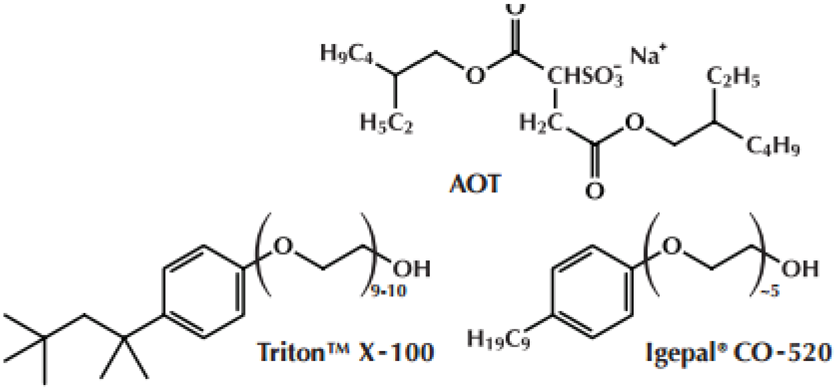


Figure 2.12: Molecular structures of commonly used surfactants in the reverse microemulsion synthesis of silica nanoparticles (Zhao et al., 2004).

Surfactants are amphiphilic long-chain organic compound (Figure 2.12), miscible in both hydrocarbons and in water, portrayed by two vital parameters which is the critical micelle concentration (CMC) and the critical micelle temperature (CMT). From these threshold value, when they are dissolved in a hydrocarbon solution, they can make a macroscopically isotropic dispersion, yet the orientation of the surfactant molecules is not random. The spherical aggregates are formed, where the hydrophilic heads are adjusted toward the centre, while the hydrophobic tails proceed reach out into the bulk oil stage.

When water is added to this framework, it places itself at the centre of these aggregates. They stabilized water droplets as small nanoreactors, where the hydrolysis and condensation of TEOS is bound together (Figure 2.13), yielding exceptionally monodisperse, spherical particles with distances across extending from 20 to 100 nm. The parameters that can control the size and monodispersity of the subsequent nanoparticles are the nature of the surfactant and the amount of water, on the grounds that obviously these are the main factors that affect the stability and dimension of the reverse micellar aggregates (Zhao et al., 2004).

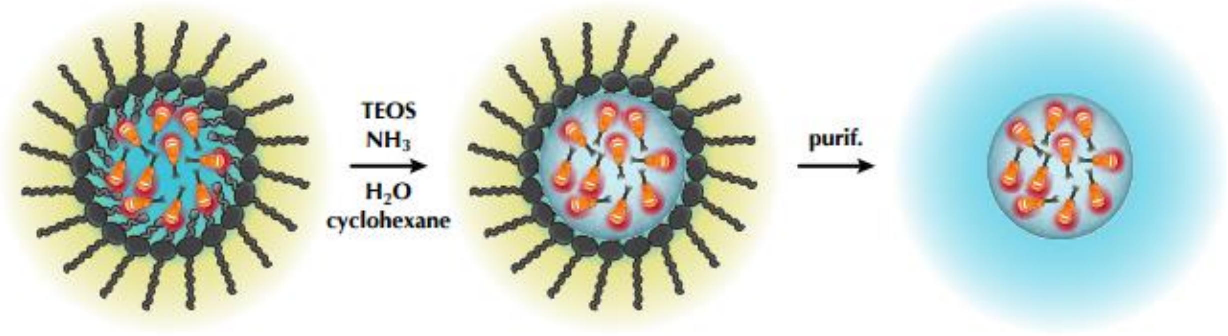


Figure 2.13: Schematic representation of the reverse microemulsion synthesis of tris(bipyridine)ruthenium(II) doped silica nanoparticles (Zhao et al., 2004).

The reverse microemulsion synthesis is favored over Stöber technique when the photoactive species is well dissolvable in water, so it can be straightforwardly included amid the development of the nanoparticles, to be physically entrapped in the structure by methods for non covalent interaction, with no need of derivatization (Arriagada et al., 1999). The addition of dye can produce fluorescent nanoparticles with multiple emission that is beneficial for multiplex bioanalysis (Wang et al., 2005). Dyescan be modified with