CHAPTER II

THEORETICAL BACKGROUND

2.1 Nanochemistry

Generally, literatures in nanochemistry were associated with the developing new methods for synthesizing, modifying, and studying the phenomena related to nanoparticles or nanostructure. Nanoparticle research is currently an area of intense scientific research, due to a wide variety of potential applications in biomedical, optical, and electronic fields.

Nanochemistry has two important aspects. One of these is associated with understanding the chemical and physical properties of particles as well as lays new foundations for these properties. The other is connected to nanotechnology, which consists of the application of nanochemistry to the synthesis, modification, and stabilization of nanoparticles and also for their directed self-assembling to give more complex nanostructures.

A nanoparticle (or nanopowder or nanocluster or nanocrystal) is a small particle with at least one dimension in range 1-100 nm. Table 2.1 classifies nanoparticles based on the diameter and number of atom in one particle [1].

Table 2.1 Classification of particles by their sizes [1]

Chemistry	Nanochemistry			Chemistry of solid state
	Number of atom in one particle			
Single atom	10 10^2	10^3	10^4 10^6	bulk
Diameter (nm)	1	2 3	5 7 10	>100

In nanochemistry, which is in a stage of development, some definition and terms are still unclear. The differences between such term as "cluster" and "nanoparticles" have not yet been formulated in literatures. The term "cluster" was largely used for a particle which includes small number of atoms, while the term "nanoparticle" was applied for larger aggregates [1].

2.2 Silver

Silver is a white lustrous transition metal as shown in Figure 2.1. Silver occurs as a free metal as well as various mineral such as lead, zinc, copper, gold, and copper-nickel ores. Most silver is produced as by-product recovered from electrolytic refining of copper, zinc and gold. Commercial silver with purity of 99.99% is commercially available.

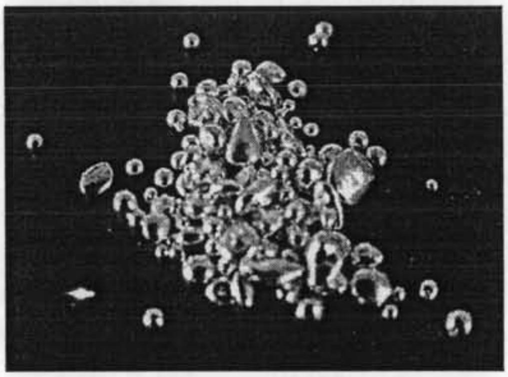

Figure 2.1 Appearance of silver metal

Among metals, silver has the whitest color, the highest thermal conductivity, the lowest contact resistance, and the highest optical reflectivity. Therefore, it is used for jewelry, silverware, and electrical devices. However, for electrical propose, copper was widely used because silver is more expensive and tarnish when exposed to air. Some physical constants of silver are shown in Table 2.1.

Table 2.2 Physical properties of silver

Properties	Value
Symbol, Atomic number	Ag, 47
Atomic weight ($g.mol^{-1}$)	107.87
Electron configuration	$[Kr]\ 4d^{10}\ 5s^{1}$
Density ($g.cm^{-3}$, near rt.)	10.49
Melting Temperature (T_m ℃)	961.78
Boiling Temperature (T_b ℃)	2162
Crystal structure	face-centered cubic
Oxidation state	+1
Atomic radius (pm)	160
Magnetic ordering	diamagnetic
Thermal conductivity ($W.m^{-1}.k^{-1}$)	429
Reduction potential (V)	0.799

Silver, silver ions, and silver compounds show an antibacterial activity just like lead and mercury but without the high toxicity to humans [23]. Hippocrates, the father of modern medicine, wrote that silver had beneficial healing and anti-disease properties. The Phoenicians store water, wine, and vinegar in silver bottles to prevent spoiling. In the early 1900s people would put silver coin in milk bottles to prolong the milk's freshness. Its antibacterial activity increases its value in utensils and jewelry.

2.3 Size effect in nanochemistry

The size effects in nanochemistry represent a phenomenon that produce the qualitative changing in physical, optical, and thermal properties on the number of atom or molecules at the surface of nanoparticles and take place in the range 1-100 nm. Each new property enables new applications.

2.3.1 Physical property: surface area

For nanoparticles which have a very high surface to volume ratio, surface and internal volume are indistinguishable. The reaction can be considered as a process in an infinite volume with a constant concentration. This can be used in application where high surface areas are critical. For example, in the catalytic industry; silver nanoparticles have proven to be good catalysts in redox reaction to produce hydrogen gas [24]. For gold nanoparticles which are known to be a poor catalyst, with decreasing of their size they can also be used as a catalyst in hydrogenation of hydrogen peroxide and decomposition of nitrogen peroxide [25]. Some nanoparticles show bactericidal effects such as silver or titanium nanoparticles and their antibacterial activity depend strongly on their sizes [12].

2.3.2 Optical property: absorption and scattering of light

Suspending in water, silver nanoparticles are typically yellow. Gold nanoparticles are responsible for the bright red color in stained glass windows. The changing in color of these nanoparticles has been interested for centuries, and has many researches on the optical property of these nanoparticles [26-27].

Optical property is associated with unusual spectra of electron energy level arranged in a discrete fashion. For bulk metal, the energy level merges together forming a continuous adsorption band. As the size decreases, the energy gap increases to discrete-like energy level. The discrete structure of energy states leads to a discrete absorption spectrum, which is in contrast to the continuous absorption spectrum of a bulk metal as shown in Figure 2.2 [1].

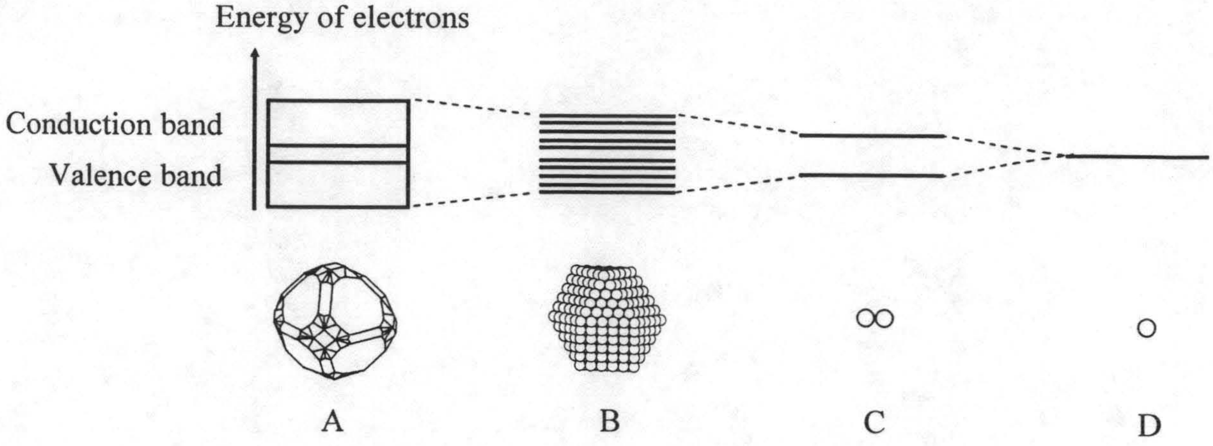

Figure 2.2 Scheme illustration of electronic states of (A) bulk metal, (B) a large cluster, (C) dimer, and (D) atom.

Generally, the interaction of nanoparticles with photons is resulted in electronic and/or vibrational excitation. This interaction can also generate decomposition and even evaporation of nanoparticles. The collective excitation leads to the oscillating of delocalized conduction electrons. The excitation of collective oscillator of conduction electrons is conventionally considered as a surface plasmon. When the incident photon frequency resonates with the collective oscillation of the conduction electron in the metal nanoparticles, the frequency is known as the Localized Surface Plasmon Resonance (LSPR) frequency [28].

The LSPR is a dipolar excitation between the negatively charged electrons and the positive charges in the particle as shown in Figure 2.3. UV light is an electromagnetic radiation, which can be represented as polarization, oscillation of electric and magnetic field that propagate in space. The electromagnetic radiation is composed of electric and magnetic field that are orthogonal to each other and to the propagated direction.

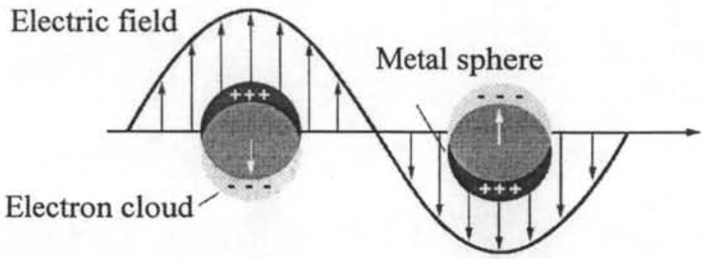

Figure 2.3 LSPR schematic illustration [28]

The LSPR depends on the size, shape, interparticle spacing and dielectric properties of the material, as well as the dielectric properties of the local environment around the nanoparticles. Since the LSPR of nanoparticles is highly depending on the local environment, the absorption maxima will be shifted when an adsorbate bind to the surface of a nanoparticle. This shifting can be monitored by UV-Visible spectroscopy. The LSPR shift induces by the adsrobates can be used as an optical biosensor to detect chemical and biological agents. Figure 2.4 shows biosensor strategy for detection of DNA with silver/gold core-shell probes [3].

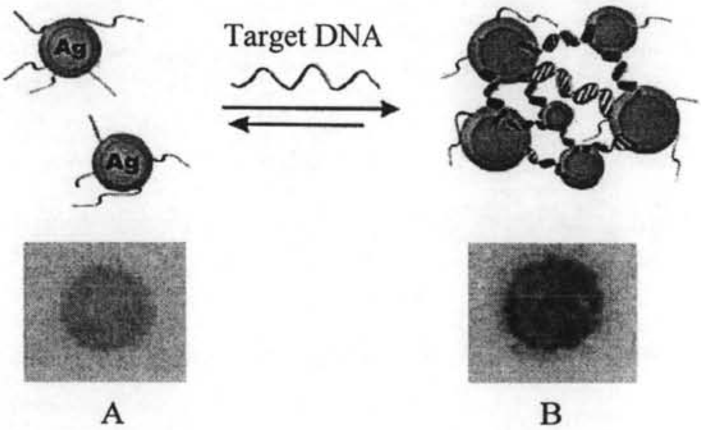

Figure 2.4 Schematic illustration for silver/gold core-shell nanoparticle-based detection probes: (A) without target, (B) with target at room temperature.

Silver/gold core-shell nanoparticles are coated with receptors that will specifically bind to the target DNA. After the addition of target DNA to the nanoparticles, the refractive index of surrounding environment is then changed inducing the shift in LSPR spectrum. As a result, the color of solution changed form yellow to black. This biosensor is moderately sensitive, rapid and low cost.

2.3.3 Thermal property: melting point

With a decreasing in size, the melting point reduces by several hundred degrees. For example, gold nanocrystals stabilized by hexane thiol with 2 nm diameters are molten at approximately 130 °C, which is incredibly lower than the bulk melting temperature of gold (1064 °C). [5]

The relationship between the melting point of metal nanoparticles and their size was explained by Lindemann. A crystal melts when the root-mean-square (standard) deviation of atoms in the crystal exceeds the average interatomic distance [1]. When increasing the temperature leads to increase in the amplitude of vibration. The surface atoms are more weakly bound, result in their wider oscillation comparing to the atoms in the bulk at the same temperature.

A decreasing in melting point with the size of metal particles can be used in many applications such as a solution-based process on a production of circuits, (metal powder sintering).

2.4 Synthesis of silver nanoparticles

Generally, all methods for synthesis of nanoparticles can be divided into two main types. The first route is generates nanoparticles from separate atom or molecules which called *"bottom up"*. The second route generates nanoparticles by mechanical dispersion or fragmentation of bulk metal which called *"top down"*. Both methods are clearly shown in Figure 2.5. The bottom up method is largely defined as chemical methods, whereas the top down method, on the other hands, is physical method.

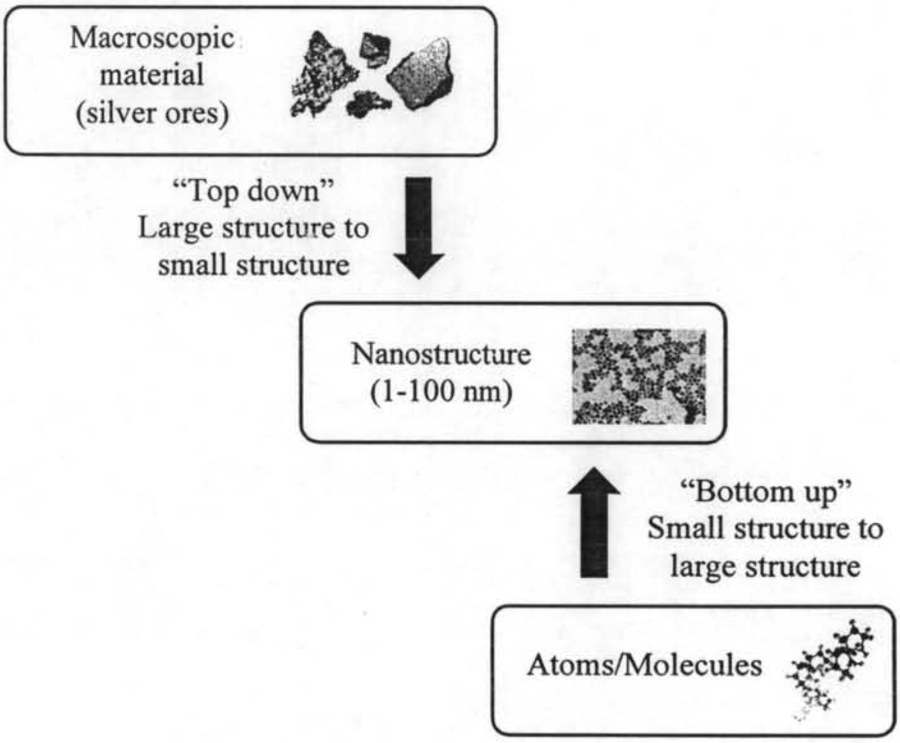

Figure 2.5 Two approaches for synthesis of silver nanoparticles. A comparison of Top down method and Bottom up method

2.4.1 Chemical method

Chemical method involved the reduction of metal salt by various reducing agents such as borohydride, citrate, ethylene glycol and aldehyde or by the active species which generate by light.

In general, the behavior of metal particles is determined by the potential difference as follows [1]:

$$\Delta E = E - E_{\mathrm{redox}} \tag{2.1}$$

where,

E = redox potential of the particles
E_{redox} = solution potential

Particles grow when $\Delta E > 0$ and dissolve when $\Delta E < 0$. This becomes more complicated by the fact that the redox potential of metal particles depends on the number of atoms. Thus, the chemical reduction is a multifactor process. It depends on the difference between the redox potentials of the metal salt and the reducing agent, concentration of its components, temperature, and also pH of a medium.

The first reproducible standard protocols for the preparation of metal colloids by chemical reduction method were established by John Turkevich [29]. He also proposed a mechanism for the stepwise formation of nanoparticles based on nucleation, growth, and agglomeration as illustrated in Figure 2.6.

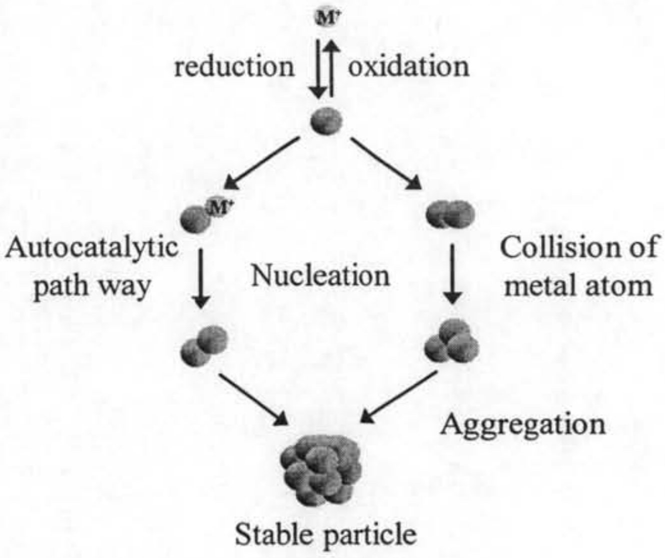

Figure 2.6 Proposed mechanism for formation of nanoparticles based on reduction, nucleation, and aggregation.

Nucleation is the process whereby a particle grows from a starting "seed" via atomic or molecular collisions. Coalescence is the merging of these nanoparticles by collision of nanoparticles and forms a larger particle, whereby the structure of the original particles is lost. Aggregation involves the interaction of the nanoparticles with each other to produce larger agglomerates where the individual nanoparticle structures are retained.

Borohydride reduction

Tetrahydroborates of alkali metal ions (MBH_4) were frequently used as a reducing agent in synthesis of metal nanoparticles in aqueous media. Borohydrides reduce metal ions with their the high reduction potential, -1.24 V in alkali solution [30] compared to the reduction potential of the transition metal ion, 0.5-1.0 V. The reactions of borohydrides occur rapidly causing the immediat nucleation of metal particles. The obtained metal particles by borohydride reduction are small and have a narrow size distribution. Chemical interaction associated with the transfer an electron from reducer to metal ion via the formation of complex M····H····B, which lowered the electron-transfer energy. However, it is difficult to control the reaction to obtain the larger particles and borohydride was considered as a toxic reducing agent. The metal particles obtained by borohydride reduction were not capable by means of the medical application otherwise they have to purify the synthesized nanoparticles before using. The synthesis of silver nanoparticles by borohydride reduction has been reported in many studies [31].

Nanosized silver particles were synthesized in aqueous surfactant system via the reduction of silver salt by sodium borohydride ($NaBH_4$) [31]. The concentration of silver nitrate ($AgNO_3$) was 10^{-4} mol.dm^{-3} and surfactant concentration was varied from 10^{-6} to 10^{-1} mol.dm^{-3} and used three types of the surfactant: cationic (cetyltrimethylammonium bromide, CTAB), anionic (sodium dodecyl sulfate, SDS) and neutral (poly(oxyethylene) isooctylphenyl ether, TX-100. The usual mole ratio of $NaBH_4$ to $AgNO_3$ was 6:1. The redox reaction can be written as:

$$2AgNO_3 + 2NaBH_4 + 6H_2O \longrightarrow 2Ag + 2NaNO_3 + 2H_3BO_3 + 7H_2 \quad (2.2)$$

Size and size distribution of synthesized silver nanoparticles are influenced by various stabilizers. The stability of silver nanoparticles is highest in CTAB which is about 30 days followed by SDS, and TX-100 is the least stable. This result indicated the strong interaction of CTAB with the surface of silver nanoparticles.

Citrate reduction

Citrate ion was commonly used as a reducing agent in metal colloid synthesis. This method invented by John Turkevich which widely used in synthesis spherical and monodisperse gold nanoparticles. This procedure was known as Turkevich method [32]. In this method, citrate ion was considered as a weak reducing agent which causes the slow nucleation of the silver particles. The obtained silver particles by citrate reduction method have larger particles and broader sized distribution than reduction with borohydride. Citrate ion also has an interaction with silver nanoparticles as shown in Figure 2.7 [33] and acted as a stabilizer. This interaction prevents or slows down the aggregation of particles by their steric hindrance.

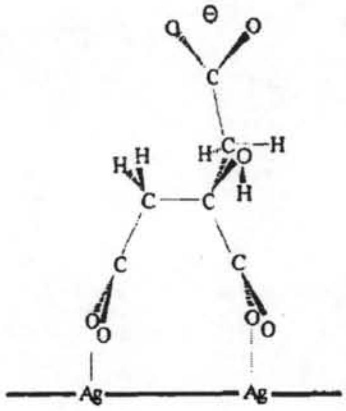

Figure 2.7 Proposed model of interaction of citrate ion at the silver nanoparticles surface.

Therefore, synthesis of silver nanoparticles by citrate reduction can be achieved without any additional stabilizer because citrate ion was acted as both a reducing agent and stabilizer [34].

Silver nanoparticles were synthesized by the reduction of silver nitrate by sodium citrate [33]. The solution of silver nitrate (1.68 mM, 500 mL) was heated rapidly to the boiling temperature. Then, add the boiling sodium citrate (0.34 M, 10 mL) into the silver nitrate solution and kept boiling for 90 min with continuous stirring.

In the initial step large silver particles was formed and then dissociated into the smaller particles as the process continues. The final silver particles have a plasmon band approximately at 402-404 nm and the full width at half maxima (FWHM) is about 53-57 nm. These results indicated the spherical silver nanoparticles with approximately 10-20 nm with a narrow size distribution.

Polyol method

The polyol process presents many advantages. It consists of the reduction in a liquid alcohol medium at moderate temperatures of metallic salts, oxide or hydroxide. Ethylene glycol was acted as a reducing agent and a solvent. This method enables an accurate control of the size and size distribution of silver nanoparticles synthesis. Silver nanoparticles were synthesized by polyol process with polyvinylpyrolidone (PVP) as a stabilizer yielding the average particles size of 15-21 nm with a narrow size distribution [18].

For the reduction of $Ni(OH)_2$ or $Co(OH)_2$ by ethylene glycol, the reduction is based on the dehydration of the ethylene glycol to acetaldehyde as follows:

$$\underset{\text{ethylene glycol}}{2HOCH_2CH_2OH} \longrightarrow \underset{\text{acetaldehyde}}{2CH_3CHO} + 2H_2O \qquad (2.3)$$

Oxidation of the acetaldehyde with formation of diacetyl:

$$\underset{\text{acetaldehyde}}{2CH_3CHO} + M(OH)_2 \longrightarrow \underset{\text{diacetyl}}{CH_3COCOCH_3} + 2H_2O + M \qquad (2.4)$$

But for the reduction of silver salt to silver nanoparticles, products were methanol and acetic acid. This indicated the different path way of oxidative process [35].

Aldehyde reduction

The use of formaldehyde or glucose to reduce silver ion is known as Tollen's reaction. This reaction is employed for constructing the silver mirror on a solid substrate. With the modified procedure, this reaction can use in synthesis of metal nanoparticles [36-37]. The fundamental reaction involved in the Tollen's reaction can be simplified as follows:

$$2[Ag(NH_3)_2]^+ + RCHO + OH^- \longrightarrow 2Ag + RCOOH + 4NH_3 + H_2O \quad (2.5)$$

Silver nanoparticles with the size ranged from 50-200 nm were synthesized by Tollen's process. The reagents were similar to common Tollen's reaction. With the appropriate condition, silver nanoparticles are obtained rather than silver film. These synthesized silver nanoparticles are very stable.

Alcohol reduction

Alcohol reduction method has been known as a reducing agent free technique for synthesis of metal nanoparticles method. In this method, metal ions are mainly reduced by reducing alcohol. Generally, alcohol/aqueous solutions such as ethanol/water mixture are frequently used as a reducing agent and a solvent for the reduction reaction in. The reaction occurs as follows:

$$C_2H_5OH + H_2O + M^+ \longrightarrow CH_3CHO + H_3O^+ + M \quad (2.6)$$

Silver-silica nanoparticles were synthesized by alcohol reduction method [17]. Precursors were silver nitrate, ethanol, and polyvinylpyrolidone (PVP) which was used as a stabilizer. The precursors were refluxed for a few hours during the formation of silver nanoparticles. The average size of silver nanoparticles was 6.9 nm on the silica sphere. This is a proper method for attaching silver nanoparticles on the surface of support materials because alcohol is a weak reducing agent which causes slow reduction rate.

Photochemical and radiation-chemical reduction

Synthesis of metal nanoparticles by photochemical (photolysis) and radiation-chemical (radiolysis) process was associated with the generation of strong reducing agent such as electrons, radicals and excited species [38-39]. These two methods are different in energy that applied to the process. Photochemical involves energies below 60 eV, but radiation-chemical uses energies around 103-104 eV. These methods have many advantages such as the absence of impurities resulting high purity nanoparticles and low temperature process.

Photochemical and radiation-chemical in solution is frequently employed for synthesizing metal nanoparticles. These nanoparticles were synthesized from solution of metal dissolved in water, alcohol, and organic solvent. Under the action of light, the active species are formed:

$$H_2O \xrightarrow{h\nu} H^+ + OH^\bullet + e^- \qquad (2.7)$$

A solvated electron interacts with metal ion to produce metal particles:

$$M^+ + e^- \longrightarrow M \qquad (2.8)$$

However, light was inducing not only the formation of nanoparticles but also the aggregation of nanoparticles. The aggregation was attributed to the exchange of electric charge which gave rise to electrical force that attracted particle to another particle.

Electrochemical

This method uses two-electrode and electrolyte solutions. The anode contains bulk metal which would be transferred into metal nanoparticles [40]. The supporting electrolyte consists of tetraalkylammonium salts, which also serve as stabilizers for the metal nanoparticles. The overall process was shown in Figure 2.3. First, the bulk metal is oxidized at the anode. Then, the metal ions migrate to the cathode, and reduction takes place at the cathode. Consequently, the formation of metal nanoparticles takes place. The obtained nanoparticles were stabilized by tetraalkylammonium to prevent the aggregation of nanoparticles and the precipitation of particles. Electrochemical is not suitable for the mass-scale production. However, this method has some advantages in high purity of the nanoparticles. Moreover, specifies particle size can be produced by adjusting current density.

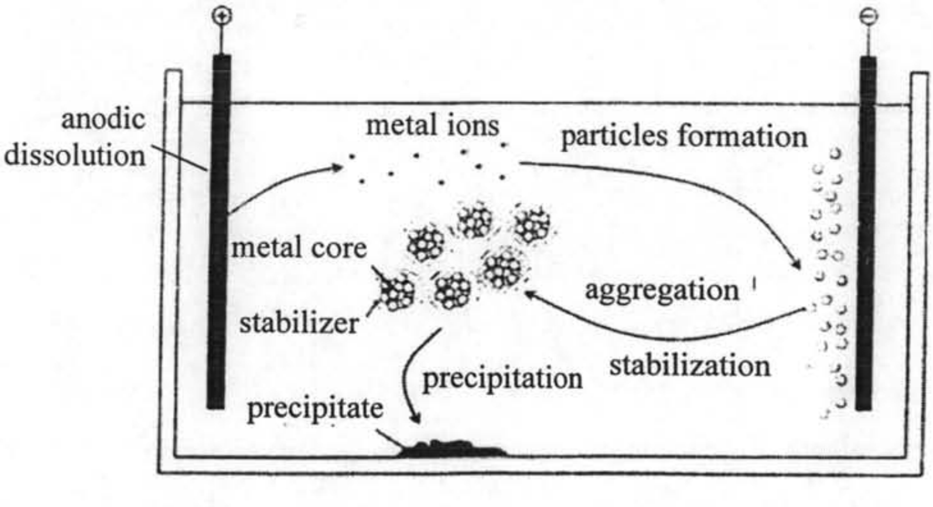

Figure 2.8 Apparatus for synthesis of silver nanoparticles by electrochemical.

Silver nanoparticles ranging from 2 to 7 nm were successfully synthesized by electrochemical technique [41]. Silver plate (anode) was dissolved in an aprotic solution of tetrabutylammonium bromide in acetonitrile and re-formation of silver nanoparticles at cathode which stabilized by tetrabutylammonium bromide. With an increase in current density from -1.35 to -6.90 mA/cm^2, the particle diameter decreases from 6 ± 7 to 1.7 ± 0.4 nm.

2.4.2 Physical method

There are many physical methods for preparation of metal nanoparticles. The major methods were based on the dispersion of bulk metal such as gas evaporation, and laser ablation or the decomposition of metal salt with high temperature (spray pyrolysis).

Inert gas condensation (IGC)

This method was based on the evaporation of metal into an inert gas flow and subsequently condensation in to a chamber at a certain temperature as shown in Figure 2.9 [21]. The IGC method mainly consists of the following steps: first, the processing chamber is evacuated to a base pressure of about 10^{-4} mbar before it is back-filled with an ultra pure inert gas (He or Ar) flowing to a pressure of typically 10-50 mbar. The source metal is fed into evaporator and then vaporized into the flowing inert gas where nanoparticles are formed. The aerosol particles are collected in a suitable medium such as metal filter.

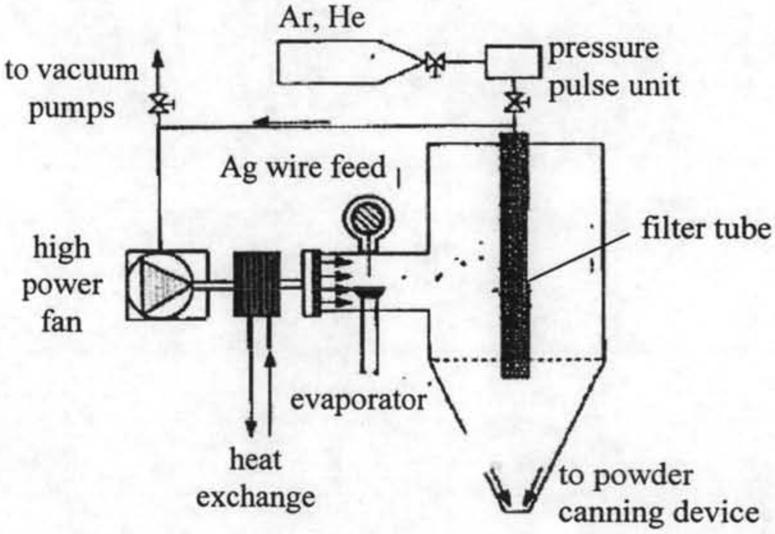

Figure 2.9 Schematic diagram for the preparation of silver nanoparticles by inert gas condensation method.

Particle size, shape and morphology depend on many parameters such as O_2 amount, wire feeding rate, gas types and pressures. The amount of O_2 affects the formation of silver oxide coated on the silver particles. Such coating decreased the growth rate of the particles resulting in small silver particles. Wire feeding rate involve the evaporation rate and vapor density. The low vapor density causes less collision of the particles and, as a consequence, smaller particles are obtained.

Laser ablation

Metal nanoparticles can be produced by irradiate metal sheet with intense laser in liquid solution with the present of stabilizer as shown in Figure 2.10. The interaction of intense laser with metal sheet results in ablation and sputtering of material. The thickness of removed layer is very small in the range of few nanometers. Thus, the removed material is aggregated into nanoparticles. It was explained in terms of self-absorption of laser radiation and thermally excites the lattice of metal particles, leading to the fragmentation of particles. Particles size can be controlled by changing the laser intensity and wavelength [42-43].

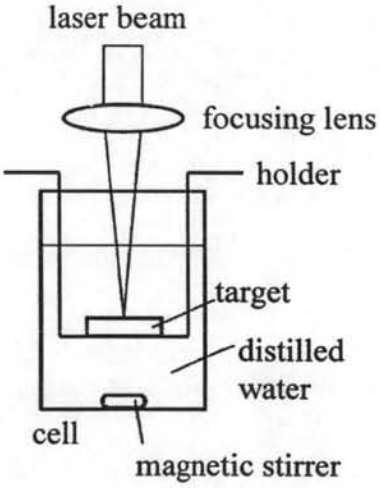

Figure 2.10 Experimental set-up for colloid preparation by laser ablation in solution.

Silver nanoparticles were prepared in water by laser ablation and using SDS as a stabilizer. The mean diameter of particles became smaller from 29 to 12 nm with decreasing of laser wavelength. The size decreases due to the extinction coefficient of the colloid at the wavelength of ablation light was increased.

Spray pyrolysis

Spray pyrolysis is widely used in production of fine-grained metal or ceramic powder because it is a continuous flow process that operated at ambient pressure and relatively inexpensive. However, the common problem for spray pyrolysis is the formation of porous particles after solvent evaporation. It is necessary to use high temperature for densifying the porous particles.

This process involved a droplet-to-particle conversion process, where a metal salt solution is atomized into droplets and sent through a hot-wall reactor. Inside the reactor the solvent evaporates and the metal salts decompose to form the product particles. In this process, it is commonly believed that the drops, when sprayed into a tubular reactor under pyrolysis conditions, serve as micro-reactors and yield one particle per drop. The droplet size, residence time, precursor solution concentration, and temperature in the tubular reactor affect the characteristics of particles. Droplets are typically generated by either jet atomization (liquid atomization by high velocity air) or ultrasonic atomization (liquid atomization from a high frequency sound wave). Jet atomization has the advantage of high throughput but also has the disadvantage of broad drop size distribution (resulting in a broad particle size distribution). On the other hand, ultrasonic atomization has the disadvantage of low throughput, but has the advantage of narrow drop size distribution (resulting in a narrows particle size distribution).

This method is used to produce solid, spherical, micron-sized silver metal particles. The experimental apparatus is shown in Figure 2.11. Droplets of a silver nitrate solution generated from aerosol generator are injected to the reactor with pressurized carrier gas. Then, the aerosol droplets are dried, decomposed and densified in the reactor. The obtained silver particles are collected on a Tuffryn membrane filter (142 mm, 0.45 μm). The effects of reaction temperature, carrier gas, precursor solution concentration, and aerosol droplet size on the characteristics of the resultant silver particles are examined.

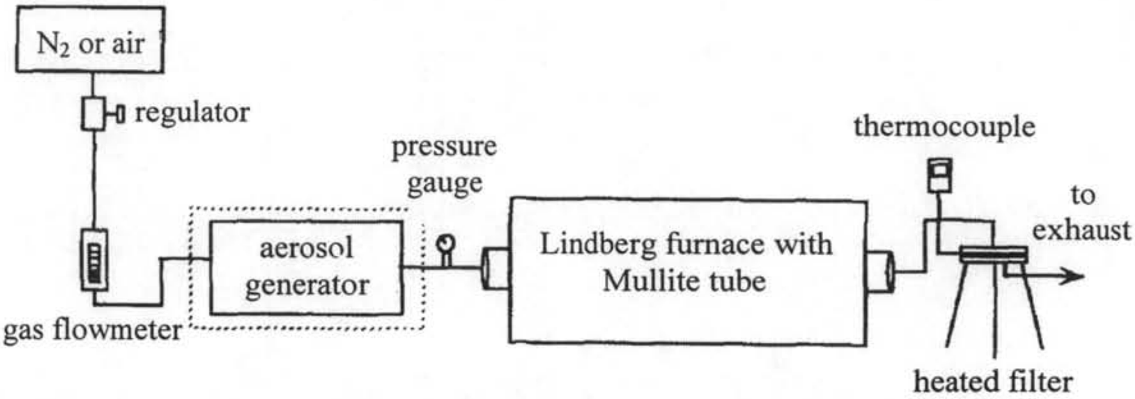

Figure 2.11 Diagram of experimental apparatus for silver production by spray pyrolysis.

The spherical silver particles with diameter ranged from 0.97–1.18 μm are produced with an ultrasonic generator at and above 600 °C under nitrogen of carrier gas [20]. This process can produce solid, dense silver particles because the long residence times (3.5-54 s) which allowed aerosol-phase densification of the porous silver particle

2.4.3 Stabilizer

When nanoparticles are dispersed into a solvent, van der Waals attraction force and Brownian motion play important roles. The van der Waals force and Brownian motion would result in the formation of larger nanoparticles because of the aggregation. As a result, a protective shell must be developed which provides electrostatic and/or steric repulsion to prevent aggregation of nanoparticles as shown in Figure 2.12. A stabilizer is a substrate, often a polymer such as polyvinylpyrolidone (PVP), sodium dodecyl sulfate (SDS) and gelatin.

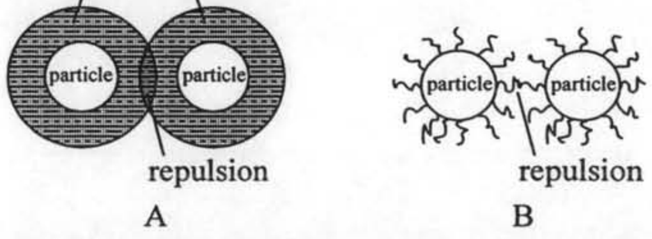

Figure 2.12 Sketch of (A) electrostatic stabilization and (B) steric stabilization

Methods for synthesis of silver nanoparticles were summarized in Table 2.3.

Table 2.3 Comparison of synthesis methods for silver nanoparticles.

Properties	Synthesis methods					
	Chemical method			Physical method		
	Chemical reduction[1]	Photolytic Radiolytic	Electrochemical	Laser ablation	IGC	Spray pyrolysis
Operation cost	Low	Medium	High	Medium	High	Medium
Mass scale production	Yes	No	No	No	No	Yes
Continuous process	No	No	No	No	Yes	Yes
Temperature	Low	Low	Low	Low	High	High
pressure	Low	Low	Low	Low	High	Low
Controllable size	No	No	Yes	Yes	Yes	Yes
Environmental friendly	No	Yes	Yes	Yes	Yes	Yes
Stabilizer	Yes	Yes	Yes	Yes	No	No
Reducing agent	Yes	No	No	No	No	No

[1] Using sodium borohydride as a reducing agent because the smallest size with a narrow size distribution was obtained.

2.5 Nebulization (atomization)

The word nebulization comes from the Latin term for "mist", which is "nebula". Generally, there are two types of nebulization, the jet nebulization and ultrasonic nebulization.

2.5.1 Generation of droplets with jet nebulization

There are many different designs of jet nebulizer based on the same basic atomization principle [44]. In jet nebulizers, the droplet generator is a two-fluid atomizer. In two-fluid atomizer, the compressed air passes through a narrow hole and entrains the solution from fluid flow capillaries by momentum transfer. The air drives the solution and force the solution passed to the orifice resulting in the disintegration of the solution to various size droplets. Larger droplet impact on the baffle (diffuser) and return to the reservoir. Only smaller droplets can pass through the baffle. A typical jet nebulizer was shown in Figure 2.12.

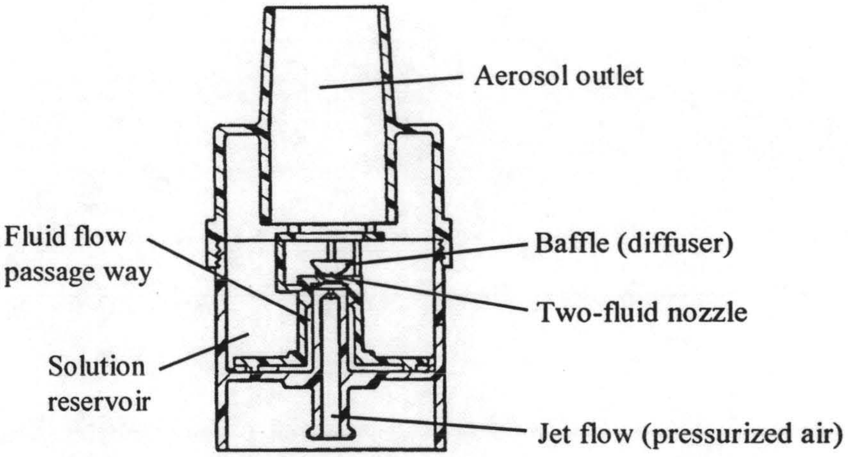

Figure 2.13 Schematic diagram of Hudson micro mist nebulizer

In case of jet nebulizers, the droplet size will not only dependent on the design of the nebulizer itself (nozzle diameter, speed of the compressed air flow), but also from the intrinsic characteristics of the liquid or suspension to be nebulized such as viscosity and surface tension. Apparently, droplet size was inversely proportional to the relative velocity between the air and the liquid and proportional to the liquid's surface tension. In 1981, Mercer derived an equation relating the primary droplet diameter (d) with the diameter of the nozzle (D_L) [45]:

$$d = 0.64 D_L \left[1 + 0.011 \left(\frac{G_I}{G_A} \right)^2 \right] \left[\frac{2\gamma}{\left(9 \rho v^2 D_L \right)} \right]^{0.45} \tag{2.9}$$

Where:

d = diameter of droplet of average volume
D_L = diameter of liquid inlet nozzle
G_I = mass flow-rate of liquid
G_A = mass flow-rate of air
γ = liquid surface tension
ρ = air density
ν = air velocity

Higher air flow rate and a smaller diameter of nozzle decrease the droplet size. The droplet size distribution depends on baffle design. Furthermore, droplet size changes after nebulization, due to the evaporation of solution, droplet aggregation, and deposition on the wall of the nebulizer and tube. The viscosity of solution has a negligible effect on droplet size distribution. Generally, the viscosity of solution resists the droplet formation, so higher viscosity was expected to increase droplet size. This was disagreed with some reports that several jet nebulizers tend to produce smaller droplets as the viscosity of solution increased [44]. However, McCallion [45] reported that the high viscosity liquid such as glycerol and mixture of propylene glycol-water produce larger droplets.

2.5.2 Generation of droplets with ultrasonic nebulization

In an ultrasonic nebulizer the vibrations of the piezoelectric crystal are transmitted to the surface of the solution in a reservoir. If the transmitted energy is sufficient, standing capillary waves are formed on the surface of the solution. Droplets are disrupted from the crests of the capillary waves [44]. In 1962, Lang published an expression relates the wavelength to droplet size D through an empirical constant. He experimentally reported:

$$D = 0.341\left(\frac{8\pi g}{\rho f^2}\right)^{\frac{1}{3}} \tag{2.10}$$

where:

D = median droplet diameter
g = surface tension of the solution
ρ = solution density
f = frequency of the surface waves

For ultrasonic nebulizers, the droplet size distributions largely depend on viscosity [45]. The droplet size is proportional to the viscosity. This can be explained from the formula for the threshold amplitude. Since the threshold is higher for increased viscosities many viscous solutions cannot be nebulized at all by this type of nebulizers. Boucher et al. reported that solutions with a viscosity above 10 cp are difficult to aerosolize with ultrasonic nebulizers. Temperature and concentration of the solution influence the viscosity of the solution and will also affect the performance of the ultrasonic nebulizers.

2.6 Thermal reduction of sprayed silver salt

The synthesis of silver nanoparticles in this work is based on a reduction of silver nitrate in spray pyrolysis condition. In spray pyrolysis, silver nanoparticles are formed by high temperature decomposition of silver ion in droplet. In order to obtain silver nanoparticles in a mild condition, the reducing agent was used. Thus, silver nitrate and reducing agent were nebulized into the tubular reactor. Presumably, the reaction occurs completely in each droplet. Then, silver atoms in a droplet are aggregated and obtained one particle per droplet just like the spray pyrolysis method. This method is called ***"Thermal reduction of sprayed silver salt"***. This will retain the advantages of spray pyrolysis such as continuous process which is demanded in industry, low cost of operation, and the size of particle can be controlled without stabilizer. The reducing agent decreases the reaction temperature making the reaction easy to operate and reduce the cost of operation. However, this method requires the process for purification to get rid of reducing agent.

There are many parameters that can be adjusted in order to control the size and size distribution of synthesized silver nanoparticles. For example, smaller silver nanoparticles can be achieved with a smaller droplet or lower concentration of silver salt. While decreasing droplet size and silver salt concentration result in the amount of silver content per drop. This lead to decrease in the size of synthesized silver nanoparticles. Temperature, flow-rate, and type of carrier gas were also affected the size and size distribution of synthesized silver nanoparticles because these parameters involve the rate of the reduction.

In this method, the jet nebulizer is used because the high aerosol output is favorable for mass scale production. Furthermore, the use of jet nebulizer is easy to invent and modify the apparatus for this synthesis method. However, jet nebulizer has the disadvantage in the broad size distribution of droplets causing the broad size distribution of synthesized silver nanoparticles.

2.7 Characterization techniques

2.7.1 UV-Visible spectroscopy

Spectroscopy is a scientific field concerning with the interaction between electromagnetic radiation and materials. When an electromagnetic radiation strikes the material, the incident beam of radiation is reflected, scattered, transmitted and absorbed by the sample [46]. These interactions can be detected by various techniques depending on experimental arrangements. The interactions of incident energy with the sample are illustrated in Figure 2.14.

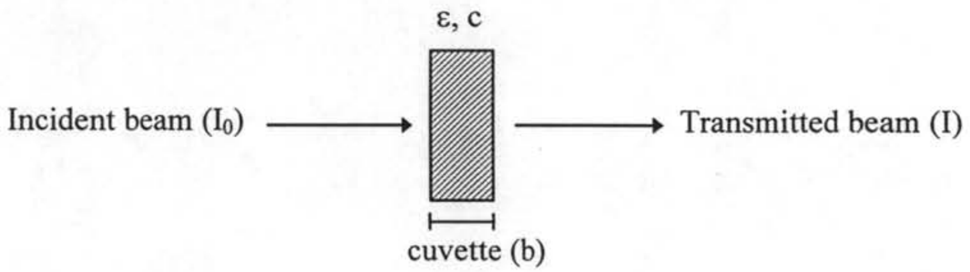

Figure 2.14 Absorption of a beam of light as it travels through a cuvette (b).

The proportional ratio of the specimen is related to the chemical concentration defined by Beer-Lambert's law given in Equation 2.11.

$$I_0 / I = e^{-A} = e^{-\varepsilon bc} \tag{2.11}$$

The linear relationship between concentration and absorbance is simple and straightforward. The Beer-Lambert law is expressed with the following equation:

$$A = \varepsilon bc \tag{2.12}$$

where,

A = absorbance

ε = absorption coefficient (L/mol·cm)

b = optical path length (cm)

c = concentration (mol/L)

UV-Visible spectroscopy is widely used to determine the optical properties of a solution. Light is traveling through the sample and the amount of transmitted light is measured. With various wavelengths absorbance is measured at each individual wavelength, a (wavelength, absorbance) graph expressing absorption at each wavelength can be drawn by means of computer software.

However, for nanoparticles, the optical properties are much more complicated and required an individually developed theory. For instance, the measured absorbance spectrum does not necessarily show the actual absorbance but the extinction of the light. The extinction intensity is the summation of absorption and scattering intensity of particles. Extinction and absorption intensity of spherical particles of arbitrary size can be calculated using Mie's theory [50] as shown in Equation 2.13 and 2.14. Mie's theory remains of great interest to this day because it is simple, exact solution to Maxwell's equations that is relevant to particles.

$$\sigma_{ext} = \frac{2\pi}{k^2}\sum_{n=1}^{\infty}(2n+1)\operatorname{Re}(a_n + b_n) \tag{2.13}$$

$$\sigma_{sca} = \frac{2\pi}{k^2}\sum_{n=1}^{\infty}(2n+1)(|a_n|^2 + |b_n|^2) \tag{2.14}$$

Transmittance spectra of spherical silver particles in glass are calculated by Mie's theory. For spherical silver with increasing the particle size from 10 nm to 100 nm with a step size of 10 nm at a fixed concentration (500 μg/cm^3) and a sample thickness of d = 1 mm [47], the calculated spectra are shown in Figure 2.15.

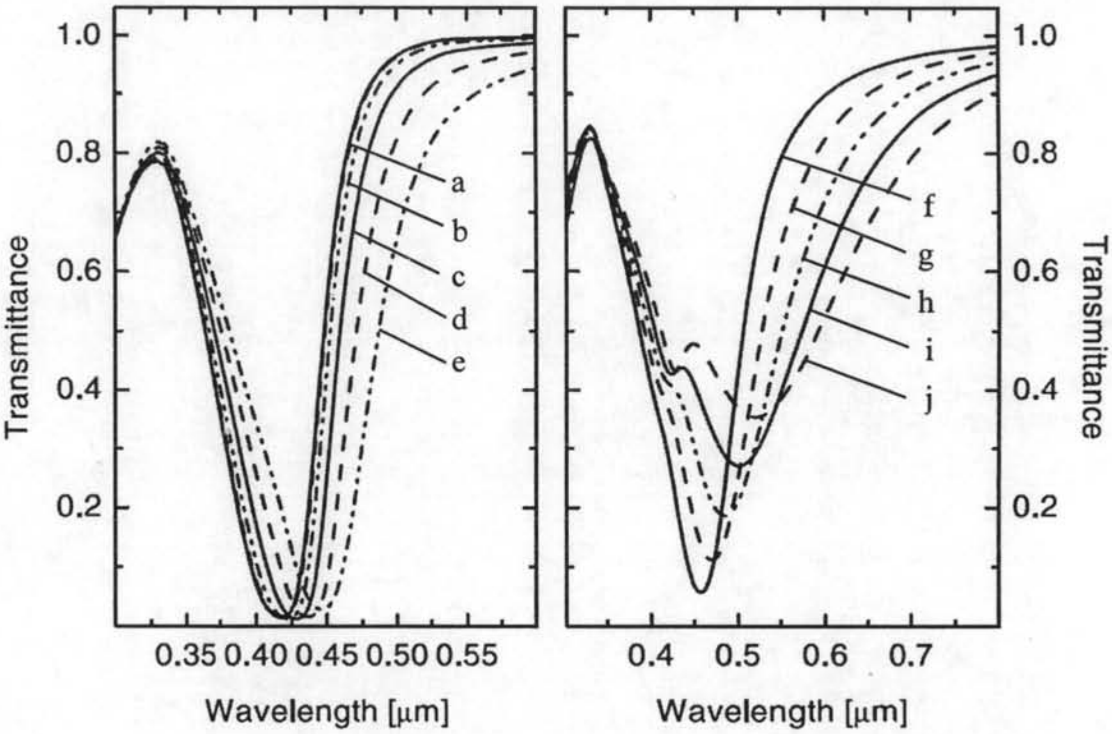

Figure 2.15 Calculated transmittance spectra of spherical silver nanoparticles in glass with increasing particle size at a fixed concentration = 500 μg/cm^3 and a sample thickness of d = 1 mm. The particle size increases from a = 10 nm to j = 100 nm with a step size of 10 nm.

In the calculated transmittance spectra of the silver nanoparticles in Figure 2.15 show the red-shift in the transmittance with increasing particle size. Shoulder around 400 nm was observed in the spectra of particles which a diameter of particles was larger than 50 nm. It is caused by the resonance excitation of the higher mode of oscillation which are quadrupole plasmon resonance where half of the electron cloud moves parallel to the applied field and half moves antiparallel.

Form the computed spectra, if the nanoparticles containing various sizes or broad size distribution. Broadening of the extinction spectra can be observed, measured by the full width at half maxima (FWHM). The low value of FWHM indicates the narrow size distribution of nanoparticles.

For particle in aggregate system, Figure 2.16(A) was the spectra of experimentally aggregated silver particles (27.4 nm) which were induced to aggregate by controlling the pH value. The calculated spectra of different aggregates silver particles and the single silver particle spectrum were shown in Figure 2.16(B). The number of single particles decreases with increasing the state of aggregation. TEM image was used in order to measure the mean sizes of aggregated silver nanoparticle.

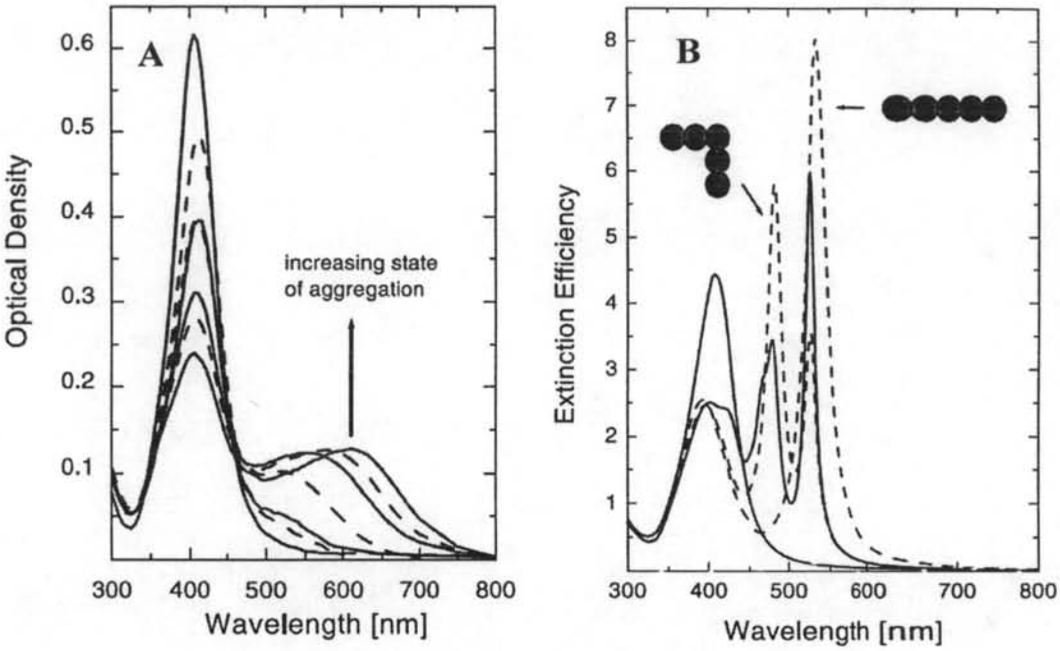

Figure 2.16 (A) Measured optical density (extinction) spectra of aggregated colloidal suspensions of silver nanoparticles with mean size = 27.4 nm

(B) Computed extinction efficiency spectra of a single silver particle (27.4 nm) and three different aggregates with 5 particles.

The extinction spectra form experiment is in agreement with the calculated spectra. When increasing the state of aggregation, the increasing of the extinction spectrum at longer wavelengths or tailing of extinction spectra was observed. The spectra of aggregated particles clearly show that the single-particle resonance splits into new resonances of the corresponding aggregate. Most of the extinction bands contribute to the extinction at longer wavelengths compared to the resonance wavelength of the single particle. In addition, the extinction at wavelengths of the dipole plasmon resonance is decreased with increasing state of aggregation.

2.7.2 Transmission electron microscopy

Transmission electron microscopy (TEM) was largely used for studying the size, size distribution and morphology of particles. TEM involves a beam of accelerated electron, 50-200 keV, emitted by a cathode in vacuum. These electrons are deflected in small angles by atoms in sample and transmitted through thin sample. Then, these electrons are magnified by magnetic lenses and hitting a fluorescent screen generating the bright field image. Schematic diagram of transmission electron microscope was shown in Figure 2.17. The interactions of electron beam with atoms in the samples are the diffraction or absorption of electron beam. The images from electron microscopes indicate the structure of a sample which can be used for determining size and morphology of metal nanoparticles.

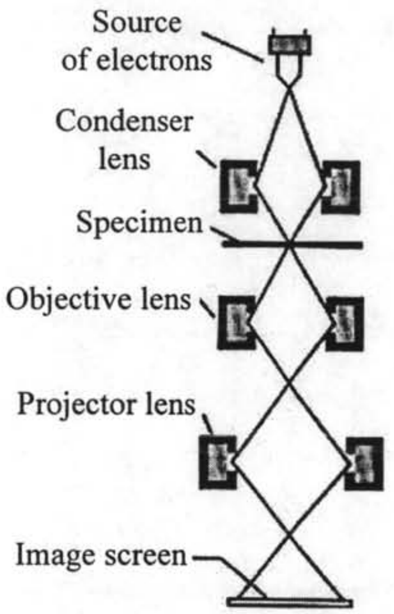

Figure 2.17 Schematic diagram of transmission electron microscope (TEM)

2.8 Antibacterial of silver nanoparticles

A series of experiments have been performed in order to investigate the antibacterial activity of silver nanoparticles. These experiments indicate that antibacterial activity of silver nanoparticles is influenced by size and morphology of the particles. Small size silver nanoparticles and have high amounted facet exhibit high antibacterial activity. The interactions between silver nanoparticles and the mechanism of the bactericidal effect of silver nanoparticles are still unclear. There are three proposed mechanisms for bactericidal of silver nanoparticles [6]:

1. Silver nanoparticles attach to the surface of cell membrane of a bacteria and disturb its function, such as permeability and respiration.

2. Silver nanoparticles are able to penetrate into the bacterial cell and interacting with sulfur- and phosphorus-compounds such as DNA.

3. Nanoparticles release silver ions, which will have an additional contribution to the bactericidal effect of the silver nanoparticles.

2.9 Effect of silver nanoparticles on human health

Silver nanoparticles were used as an antibacterial agent in many commercial product even the food packaging and cosmetic. This means that silver nanoparticles will become close contact or enter human body. Therefore, it is important to study the effect of silver nanoparticles on human health.

The toxicity of silver nanoparticles was studied by testing with the BRL 3A rat liver cell [48]. If the liver cell is affected negatively, silver nanoparticles could cause a mutation or other genetic change. This article is a study with the purpose of evaluating the suitability of a mouse liver cell as a model to evaluate the toxicity of nanoparticles in vitro. Different nanoparticles such as silver, molybdenum, iron oxide, titanium dioxide and aluminium) were tested in regard to cell morphology, mitochondrial function (MTT assay) and release of lactate dehydrogenase (LDH assay). The result showed that silver nanoparticles were the most toxic nanoparticle used in their studies.

A different aspect of the research in the health effect of silver nanoparticles was performed by Alt et. al. Silver bone cement were tested with mouse fibroblasts and human osteoblasts in vitro by the release of lactate dehydrogenase (LDH assay), total protein content, and number of vital cell [49]. The result showed that silver bone cement was not toxic. He proposed that silver nanoparticles were not toxic because the animal cells are eukaryotic cells which contrast to the bacteria cell which was prokaryotic cell. Eukaryotic cells have higher structural and functional redundancy compared to prokaryotic cells. Therefore, higher silver concentrations are required to harm the human cell compared to bacterial cells. This report provides a therapeutic window in which bacterial cells are died, but the concentration was not high enough to harm the animal cells.

Therefore, it is difficult to conclude that silver nanoparticles are a toxic or non-toxic to human health. However, there are no doubts that nanoparticles may cause many effects due to their small size. The very small size makes them highly mobile in the environment as well as in the human body. They can enter human tissue through lungs, skin and digestion and this could cause health problems. Thus, it is necessary to perform more scientific studies on the effect of nanoparticles on human health.