CHAPTER II

THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Polyelectrolyte Multilayers (PEM)

Polyelectrolyte Multilayers are polymer systems consisting of a macromolecule which anionic and cationic electrolyte groups are covalently bonded together and counterions, low molecular-weight for electroneutrality. Polyelectrolyte properties are similar to both electrolytes and polymers. Their statistical properties are being used in a wide range of technological, industrial, biology, and biochemistry fields. Example of polyions used for multilayer fabrication is presented in Figure 2.1.

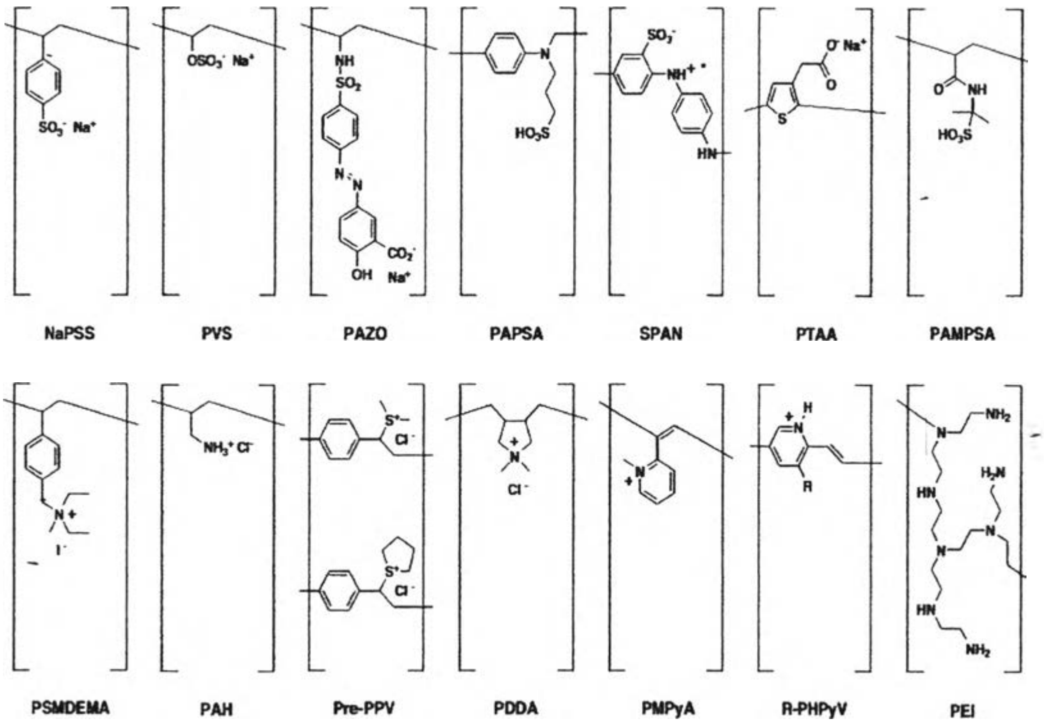


Figure 2.1 Example of polyions used for multilayer fabrication.

The commercial PEM are obtained by polymerization, polycondensation, or polyaddition process. PEM also produced from nature such as gelatin, pectin and from a chemical modification of nonionic polymers such as cellulose, starch, etc.

PEM films created by Layer-by-layer (LbL) deposition used to modify properties of surface of materials. The parameters controlling the growth of PEM was important for the multilayer formation included type of polyelectrolyte, which affected the total thickness, polymer charge density, required for minimum to unchanging the chemical structure, and influence of ionic strength because the total multilayer thickness can be controlled by adding salt to the polyions solution. The effect of ionic strength by adding salt to the thickness of PEM showed in Figure 2.2.

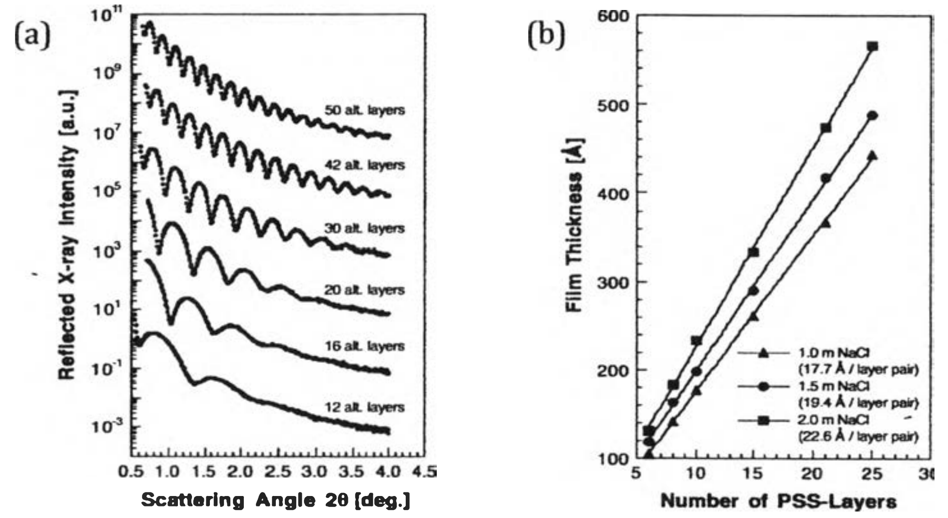


Figure 2.2 Fine-tuning the film thickness by X-ray reflectometry: (a) X-ray intensity at different scattering angle as a function of number of layers (b) film thickness with increasing number of layers by ionic strength. (Decher *et al.*, 1992).

In 2003, Mermut *et al.* used Atomic Force Microscope (AFM) to study the mechanical properties of PEM thin films of poly(allylamine hydrochloride) (PAH) and an azobenzene containing polyelectrolyte (P-Azo) showed that the relative Young's modulus was determined as a function of the ionization fraction of PEM films to find thickness. The elasticity of PEM films as a function of polymer charge density, which high charge density exhibited an elastic modulus and adhesion values depend on the ionic cross-link density of the films, which high ionic cross-link exhibit high adhesion.

2.1.1 Synthetic PEM by PDADMAC/PSS

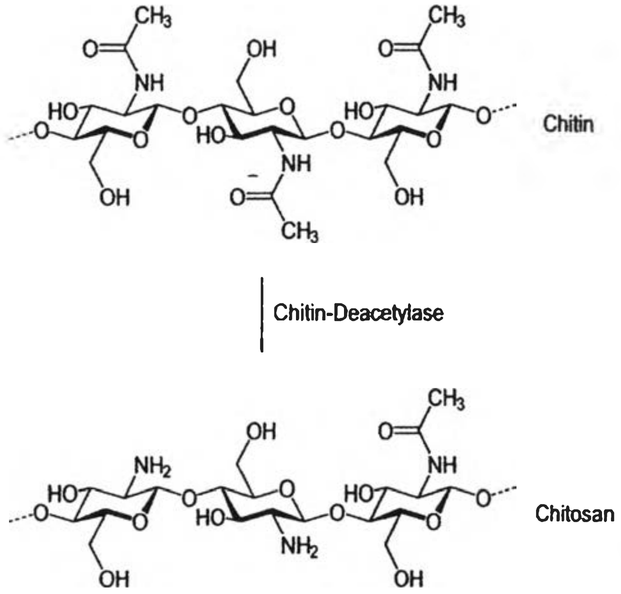
Poly(diallydimethylammonium chloride), PDADMAC and Poly (sodium 4-styrene sulfonate), PSS are macroion and counterion on aqueous solution in all range of pH between 0-14, remain as dissociated polyacid and polybase in all range. Chemical structure of PDADMAC and PSS showed in Figure 2.3.

Figure 2.3 Chemical structure of poly(diallydimethyammonium chloride), PDADMAC and poly(sodium 4-styrene sulfonate), PSS.

In 1999, Dubas *et al.* studied the driving forces behind PEM formation of the strong polycation/polyanion pair, PDADMAC/PSS, stated that the film thickness is proportional to salt concentration, the number of layers, and hydrophobicity and solvent quality. Kumlangdudsana *et al.* (2011) used PDADMAC/PSS PEM coating on poly(methyl methacrylate) (PMMA) substrate to produced the organic solvent resistant microfluidic chips. The more hydrophilic properties of PDADMAC/PSS decreased rate of organic solvent penetration.

2.1.2 Natural PEM by Chitosan/alginate

Chitosan is derived from chitin (poly[b-(1→4)-2-acetamido-2-deoxy-D-glucopyranose]) which is a waste from crustaceous shells such as shrimp and crab, squid pen, and cell wall of some bacteria or fungi, that modified to value-added products. Chitosan (poly[b-(1→4)-2-amino-2-deoxy-D-glucopyranose]), a copolymer of glucosamine and N-acetyl glucosamine formed by the deacetylation of chitin, is a polycationic, biocompatible and biodegradable polymer. In addition, chitosan has different functional groups that can be modified with a wide array of ligands. Because of its unique physicochemical properties, chitosan has great potential in a range of biomedical applications, including tissue engineering, non-viral gene delivery, enzyme immobilization, wound dressing, and control drug release. Moreover, chitosan can be used in several applications such as seed coating in agriculture, film and membrane, and packaging (No *et al.*, 1997). The *N*-deaceylation of chitin converse to chitosan showed in Scheme 2.1.



Scheme 2.1 *N*-deaceylation of chitin converse to chitosan.

The effect of chitosan coating on storage the properties and quality of fresh fruits to extended their shelf life such as in peaches, pears and kiwis by chitosan film, cucumbers, bell peppers, strawberries, and tomatoes could be stored for longer time after coating with chitosan. These results can be attributed to the reduced respiration rate, inhibition of fungal development and delay of ripening process which is caused by ethylene and carbon dioxide development (Shahidi *et al.*, 1999).

Alginate or Alginic acid is an anionic polysaccharide which extracted from cell walls of brown seaweeds (family *Phaeophyceae*), exists as a salt of sodium, calcium, magnesium, strontium, and barium in gelled form. (Mantilla *et al.*, 2013). Alginate is linear, unbranched copolymers made of two uronic acids that contains β-D-mannuronic acid (M) and α-L-guluronic acid (G). The structure of monomer of alginate and alginate chains are shown in Figure 2.4.

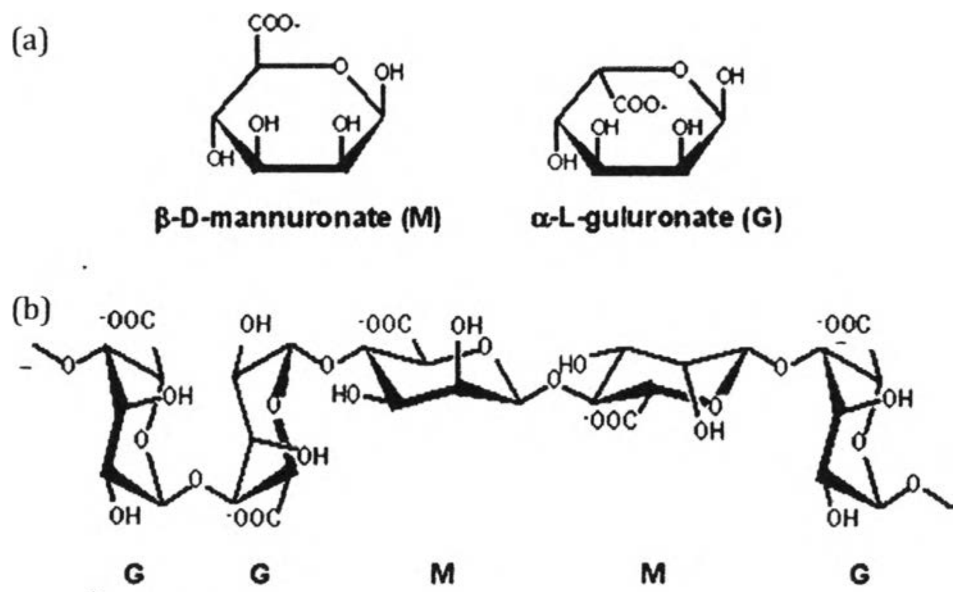


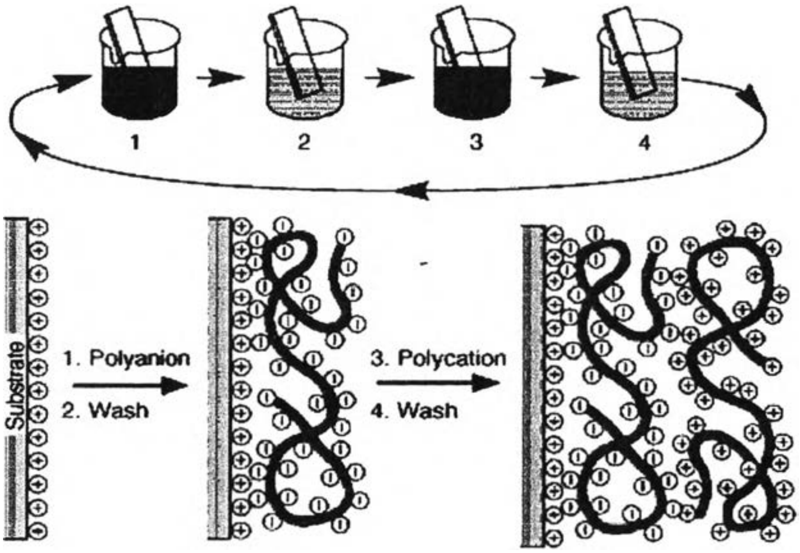
Figure 2.4 Structural alginates: (a) the monomers in alginate (b) the alginate chain (www.apspharmscitech.org).

Sodium alginate is a block polymer that comes along with sodium ions of alternating of both sugars, sodium poly(L-guluronate) and sodium poly(D-mannuronate), which can be soluble in water or mixture of water with up to 20% ethanol of 70% glycerol if the pH is greater than 3 (Sime, 1990). Sodium alginate is a hydrophilic biopolymer that has a coating function because of the properties such as high mechanical stability, high porosity, thickening, and its gelation form, which has been effective to used for maintain post harvest quality of fruit.

2.2 The Layer-by-Layer (LbL) Technique

The layer-by-layer (LbL) assembly is produced by depositing alternating layers of two or more species that require electrostatic interactions between two opposite charges, positive and negative charge by sequential dipping a solid substrate into the cationic and anionic solution (Kumlangdudsana *et al.*, 2011). The bilayers can be preformed in different ways such as dipping, spinning, spraying, and flow base technique. After deposition of each layer the excess of molecules can be remove by immersing the substrate into the washing solution and dried under air or nitrogen.

The experimental parameters that can affect the roughness, thickness and porosity of multilayers are pH of solution, ionic strength, polyelectrolyte concentration, molecular weight, salt concentration and type, solvent quality, and deposited time (Dubas *et al.*, 1999). There are the several advantages of LbL technique for example extremely simple and cheap, wide range of material that can be used of materials including polyions, metals, ceramics, nanoparticles, and biological molecules, high degree of control thickness. Characterization of LbL coating deposition is done by optical techniques such as Polarization interferometry or Ellipsometry of mechanical techniques such as Quartz crystal microbalance (QCM). The LbL strategy has become an important step for the fabrication of layered, nanostructured devices for electrochemical applications because its can control the molecular level of layers (Crespiho *et al.*, 2006). The LbL assemble technique showed in Scheme 2.2.



Scheme 2.2 The layer-by-layer (LbL) assemble technique (G. Decher, 1997).

The nanocoating technology, LbL deposition provided surface modification, interaction between the substrate and the environment, and fabrication of thin film permitting multimaterial fabrication include proteins and colloids. There are several applications of nanocoating LbL for example anticorrosion, antireflective coating, biocompatibilisation, biosensors, implants, optical waveguides, electroluminescent devices, microreactors, etc.

In 2012, Medeiros *et al.* characterized the properties of nanomultilayer coating on 'Tommy Atkins' mangoes, based on food-grade materials, using electrostatic LbL self-assembly with five alternative layers of pectin and chitosan The report showed that the mangoes better external appearance, lower mass loss, lower total soluble solids (TSS), lower titratable acidity (TA), less dehydrated surface, inhibit fungal growth, and much more maintain flesh. This study suggest that the combination of chitosan, as an antimicrobial and good gas barrier properties, and pectin, a low oxygen permeability layers, were efficient in decreasing gas flow leading to the extension of the shelf-life of mangoes.

Pinheito *et al.* (2012) prepared multilayer nanocoatings composed of K-carrageenan and chitosan produced by LbL deposition method onto a polyethylene terephthalate (PET). Methylene Blue (MB), that was used as model cationic compound, was incorporated in different positions of nanolayered coating to measured the loading and release behavior. UV-VIS spectroscopy and quartz crystal microbalance analysis showed that the increase in amount of MB loaded with the distance from the first layer because MB can diffuse into inner nanolayers coating interpenetration. The Linear Superimposition Model explained that MB released with an abnormal behavior depended on the position of MB incorporated on nanolayered coating or the temperature and pH of substrate. The structure of nanolayered can be useful for the development of edible coating no fresh fruit with bioactive compounds to understanding the loading and release phenomena of bioactive compounds.

2.3 The Edible Coatings

Edible coatings are thin layers applied on surface product, which act as a natural protective waxy coating to provide a barrier to gas, moisture and solute movement. An ideal coating characteristics are that it can extend storage life of fruit without anaerobiosis and decrease decay with out effected its quality by reducing moisture and solute migration, gas exchange, respiration and oxidative reaction rates, and reducing physiological disorders (Baldwin *et al.*, 1996). Major advantage of edible coating is it can be loaded with several active ingredients such as antibrowning agent, colorants, flavors, nutrients, and antimicrobial compounds, that can extend product shelf life and decrease the risk of pathogen growth, into the polymer matrix and can used with the food, hence promoted safety or even nutritional and sensory quality. Moreover, the coating help to reduce the synthetic packaging waste because its composed of biodegradable raw material. Biodegradable coatings must exhibit special functional requirements such as water/lipid solubility, color and appearance, mechanical characteristics, nontoxicity, and gas barrier. The characteristics required for coating depend on the product matrix based on moisture content and deterioration process (Guilbert *et al.*, 1996). Effect of coating on fruits depends on temperature, alkalinity, thickness and type of coating, and variety and condition of fruits.

The edible coating can be loaded with potential active ingredients such as antimicrobial agents to extend the microbial stability of fruits. The limit on application of antimicrobial agents directly on fruit surface is the active substances are neutralized or diffused from surface into the fruits so, limiting the effective of the agents. There are several antimicrobials that can used in edible coating for example organic acids (acetic, benzoic, lactic, propionic, sorbic), fatty acid esters (glyceryl monolaurate), polypeptides (lysozyme, nisin, lactoferrin, peroxidase), plant essential oils (EOs) (cinnamon, oregano, lemongrass), nitrites and sulfites, etc (Franssen *et al.*, 2003). The alternative chemical preservatives such as EOs and their active constituents can used on fruits to against microorganisms, including several pathogens but, its using limited due to their impact on organoleptic fruit properties and their interacted with fruit components. The development of antimicrobial edible coating to promoted the properties of fruits and effectiveness of antimicrobial had the drawback about their strong flavor lead to changing the original taste of fruits.

Chien *et al.* (2007) analyzed the effect of coating chitosan with low and high molecular weight on Murcott tangor. The studies showed that increasing the concentration of low molecular weight chitosan promoted the retention of firmness and water content. Coating samples exhibit effective antifungal activity by compared between high molecular weight chitosan and the fungicide thiabendazole (TBZ), improved firmness, titratable acidity, ascorbic acidity, and water content. Therefore, reducing water loss from fruit during storage or ripening helps to maintain the quality of fruit and extended its storage life.

The studied about the edible film based on over-ripe banana puree added pectin and glycerol as plasticizer by Martelli *et al.* (2013) reported that edible film can promoting elongation, better plasticity and handability. Small amounts of chitosan nanoparticles (0.2 wt.%) were found to be as an adequate reinforcement material, raise mechanical properties. In the other hand, the nanoparticles lead to lower water vapor permeation and had no effect on antibacterial activity.

2.4 Essential Oils (EOs)

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds that is generally distilled from plants. EOs are known as volatile oils, ethereal oils, or aetherolea, or simple as the "oil of" the plant from which they were extracted, such as oil of clove. Most EOs are clear, but some oils such as patchouli, orange and lemongrass are yellow. EOs are extracted by stream distillation, expression, or solvent extraction. They are used in perfumes, cosmetics, soaps, and other products. The interesting in EOs had revived in recent decades with the popularity of aromatherapy, a branch of alternative medicine that demand that essential oils and other aromatic compounds have healthful effects. Example of EOs that can incorporated with the edible coating on fruits surface are cinnamon (CM), limonene (LIM), oregano (ON), peppermint (PM), thyme (T), and eugenol (EN).

Rojas-Grau *et al.* (2007) incorporated oregano and lemongrass oils and vanillin on apple puree-alginate edible coatings to extended the shelf-life of fresh-cut 'Fuji' apples, reported that coating containing EOs exhibited the strong antimicrobial activities, reduced respiration rate and ethylene production, severe softening, and the best sensory quality.

In 2011, Vu *et al.* developed an active edible coating preparation in order to increase the shelf-life of fresh food. The strong active antimicrobial against moulds and total flora isolated from strawberries are red thyme (RT), oregano (OR), limonene (LIM), and peppermint (PM) that act as bioactive compounds was sprayed onto strawberries. Moreover, the study showed that the functionalized chitosan by acylation with palmitoyl chloride can increase its hydrophobicity properties to controlled release and improved its stability adhesion to fruit surface.

2.5 Curcumin

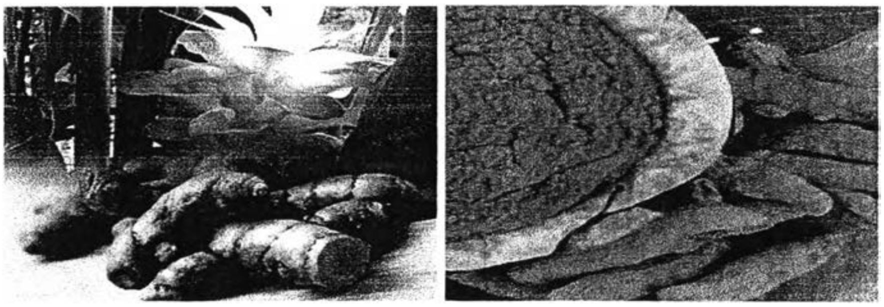


Figure 2.5 (Left) Rhizome of *Curcumin longa* Linn. (Right) Curcumin extract powder.

Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione], a yellow pigment from rhizomes of *Curcuma longa* Linn. (Figure 2.5), is a therapeutic compound in several medical applications such as antioxidative, anticancer activity, anti-inflammatory activity, antimicrobial activity, cholesterol level, rheumatoid arthritis, cataract, induce stress, Alzheimer disease, cystic fibrosis, wound healing, skin lightening and ease pain (Pandey *et al.*, 2011). However, curcumin is poorly soluble in water at neutral pH and room temperature that is decreases its bioavailability. In 2012, Jagannathan *et al.* studied the temperature-dependent on the solubility and stability of curcumin in aqueous medium, the result was shown that at higher temperature, the breaking of intramolecular hydrogen bonding leads to the dissolution of curcumin. Chemical structure of curcumin is shown in Figure 2.6.

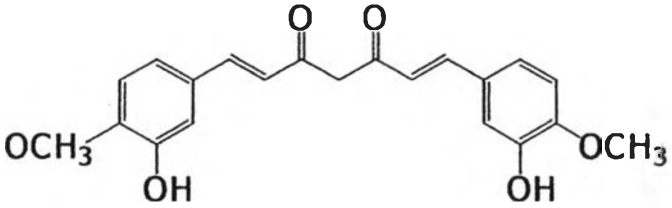


Figure 2.6 Chemical structure of curcumin.

Kittitheeranum *et al.* (2010) studied the kinetics and parameters controlling the loading of 0.01% w/v curcumin in PEM, non-pH-dependent PDADMAC and PSS, thin films as a function of the solvent composition, curcumin concentration and film thickness by UV-vis spectroscopy. They found that the partitioning coefficient between 80/20% of water/ethanol solvent and PEM film is equal to 2.07×10^5. The extinction coefficient of curcumin loaded into PEM was found to 64000 $M^{-1}cm^{-1}$.

2.6 Surfactants

Surfactants in form of micelle or vesicles are useful for solubilizing and stabilizing hydrophobic molecules that are poorly soluble in water. Addition of surfactant to the dissolution medium improves the dissolution of hydrophobic molecules by faciliting the molecules release process at the solid-liquid interface and micelle solubilization in the bulk. The solubility of molecules can be improved by confirming that the surfactant concentration is above the critical micelle concentration (CMC). The CMC of surfactant in recent literature has been defined as the concentration of surfactant solution at which the molecules self aggregate to form micelle. In 2006, Wang *et al.* investigated the use of micelles and vesicles from ionic surfactants for stabilized and solubilized curcumin, a natural product that undergoes alkaline hydrolysis in water.

2.6.1 Sodium Dodecyl Sulfate (SDS)

Sodium dodecyl sulfate (SDS) or sodium lauryl sulfate (SLS) is anionic surfactant used in many cleaning applications which is consisting of 12 carbons tail linked to sulfate group, lead to amphiphilic properties required of detergent for laundry. The CMC is about 6-8mM. The chemical structure of SDS is shown in Figure 2.7.

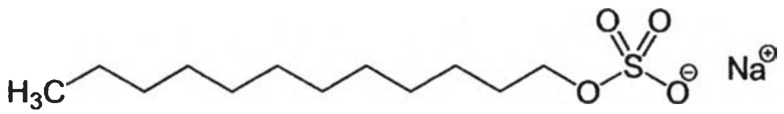


Figure 2.7 Chemical structure of sodium dodecyl sulfate (SDS).

In 2009, Rahman *et al.* developed a new dissolution medium for improve solubility of curcumin at 37 °c adding with surfactant (0.5% sodium lauryl sulfate, SLS, in water) as a dissolution medium. Their result showed that dissolution rate of curcumin increased with increasing SLS concentration.

2.6.2 Hexadecyltrimethylammonium Bromide (CTAB)

Hexadecyltrimethylammonium bromide (CTAB) or cetrimethyl ammonium bromide is cationic surfactant used to providing a buffer solution for extract DNA, synthesis of gold nanoparticles, widely used in household products such as shampoos or cosmetics and it is an antiseptic against bacteria and fungi. Their CMC is about 1mM. The chemical structure of CTAB is shown in Figure 2.8.

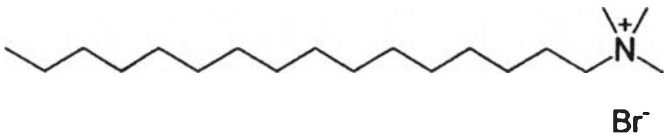


Figure 2.8 Chemical structure of cetrimethylammonium bromide (CTAB).

In 2004, Gamboa *et al.* studied the effect of polymer structure on the polyelectrolyte/micelle interaction. This interaction occurs between the anionic groups of the polymer chain and the cationic head of the CTAB micelle. In this process the bromide counterions which are associated form CTAB will be released to the solution and can measured the amount of free bromide ion concentration by potentiometer. They found that the bromide concentration increase with increasing the interaction between polymer and CTAB relate to the ionization of CTAB micelles.