CHAPTER II

LITERATURE REVIEW

A. Botanical, Chemical and Pharmacological Aspects of *Garcinia mangostana*

1. Botanical Aspects of *Garcinia mangostana*

Garcinia mangostana Linn. is in the family of Guttiferae, its common name is mangosteen (Figure 1). It is a very slow-growing tree, erect, with a pyramidal crown, attains 20 to 82 feet (6-25 m) in height, has dark-brown or nearly black, flaking bark, the inner bark containing much yellow, gummy and bitter latex. The evergreen, opposite, short-stalked leaves are ovate-oblong or elliptic, leathery and thick, dark-green, slightly glossy above, yellowish-green and dull beneath, 3 1/2 to 10 inches (9-25 cm) long, 1 3/4 to 4 inches (4.5-10 cm) wide, with conspicuous, pale midrib. New leaves are rosy. Flowers, 1 1/2 to 2 inches (4-5 cm) wide and fleshy, may be male or hermaphrodite on the same tree. The former are in clusters of 3-9 at the branch tips, there are 4 sepals and 4 ovate, thick, fleshy petals, green with red spots on the outside, yellowish-red inside, and many stamens though the aborted anthers bear no pollen. The hermaphrodite are borne singly or in pairs at the tips of young branchlets, their petals may be yellowish-green edged with red or mostly red, and are quickly shed.

The fruit, capped by the prominent calyx at the stem end and with 4 to 8 triangular, flat remnants of the stigma in a rosette at the apex, is round, dark-purple to red-purple and smooth externally, 1 1/3 to 3 inches (3.4-7.5 cm) in diameter. The rind is 1/4 to 3/8 inch (6-10 mm) thick, red in cross-section, purplish-white on the inside. It contains bitter yellow latex and a purple, staining juice. There are 4 to 8 triangular segments of snow-white, juicy, soft flesh (actually the arils of the seeds). The fruit may be seedless or have 1 to 5 fully developed seeds, ovoid-oblong, somewhat flattened, 1 inch (2.5 cm) long and 5/8 inch (1.6 cm) wide, that cling to the flesh .

Production normally begins in the eighth year after planting, producing a high yield and the maximum production is reached in the 24th year of planting. It is a seasonal fruit, producing normally from June to August. Traditionally seeds have propagated mangosteen, but recently budgrafting has been successful in propagating the plants. The plants mostly proliferate in hot and humid climate, preferably with a short dry season such as in India, Thailand, Indonesia and Philippines (Morton, 1987).

Figure 1 *Garcinia mangostana* Linn.

2. Chemical Components of *Garcinia mangostana*

Xanthones, terpenoids and anthocyanin glycosides have been reported from the fruit rind and other parts of *Garcinia mangostana* and some of them have shown a variety of biological activities (Mahabusarakam, Wiriyachitra and Taylor, 1987; Parveen et al., 1991; Nilar and Harrison, 2002).

Xanthones are highly active plant phenols found in a limited number of plants. They are formed by the condensation of a phenylpropanoid precursor. *Garcinia mangostana* has the largest known number of xanthones from one source, over 20 different varieties. More importantly, the kinds of xanthones in *Garcinia mangostana* show considerable biological activities such as antimicrobial, anti-inflammatory and anti-tumor activities (Bruneton, 1995). The major xanthones are α-mangostin, β-mangostin, γ-mangostin, gartanin, 8-deoxygartanin and garcinones A, B and C (Sen et al., 1980; Mahabusarakam et al., 1987). Chemical structures of α-mangostin and γ-mangostin are shown in Figure 2.

Mangostin or α-mangostin (1,3,6-trihydroxy-7-methoxy-2,8-bis-(3-methyl-2-butenyl)-9*H*-xanthen-9-one) is the most active component. It is isolated from various parts of *Garcinia mangostana*. It appears as yellow crystals with melting point of 181.6-182.6 °C and molecular weight of 410.46. It is soluble in alcohol, ether, acetone, chloroform and ethyl acetate but practically insoluble in water (Budavari, 2001).

Moreover, various chemical compounds from fruit rind were reported as follows: (Sen et al., 1981; Balasubramanian and Rajagopalan, 1988; Asai et al., 1995; Chairungsrilerd, Takeuchi et al., 1996; Gopalakrishnan and Balaganesan, 2000; Huang et al., 2001; Suksamrarn et al., 2002).

- 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2(H), 6(H)-pyrano-(3,2,6)-xanthen-6-one
- BR-xanthone-A
- BR-xanthone-B
- 3-isomangostin
- 3-isomangostin hydrate

- 1-isomangostin
- 1-isomangostin hydrate
- kolanone
- mangostin-3,6-di-O-glucoside
- normangostin
- mangostin triacetate
- garcimangosone A
- garcimangosone B
- garcimangosone C
- garcimangosone D
- mangostanol
- mangostenol
- mangostenone A
- mangostenone B
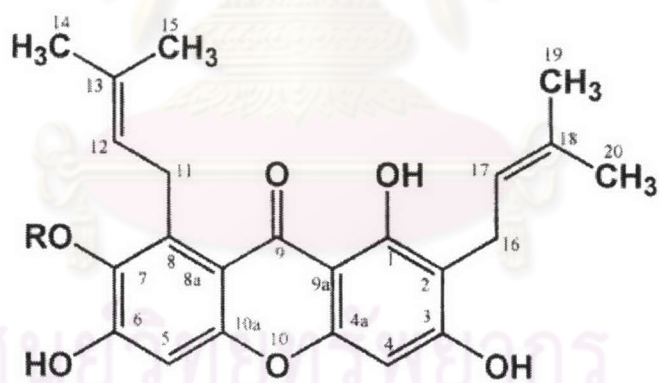
R = CH$_3$ α-Mangostin
R = H   γ-Mangostin

Figure 2 Chemical structures of α-mangostin and γ-mangostin

3. Pharmacological Activities and Toxicities of *Garcinia mangostana*

3.1 Pharmacological Activities

There are many investigations on pharmacological activities of xanthones from *Garcinia mangostana*.

3.1.1 Antibacterial Activity

There are many reports on the antibacterial activity of fruit rind and other parts. Mangostin and its derivatives exhibited inhibitory effect against *Staphylococcus aureus*, bacteria causing wound infection (Mahabusarakam, Phongpaichit, Jansakul et al., 1983; Sundaram et al., 1983). Extracts of *Garcinia mangostana* were investigated against methicillin-resistant *Staphylococcus aureus* (MRSA), it was found that α-mangostin had a minimum inhibitory concentration (MIC) of 1.57-12.5 μg/ml. Other related xanthones were also examined their anti-MRSA activity (Iinuma et al., 1996). Other studies showed that α-mangostin was found to be active against vancomycin resistant *Enterococci* (VRE) and methicillin-resistant *Staphylococcus aureus*, two of the leading causes of nosocomial infections (Phongpaichit et al., 1994; Sakagami et al., 2005).

The alcoholic extract from *Garcinia mangostana* was tested the activity against *Streptococcus mutans*, bacteria causing dental caries, and exhibited significant antibacterial activity (Gritsanapan et al., 2002; Rojanapanthu et al., 2002).

Moreover, the activity against dysenteric bacteria and diarrheal infecting bacteria such as *Escherichia coli, Salmonella agona, Salmonella typhi, Salmonella typhimurium, Salmonella stanley, Salmonella virchow, Salmonella welterverden, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Vibrio cholerae* and *Vibrio parahaemolyticus* have been reported (Sindermsuk and Deekijsermphong, 1989).

3.1.2 Antimycobacterial Activity

Prenylated xantnones isolated from the fruit hulls, edible arils and seeds of *Garcinia mangostana* were tested for their antituberculosis potential. α-mangostin, β-mangostin and garcinone B exhibited strong inhibitory effect against *Mycobacterium tuberculosis* with the MIC value of 6.25 μg/ml (Suksamrarn et al., 2003).

3.1.3 Antifungal Activity

Mangostin and its derivatives showed the activity against *Trichophyton mentagrophytes*, *Microsporum gypseum* and *Epidermophyton floccosum* at a concentration of 1 mg/ml but all the test compounds had no effect on *Candida albicans* (Mahabusarakam, Phongpaichit and Wiriyachitra, 1983; Sundaram et al., 1983). Moreover, the activity against three phytopathogenic fungi, *Fusarium oxysporum vasinfectum, Alternaria tenuis and Dreschlera oryzae*, have been reported (Gopalakrishnan, Banumathi and Suresh, 1997).

3.1.4 Central Nervous System Depressant Activity

Mangostin and its derivatives from the fruit rind had screened for various pharmacological effects in experimental animals. All the test compounds produced CNS depression characterized by ptosis, sedation, decreased motor activity, potentiation of pentobarbital sleeping time and ether anesthesia in mice and rats. None of the compounds exhibited analgesic, antipyretic and anticonvulsant effects (Shankaranarayan, Gopalakrishnan and Kameswaran, 1979).

3.1.5 Cardiovascular Effect

Mangostin-3,6-di-O-glucoside produced significant effects on the cardiovascular system of frogs and dogs. It produced myocardial stimulation and increased in blood pressure which was partially blocked by propranolol (Shankaranarayan et al., 1979).

3.1.6 Anti-inflammatory Activity

Mangostin, 1-isomangostin and mangostin triacetate produced anti-inflammatory activity both by intraperitoneal and oral routes in rats when tested by carrageenin-induced hind paw edema, cotton pellet implantation and granuloma pouch techniques. These compounds did not produce any mast cell membrane stabilizing effect and the degranulation effect of polymyxin B, diazoxide and Triton X-100 on rat peritoneal mast cells *in vitro* was not prevented. These compounds did not alter the prothrombin time of albino rats. Only mangostin produced significant anti-ulcer activity in rats (Shankaranarayan et al., 1979).

The crude extracts from fruit hulls were examined the effect on prostaglandin E_2 (PGE_2) synthesis. The study found that the extracts potently inhibited A23187-induced prostaglandin E_2 synthesis in C6 rat glioma cells (Nakatani, Atsumi et al., 2002). In further study, γ-mangostin was isolated and examined the effect on arachidonic acid cascade in C6 rat glioma cells. The results indicated that γ-mangostin exhibited inhibitory activity of prostaglandin E_2 release induced by A23187, a Ca^{2+} ionophore and competitively inhibited the activities of both constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2) (Nakatani, Nakahata et al., 2002). These results suggest that γ-mangostin is a new useful lead compound for anti-inflammatory drug development.

3.1.7 Antihistamine Activity

A crude methanolic extract inhibited the contractions of isolated thoracic rabbit aorta induced by histamine and serotonin. The extract has been fractioned by silica gel chromatography. The active compounds were α-mangostin and γ-mangostin. The results suggested that α-mangostin and γ-mangostin are a histaminergic and a serotonergic receptor blocking agent, respectively. Further study found that in the presence of chlorpheniramine, a histamine H_1 receptor antagonist, α-mangostin did not affect the relaxation of the rabbit aorta induced by histamine. It is suggested that α-mangostin is novel competitive histamine H_1 receptor antagonist (Chairungsrilerd, Furukawa et al., 1996a, 1996b).

3.1.8 Anti-tumor Activity

The xanthone compounds from *Garcinia mangostana* were tested the cytotoxic effects on a panel of 14 different human cancer cell lines. The results have shown that garcinone E has potent cytotoxic effect on hepatocellular carcinoma cell lines and suggest that garcinone E may be potential useful for the treatment of certain types of cancer (Ho, Huang and Chen, 2002).

The extracts from fruit rind were investigated the *in vitro* antileukemic activity. The effects on the cell growth inhibition of human leukemia cell line HL60 were examined by Hoechst 33342 nuclear staining and nucleosomal DNA gel electrophoresis. All compounds displayed growth inhibitory effects. Among them, α-mangostin showed complete inhibition at 10 μM through the induction of apoptosis (Matsumoto et al., 2003). In another research, the cytotoxic effect against K562, P3HR1, Raji and U937 leukemia cells was evaluated by the colorimetric XTT assay. Results indicated that the extract showed a significant cytotoxic effect against K562 and Raji cells ($P<0.05$). It also possessed moderate anti-U937 activity, but was less effective against P3HR1 cells (Chiang et al., 2004).

The crude methanolic extract of *Garcinia mangostana* was determined of the antiproliferative, apoptic and antioxidative properties using human breast cancer (SKBR3) cell line as a model system. These investigations suggested that the methanolic extract had strong antiproliferaion, potent antioxidation and induction of apoptosis. Thus, it indicates that this substance has potential for cancer chemoprevention which was dose dependent as well as exposure time dependent (Moongkarndi, Kosem, Kaslungka et al., 2004; Moongkarndi, Kosem, Luanratana et al., 2004).

The crude α-mangostin was examined the short-term chemopreventive effects on putative preneoplastic lesions involved in rat colon carcinogenesis. This finding showed that crude α-mangostin has potent chemopreventive effects in short-term colon carcinogenesis bioassay system and suggests that longer exposure might result in suppression of tumor development (Nabandith et al., 2004).

3.1.9 Anti-HIV Activity

The ethanol extract of *Garcinia mangostana* showed potent inhibitory activity against HIV-1 protease. The activity-guided purification of the extract resulted in the isolation of two active, known compounds. The isolated compounds were mangostin and γ-mangostin with the IC_{50} values of 5.12 ± 0.41 microM and 4.81 ± 0.32 microM, respectively (Chen, Wan and Loh, 1996).

3.1.10 Antioxidant Activity

The methanol extract of the fruit hulls was found to exhibit a potent radical scavenging effect. By monitoring the radical scavenging effect, two xanthones, α-mangostin and γ-mangostin were isolated. The antioxidative activity of these two xanthones was measured by the ferric thiocyanate method. It was found that γ-mangostin showed more potent antioxidative activity than BHA and α-tocopherol (Yoshikawa et al., 1994).

Mangostin was investigated the antioxidant effects on metal ion dependent (Cu^{2+}) and independent (aqueous peroxyl radicals) oxidation of human low density lipoprotein (LDL). Mangostin prolonged the lag time to both metal ion dependent and independent oxidation of LDL in a dose dependent manner. Formation of thiobarbituric reactive substances (TBARS), generated in LDL after oxidation, was inhibited with 100 microM of mangostin. Mangostin (100 microM) significantly inhibited the consumption of alpha-tocopherol in the LDL during Cu^{2+} initiated oxidation. From these results, conclude that mangostin is acting as a free radical scavenger to protect the LDL from oxidative damage which is a critical role in cardiovascular and other chronic diseases (Williams et al., 1995). In further study, mangostin and structural modification of mangostin were tested the antioxidant activity on an isolates LDL and plasma assay. The results of this study show that structural modification of mangostin can have a profound effect on antioxidant activity. Derivatisation of C-3 and C-6 with aminoethyl derivatives enhances antioxidant activity, which may be related to changes in solubility (Mahabusarakam, et al., 2000).

3.1.11 Other Activity

Polysaccharides from the pericarps of *Garcinia mangostana* were composed of mainly D-galacturonic acid and a small amount of neutral sugar (L-arabinose as the major one and L-rhamnose and D-galactose as the minor ones). The extract of polysaccharides was studied for immunopharmacological activities by phagocytic test to intracellular bacteria (*Salmonella enteritidis*) and superoxide generation tests. The results showed that the number of *S. enteritidis* in cultured monocyte with extract of pericarp was killed. Superoxide generation test was also done by color reduction of cytochrome C. The extract was stimulating superoxide production. This study suggests that polysaccharides in the extract can stimulate phagocytic cells and kill intracellular bacteria (*S. enteritidis*) (Chanarat et al., 1997).

3.2 Toxicological Studies

Toxicological study of mangostin was performed by Sornprasit et al. (1987). The effects of mangostin on the activities of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) enzymes were tested by treated the rats with high dose of mangostin (200mg/kg body weight) by intraperitoneal injection. The activities of these two enzymes were increased and reached the maximal level after 12 hr injection. The activities of enzymes were dose dependent. Another experiment in this study, the effect of mangostin which were forced fed to rats was compared with paracetamol in the same dose (1.5g/kg body weight). It was found that paracetamol increased the activities of SGOT and SGPT much more than mangostin and the amounts of total liver protein of paracetamol treated rats significantly decreased, whereas mangostin treated rats did not change total liver protein. The results indicate that mangostin has lower hepatotoxicicty than paracetamol.

Hepatotoxic effects of xanthones extracted from fruit rind of *Garcinia mangostana* were studied in isolated rat hepatocytes using the release of cellular transaminase (SGOT and SGPT) as the criteria for loss of cell membrane integrity. Malondialdehyde (MDA) formation, glutathione (GSH) content and aminopyrine N-demethylase activity were investigated for the preliminary information of xanthones

hepatotoxic mechanism. Carbon tetrachloride (CCL_4) was selected as the reference hepatotoxin. Xanthones demonstrated the dose-related hepatotoxic effect, at 200 µg/ml, by increasing the release of cellular transaminases, decreasing MDA formation and GSH content with no change in aminopyrine N-demethylase activity. CCL_4 (0.08mM) showed different result, especially the increase in MDA formation, illustrating its different hepatotoxic mechanism. Coadministration of xanthones and CCL_4 exhibited no additive cytotoxic effects. Pretreatment of xanthones (100 mg/kg/day, p.o., 5 days) prior to isolation of hepatocytes, caused no changes in transaminase activities and MDA formation with the increase in GSH content. In the presence of CCL_4, hepatocytes from xanthone and Tween (as the solvent used to dissolve xanthones) treated rats demonstrated higher production of MDA than the control hepatocytes. Lowering of GSH content by CCL_4 was also observed, with similar reduction in hepatocytes from the control and Tween treated groups. In xanthone treated hepatocytes, the GSH content was similar to control level after incubation with CCL_4. In conclusion, the hepatotoxic mechanisms of xanthones may be different from CCL_4 and may involve cellular GSH level (Sapwarobol, 1997; Pramyothin, Sapwarobol and Ruangrungsi, 2003).

4. The Medicinal Uses of *Garcinia mangostana*

Garcinia mangostana has traditionally been used for a long time for treatment of wounds and diarrhea. The medicinal uses are as follows:

1. Root may be given to treat irregular menstruation.
2. Bark has been used for wound healing and treatment of diarrhea.
3. Leaves are useful for treatment of dysentery.
4. Fruit rind is an astringent and has been used for treatment of both normal and infected wounds including chronic ulcers. It was also used for diarrhea and dysentery.

As the traditional recipes of *Garcinia mangostana* in treatment of diarrhea, the dried fruit rind was boiled with water then the extract was taken. The dose for children is 1-2 teaspoonfuls every 4 hr and the adult dose is 1 tablespoonful every 4

hr. For treatment of wounds, the dried fruit rind was rubbed with a special stone using saturated calcium hydroxide solution as a solvent. The suspension was applied over the wound areas (Perry and Metzger, 1980; Farnsworth and Bunyapraphatsara, 1992).

Considering information received from the pharmacological activities of *Garcinia mangostana*, this medicinal plant exhibits many interesting activities. The extracts from this plant can be developed into the pharmaceutical dosage forms for treatment of the diseases. However, only few studies have been developed from this plant such as Hiranras (2001) designed the buccal mucoadhesive films for treatment of aphthous ulcers and investigated the release mechanism and stability study. The release mechanism was demonstrated to follow diffusion-controlled model. All formulations were stable under the storage at 40 ℃ and 75% relative humidity for 3 months. Panchinda (1992) evaluated the efficacy of 1.5% mangostin cream for chronic wound treatment and reported that it was effective for wound healing and no serious side effect. Kumjorn (2003) compared the healing of foot ulcers in diabetic patients by dressing with mangostin cream and normal saline wet dressing. The results showed that patients who received mangostin cream dressing treatment earned higher healing rates when compared with normal saline dressing treatment and recommended that the mangostin cream is a new alternative dressing for promoting wound healing.

B. Dental Caries and Periodontal Diseases

Dental caries and periodontal diseases are the most common oral of diseases and the major cause of tooth loss in population worldwide. Dental caries and periodontal diseases are infectious diseases, caused by dental plaque. Bacteria in dental plaque ferment dietary sugars and release acids, which remove minerals from the tooth structure and result in dental caries. The further accumulation of plaque around the gingival margin and subgingival region shifts its microbial composition from *Streptococcus*-dominated to a larger number of *Actinomyces spp.* and subsequently spirochete and gram-negative bacilli. These changes are associated with

the development of periodontal diseases including gingivitis and periodontitis (Rosan and Lamont, 2000).

1. Dental Caries

1.1 Etiology

Dental caries, which is also called tooth decay, is the gradual destruction of enamel of a tooth and thus open a path for bacteria to reach the pulp (Figure 3). Dental caries development is considered to involve three major factors: dental plaque, carbohydrate and susceptible tooth surface. The essential process involves demineralization of the tooth structure by high concentration of organic acid produced by specific bacteria in the dental plaque from dietary carbohydrate (Cawson and Odell, 2002; Fejerskov and Kidd, 2003).
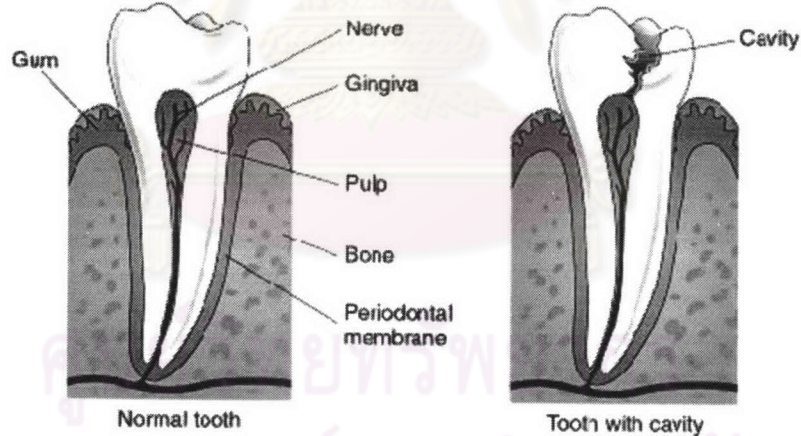
Figure 3 Illustrations of normal tooth and tooth with cavity

1.2 Microbiology

Although dental plaque is composed of a variety of microorganisms, the primary microorganism appears to be *Streptococcus mutans*. *Streptococcus mutans* possess the ability to synthesize extracellular polysaccharides including insoluble-glucans and fructans from dietary sucrose by using glucosyltransferase. This process may increase cariogenicity by enhancing plaque mass, promoting the bacterial colonization on the tooth surface and changing the diffusion properties of the plaque matrix. *Streptococcus mutans* are acidogenic and aciduric. It is able to form acids by fermenting dietary sugars and maintain sugar metabolism under extreme acidic environment such as in carious lesion. The cariogenicity of *Streptococcus mutans* depends on its ability to form insoluble extracellular glucans and ability to produce acid. Glucans from streptococci can adhere to the tooth surface, probably via specific receptors. In this way, *Streptococcus mutans* and its glucans may initiate their attachment to the teeth and build up the critical masses of plaque (Loesche, 1986; Willett, Rosen and White, 1991; Fejerskov and Kidd, 2003).

Table 1 Essential property of cariogenic bacteria

Essential property of cariogenic bacteria
• Acidogenic
• Able to produce a pH low enough (usually pH < 5) to decalcify tooth substance
• Able to survive and continue to produce acid at low levels of pH
• Possess attachment mechanisms for firm adhesion to smooth tooth surfaces
• Able to produce adhesive, insoluble plaque polysaccharides (glucans)

1.3 Dental Plaque

Dental plaque is a soft deposit that accumulates on the tooth surfaces. Plaque can be defined as a complex microbial community, it has been estimated that as many as 400 distinct bacterial species may be found in plaque. In addition to the bacterial cells, plaque contains a small number of epithelial cells, leukocytes, and macrophages. The cells are contained within an extracellular polysaccharide matrix, which is formed from bacterial products and saliva. Inorganic components are also

found in dental plaque; largely calcium and phosphorus which are primarily derived from saliva. The inorganic content of plaque is greatly increased with the development of calculus. The process of calculus formation involves the calcification of dental plaque. The practical consequences of calculus formation are that the deposit is significantly more difficult to remove once calcified, and it leaves a rough surface on the root which is easily colonized by plaque. The calculus on the tooth surface as shown in Figure 4 is brown coloration.
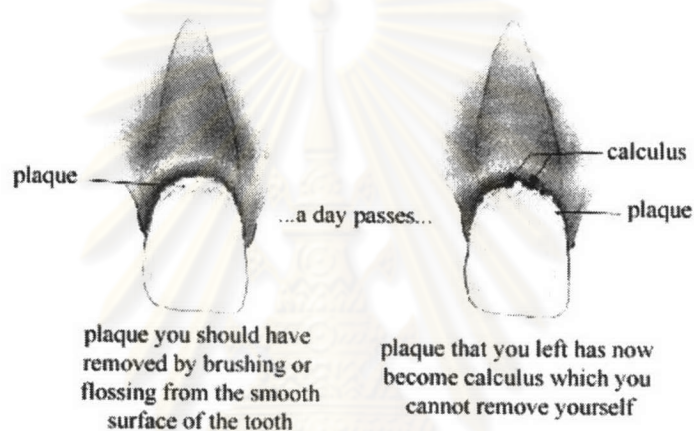

Figure 4 Dental plaque and calculus on the tooth surface

After the initial colonization of the tooth surface, plaque increases by two distinct mechanisms: 1) the multiplication of bacteria already attached to the tooth surface and 2) the subsequent attachment and multiplication of new bacterial species to cells of bacteria already present in the plaque mass. The secondary colonizers include gram-negative species such as *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Capnocytophaga* species. A key property of these microorganisms appears to be the ability to adhere to gram-positive species already present in the existing plaque mass. These organisms would typically be found in plaque after 1 to 3 days of accumulation.

After one week of plaque accumulation, other gram-negative species may also be present in plaque. These species represent what is considered to be the "tertiary colonizers" include *Porphyromonas gingivalis*, *Eikenella corrodens*,

Actinobacillus actinomycetemcomitans, Campylobacter species and the oral spirochetes (*Treponema* species). The structural characteristics of dental plaque in this time period reveal complex patterns of bacterial cells of cocci, rods, fusiform, filaments, and spirochetes. The structural interactions of the bacteria probably are a reflection of the complex metabolic interactions that are known to occur between different plaque microorganisms. One example of this is the production of succinic acid from *Campylobacter* species that is known to be used as a growth factor by *Porphyromonas gingivalis. Streptococcus* and *Actinomyces* species produce formate, which may then be used by *Campylobacter* species. *Fusobacterium* species produce both thiamine and isobutyrate that may be used by spirochetes to support their growth. The metabolic and structural interactions between different plaque microorganisms are a reflection of the incredible complexity of this ecological niche.

The overall pattern observed in dental plaque development is a very characteristic shift from the early predominance of gram-positive facultative microorganisms to the later predominance of gram-negative anaerobic microorganisms, as the plaque mass accumulates and matures. This developmental progression is also reflected in the shifts in predominant microorganisms that are observed in the transition from health to disease (Rosan and Lamont, 2000).

Table 2 Selected bacterial species found in dental plaque

	Facultative	Anaerobic
Gram-positive	*Streptococcus mutans*	
	Streptococcus sanguis	
	Actinomyces viscosus	
Gram-negative	*Actinobacillus actinomycetemcomitans*	*Porphyromonas gingivalis*
	Capnocytophaga species	*Fusobacterium nucleatum*
	Eikenella corrodens	*Prevotella intermedia*
		Bacteroides forsythus
		Campylobacter species
Spirochetes		*Treponema denticola*

2. Periodontal Diseases

2.1 Etiology

Periodontal disease is the general description applied to the inflammatory response of the gingiva and surrounding connective tissue to the bacterial or plaque accumulations on the teeth. These inflammatory responses are divided into two general groupings: gingivitis or periodontitis. Gingivitis is extremely common, and is manifested clinically as bleeding of the gingival or gum tissues without evidence of bone loss or deep periodontal pockets. Pocketing is the term given to the pathologic loss of tissue between the tooth and the gingiva, creating spaces that are filled by dental plaque. Periodontitis occurs when the plaque-induced inflammatory response in the tissue results in actual loss of collagen attachment of the tooth to the bone, to loss of bone and to deep periodontal pockets.

Immunological mechanisms are involved in periodontal disease. The bacterial plaque have more virulent factor that activated host cells and initiated inflammation. Neutrophils are recruited to the periodontal pocket because of attracting molecules, released by the bacteria, called chemotactic peptides. Furthermore, as bacteria damage the epithelial cells, they cause epithelial cells to release molecules termed cytokines that further attract leukocytes to the crevice. The neutrophils within the crevice can phagocytosis and digest bacteria and therefore, remove these bacteria from the pocket. The neutrophil defense may in some instances operate well and reduce the bacterial load and can be considered important in preventing the gingivitis lesion from becoming established. If, however there is an overload of microbial plaque, then the neutrophils and the barrier of epithelial cells will not be sufficient to control the infection. In such instances, the gingival tissue will become inflammation and alteration in the blood vessel network and many capillary beds are open. Serum transudate and proteins from the blood cause swelling and there is an influx of inflammatory cells into the tissue. The inflammatory cells include lymphocytes, macrophages and neutrophils. Macrophages and neutrophils are phagocytic cells that engulf and digest bacteria, whereas lymphocytes are more

involved in mounting an immune response the microbes (Kinane, 2001; Wilson and Kornman, 2003).
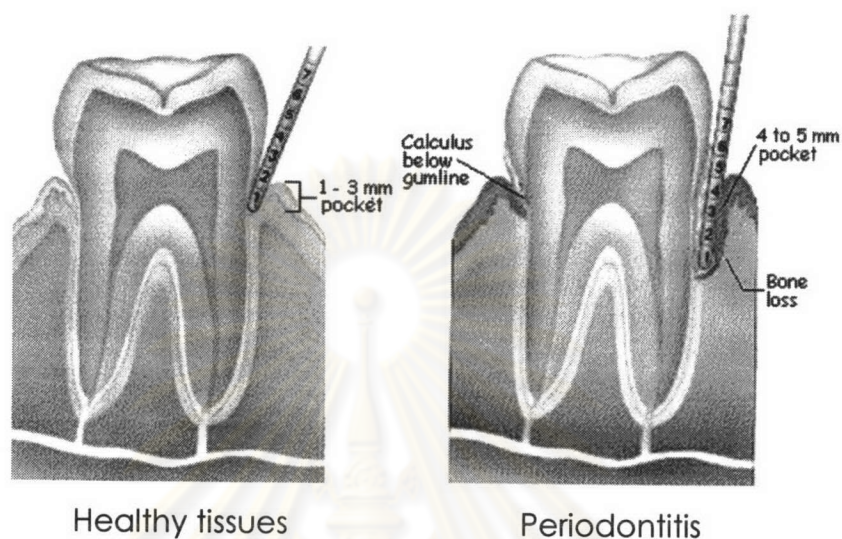

Figure 5 The depth of periodontal pocket is measured by periodontal probe

2.2 Microbiology

About 400 bacterial species are found in the human subgingival plaque samples. Of that number, possibly 10-20 species may play a role in the pathogenesis of destructive periodontal disease. The microbes involved in periodontal disease are largely gram-negative anaerobic bacilli with some anaerobic cocci and a largely quantity of anaerobic spirochetes. The main organisms linked with deep destructive periodontal lesions are *Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus* and *Actinobacillus actinomycetemcomitans. Porphyromonas gingivalis* is more frequently detected in severe adult periodontitis. They are reduced in numbers in successfully treated sites but are seen in sites with recurrence of disease after therapy. It has been shown that *Porphyromonas gingivalis* induced elevated systemic and local antibody responses in subjects with various forms of periodontitis. *Bacteroides forsythus* has been found in higher numbers in sites exhibiting destructive periodontal disease than in gingivitis or healthy sites. *Actinobacillus actinomycetemcomitans* appears to be the predominant periodontal pathogen. The

above mentioned putative pathogens are always part of a large and varied microflora found in the subgingival plaque. Some are not detected in certain sites with periodontitis and can even be completely absent in cultures (Haffajee and Socransky, 1994; Kinane, 2001; Wilson and Kornman, 2003).

Table 3 Microbial species associated with various clinical forms of periodontitis

Species	Juvenile periodontitis	Early onset periodontitis	Adult periodontitis	Refractory periodontitis
Actinobacillus actinomycetemcomitans	+++	++	++	+ to ++
Porphyromonas gingivalis	±	+++	+++	++
Prevotella intermedia	++	+++	+++	+++
Fusobacterium nucleatum	+	++	+++	++
Eikenella corrodens			+++	
Bacteroides forsythus	±	++	+++	++

± occasionally isolated; + less than 10% of the patients positive; ++ less than 50% of the patients positive; +++ more than 50% of the patients positive
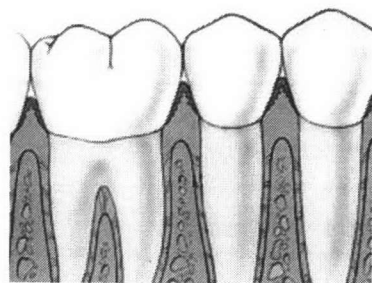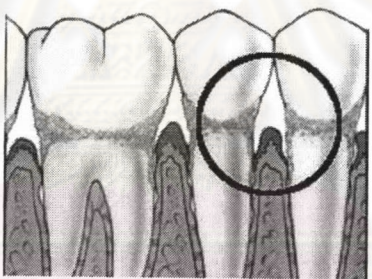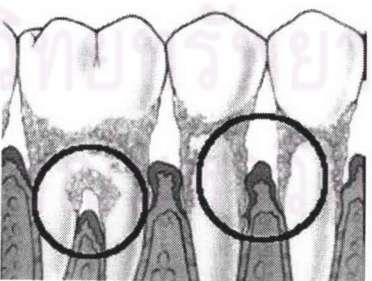
Normal Gums and Bone
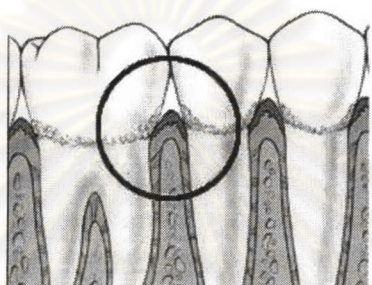
Hard Deposits and Inflammation Developing

Significant Bone Destruction, Hard Deposits, & Infected Gums

Inflammation and Bone Loss are Severe.
The teeth become loose.

Figure 6 The progression of periodontal disease

3. Methods in Dental Plaque Control

Both dental caries and periodontal diseases are infectious diseases, caused by dental plaque. Therefore, elimination of bacteria in dental plaque is an essential factor in prevention and treatment of the diseases. Therapeutic approaches are mechanical scaling and root planing to remove subgingival plaque and calculus. As a result of treatment, there is a decrease of subgingival pathogens and inflammation (Kaldahl, Kalkwarf and Patil, 1993). Unfortunately, in some instances, the complex anatomy of the root and periodontal pocket lead to inability and difficult to eradicated all pathogens. In addition, control of supragingival plaque is essential in order to prevent recolonization of the subgingival pathogens. Clinical study indicated that scaling and root planing, in combination with optimal oral hygiene, results in an alteration of the subgingival plaque which is sufficient to stop periodontal destruction (Suomi et al., 1971). Thus, oral hygiene is important for the clinical outcome of the treatment. However, severe forms of periodontitis often cannot be arrested by mechanical treatment alone.

Systemically applied antimicrobials have been advocated for the treatment of severe forms of periodontitis. However, side effects including hypersensitivity, gastrointestinal intolerance and the development of bacterial resistance have been described (Bollen and Quirynen, 1996). Some studies also reported poor results due to the active product could not achieve an adequate concentration at the site of action and the inability of the active product to be retained locally for a sufficient period of time (Vandekerckhove, Quirynen and Steenberghe, 1997). These led to use of locally antimicrobial agents. The concentration of drug in local tissue can be enhanced by incorporating the active agent into controlled release delivery systems and placed directly in the periodontal pocket. Sustained local delivery systems might also be recommended for sites considered as difficult to instrument because of depth or an anatomical complexity (Kornman, 1993). Many studies suggest that the controlled delivery devices are a useful adjunct to conventional treatments but are no substitute for these treatments (Schwach-Abdellaoui, Vivien-Castioni and Gurny, 2000).

C. Drug Delivery Devices in Periodontics

Local delivery of antimicrobial agents by controlled delivery systems has been attractive for pharmaceutical development. In recent years, there have been introduce new commercial products available in United States and Europe such as Actisite® (25% tetracycline in ethylene vinyl acetate fibers), PerioChip® (33% chlorhexidine in a gelatin-based chip), Elyzol® (25% metronidazole in glyceryl monooleate and sesame oil) and Atridox® (10% doxycycline in poly(DL-lactide) dissolved in a biocompatible solvent N-methyl-2-pyrrolidone). The attractiveness of treating periodontal disease by the sustained release of antimicrobial agents in the periodontal pocket is based on the prospects of maintaining effective high levels of drug in the gingival crevicular fluid (GCF) for a sustained period of time to produce the desirable clinical benefits of attachment level gain, pocket depth reduction and bleeding on probing reduction. In addition, a local delivery device should have a high patient acceptance and a method of application acceptable to the dentist's practice (Southard and Godowski, 1998).

Table 4 Advantages and disadvantages of controlled delivery systems for the treatment of periodontitis

Advantages	Disadvantages
• Maintenance of drug levels in a therapeutically desirable range	• Toxicity or lack of biocompatibility of the polymer material
• Reduction or elimination of harmful side effects of drugs	• Pain caused by the presence of the implant
• Protection from degradation of drugs with short *in vivo* half-life	• Production of harmful by-products from a polymer if it is biodegradable
• Elimination of patient discomfort compared to parenteral administration	• Need of surgical procedure to implant the device in the appropriate location
• Improved patient compliance	• Expense of a particular polymer-drug formulation

1. Fiber

1.1 Hollow Fiber

The reservoirs without rate control delivery devices such as hollow fibers filled with a therapeutic agent in which the agent is released simply by diffusion through the reservoir wall (Kornman, 1993).

The first delivery devices involved hollow fibers of cellulose acetate filled with tetracycline. Although GCF levels of tetracycline remained in the therapeutic range for 24 hr and some effects on spirochetes were reported. These fibers released tetracycline at a first order rate with 95% of the drug released in the first 2 hr. The study should be viewed primarily as an evaluation of drug delivery (Goodson, Haffajee and Socransky, 1979; Lindhe et al., 1979).

1.2 Ethylene Vinyl Acetate Fiber

After the poor control of drug release from hollow fibers, Goodson et al. (1983) evaluated the delivery of tetracycline incorporated into different polymers. Ethylene vinyl acetate fiber, a non-biodegradable copolymer, was found to be flexible and allow drug delivery for up to 9 days *in vitro*. The ethylene vinyl acetate fibers containing 25% tetracycline hydrochloride commercialized under the trademark Actisite® were placed circumferentially into the pockets with an applicator, and secured with cyanoacrylate adhesive.

A study conducted on 20 patients evaluated the safety and clinical efficacy of tetracycline fibers applied for 10 days after scaling and root planing. Analysis of data indicated that a significant decrease in probing depth and gain in attachment were present at all follow-up visits. The proportion of bleeding pockets was reduced from 77 to 27 percent during the experimental period (Vandekerckhove, Quirynen and Steenberghe, 1997). In a study enrolling 122 patients from three dental centers, ten adverse events related to the fiber treatment were reported including three cases of oral candidiasis and among the seven remaining, severe gingival redness, tongue pigmentation and glossitis (Drisko et al., 1995).

2. Strip

Strips containing 25% tetracycline hydrochloride or metronidazole in poly(hydroxybutyric acid) as a biodegradable polymer matrix showed sustained release over 4-5 days with a significant burst effect at first day. A favorable alteration occurred in the microbial flora of pockets treated with strips containing metronidazole compared to those treated by root planing. The clinical improvement was a short duration as the results were not maintained over time once the active treatment was terminated (Deasy et al., 1989).

A controlled release strip coded PT-01, made of poly(methacrylic acid) and hydroxypropylcellulose containing 10% ofloxacin has been reported by Kimura et al. (1991). Data showed that ofloxacin could be found in higher concentrations than the MIC of most periodontopathic bacteria in GCF over 7 days by a single application of PT-01. Although the weekly application of PT-01 on days 0-35 showed some further shift in the proportion and reduction in subgingival microorganisms, statistically no significant differences in the microbiological results between the strip group and the control groups were found.

So far, no product has been marketed because of the only temporary clinical improvements after treatment completion.

3. Film

Films based on synthetic biodegradable polymers, poly(lactide/glycolide) (PLGA) containing 25% tetracycline hydrochloride were developed and evaluated *in vitro* and clinical study. *In vitro* study showed an incomplete release of tetracycline, only 30-60% of total tetracycline was released. This result was explained by the presence of drug particles entrapped within the hydrophobic, PLGA matrix. Preliminary results from 8 patients indicate that the films were effective in decreasing the bacterial count in GCF and demonstrated a significant microbial inhibition for two weeks over the control placebo film (Agarwal et al., 1993).

Ethyl cellulose films containing either 20% metronidazole or 20% chlorhexidine were compared to short-term use of systemic antibiotic in patients with advanced forms of periodontal disease in order to prevent the surgery. All teeth treated with the ethyl cellulose films were scaled just before the insert of the films. There was 93% reduction in the need for periodontal surgery for individual teeth and 81% reduction in the need for tooth extractions (Loesche et al., 1996).

4. Chip

More recently, a chip composed of cross-linked hydrolyzed gelatin matrix for local delivery of chlorhexidine gluconate has been developed and commercialized under the trademark PerioChip® (Figure 8). In a pharmacokinetic study, there was an initial peak concentration of chlorhexidine in the GCF at 2 hr after chip insertion with slightly lower concentrations maintained over the next 4 days. The chlorhexidine concentration then progressively decreased until study conclusion after 10 days. The mean concentration of chlorhexidine in the GCF was 2007 µg/ml at 2 hr and remained in the range of 1400-1900 µg/ml for the next 70 hr. Chlorhexidine remained at clinically effective levels (MIC 125 µg/ml) in the GCF of the periodontal pockets for over 1 week with no detectable systemic absorption (Soskolone et al., 1998).

The meta-analysis indicated that randomized clinical trials of the chlorhexidine chip have been shown to enhance effects of scaling and root planing. Chlorhexidine chip in conjunction with scaling and root planing, when compared to scaling and root planing alone, has shown significant improvement in probing pocket depth reduction, probing attachment level and bleeding on probing. This local delivery system, in combination with scaling and root planing, has also resulted in significantly more probing depth reductions of 2 mm or more. The system is safe and effective. Placement of the chip is usually done in less than 1 min and the chip is completely degraded by enzymes within 7 to 10 days and does not need to be removing (Killoy, 1998).
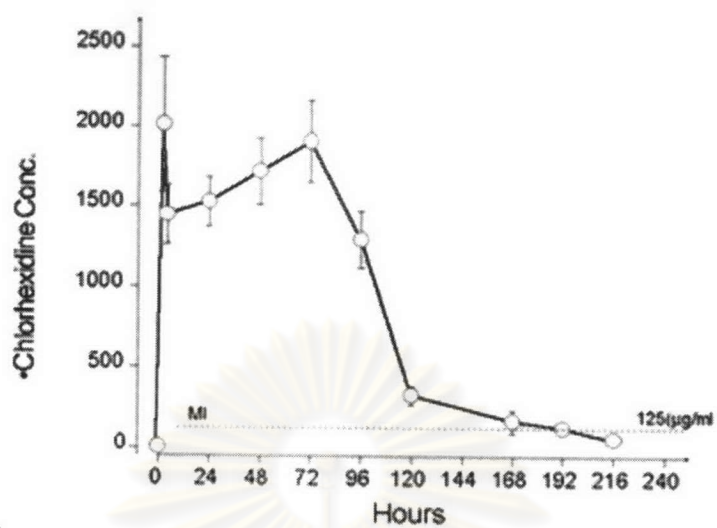
Figure 7 Release profile of chlorhexidine chip

Figure 8 PerioChip® (chlorhexidine gluconate)

The most frequently observed adverse events in the clinical trials of the chlorhexidine chip were transient toothache, upper respiratory tract infection and headache. Toothache was the only adverse reaction that was significantly higher in the treatment group when compared to placebo ($P=0.042$). Most oral pain or sensitivity occurred within the first week of the initial chip placement, was mild to moderate in nature, and spontaneously resolved within days (Jeffcoat et al., 1998).

5. Injectable System

Injectable systems are particularly attractive for the delivery of antibiotic agents into the periodontal pocket. The application can be easily and rapidly carried out, without pain, by using a syringe. Thus, the cost of the therapy is considerably reduced comparing to devices that need time to be placed. Moreover, an injectable delivery system should be able to fill the pocket, thus reaching a large proportion of pathogens.

Two types of injectable delivery systems have been assessed in the treatment of periodontal diseases, biodegradable microspheres and gels.

5.1 Microspheres

Minocycline microspheres were developed by incorporating minocycline into poly(lactide/glycolide) (PLGA), a bioresorbable polymer and investigated the efficacy and safety in clinical study. Patients with moderate to advanced periodontitis were enrolled and randomized to 1 of 3 treatment arms: 1) scaling and root planing alone, 2) scaling and root planing plus vehicle, or 3) scaling and root planing plus minocycline microspheres. The results showed that minocycline microspheres plus scaling and root planing provided substantially more probing depth reduction than other two groups. The difference reached statistical significance after the first month and was maintained throughout the 9 months trial. There was no difference in the incidence of adverse effects among treatment groups (Williams et al., 2001).

Minocycline microspheres were also evaluated the efficacy in smokers with chronic periodontitis. Significantly greater pocket depth reductions with scaling and root planing plus adjunctive minocycline microspheres treatment were observed at 1, 6 and 9 months ($P<0.05$) versus control treatments. The data indicated that locally delivered minocycline microspheres was more effective than scaling and root planing alone in reducing pocket depths in smokers with chronic periodontitis (Paquette et al., 2003).

Recently, minocycline microspheres was approved for marketing by the U.S. Food and Drug Administration under the trademark Arestin® and will be available in the market soon. Arestin® is provided as a dry powder, packaged in a unit-dose cartridge (Figure 9). Arestin® is a variable dose product, dependent on the size, shape and number of pockets being treated. The duration of dosage is 3-month intervals. Arestin® does not have to be removed as it is bioresorbable.

Figure 9 Arestin® (minocycline hydrochloride)

5.2 Gel

Mucoadhesive, metronidazole-containing gel systems designed for periodontal treatment, based on hydroxyethylcellulose, carbopol 974P and polycarbophil have been described. *In vitro* drug release was significantly decreased as the concentration of each polymeric component was increased, due to both the concomitant increased viscosity of the formulation and additionally, the swelling kinetics of polycarbophil following contact with dissolution fluid. Increasing the concentrations of each polymeric component significantly increased formulation hardness, compressibility, adhesiveness and syringeability due to polymeric effects on formulation viscosity. The optimal choice of bioadhesive formulation for use in periodontal disease will involve a compromise between achieving the necessary release rate of the drug and the mechanical characteristics of the formulation (Jones et al., 1997).

The semi-solid systems based on auto-catalyzed poly(ortho esters) (POEs) containing tetracycline free base were developed and studied for *in vitro* drug release and preliminary trial in humans. By modifying the proportion of lactic acid in the polymer, viscous or solid materials having different degradation rate can be produced. Tetracycline free base was released within 10-14 days depending on polymer structure (Schwach-Abdellaoui et al., 2001). Formulations containing 10% or 20% tetracycline were evaluated in a panel of 12 patients with severe and recurrent periodontitis. In the first trial including 6 patients were treated immediately after scaling and root planing. Patients tolerated both formulations well, experienced no pain during application and showed no signs of irritation or discomfort during the observation period. However, retention of the formulation was minimal in this first study. An improved clinical protocol followed in the second study (stopping bleeding after scaling and root planing) prolonged the retention of the formulations in the inflamed periodontal pockets. For up to 11 days, tetracycline concentrations in the GCF were higher than the MIC of tetracycline against most periodontal pathogens (Schwach-Abdellaoui et al., 2002).

An injectable lipid-like based on glyceryl monooleate and sesame oil containing 25% metronidazole (Elyzol®) has become available with supportive

evidence of efficacy (Figure 10). This system is based on the ability of mixtures of monoglycerides and triglycerides to form liquid crystals in contact with water. It is formulated as a suspension, which transforms to a controlled release, semi-solid on contact with GCF (Norling et al., 1992). As a gel is made from monoglycerides and triglycerides which are subject to lipolysis and other types of esterase activity. The dental gel will be rapidly eliminated from the periodontal pocket (Stoltze, 1995). The metronidazole concentration was monitored in GCF after one application of a 25% dental gel. The concentrations obtained were generally above MIC_{50} for susceptible periopathogens 24 hr after one application of a 25% metronidazole gel. Therapeutic levels were maintained for a period of 2-3 days (Stoltze, 1992). The clinical trial was designed to compare the clinical efficacy of scaling with application of 3 different preparations/dose frequencies of topical metronidazole in the treatment of adult periodontitis. The 4 treatments were: (A) metronidazole 25% dental gel applied 1 × a week for 2 weeks; (B) metronidazole 15% dental gel applied 1 × a week for 2 weeks; (C) metronidazole 15% dental gel applied 2 × a week for 2 weeks; (D) subgingival scaling, performed 1 × only. Data indicated that all 3 antibiotic treatments (A, B, C) reduced the symptoms of periodontal pathology comparable to subgingival scaling (D). When using a topical drug therapy, it seems important to use a preparation that requires as few applications as possible. The best candidate for drug therapy would therefore be treatment (A) metronidazole 25% applied 1 × a week for 2 weeks (Klinge et al., 1992). Many clinical trials were developed to compare the effects of topical application of metronidazole 25% dental gel and subgingival scaling in the treatment of periodontitis. Data indicated that both treatments were effective in reducing probing pocket depth and bleeding on probing. Metronidazole trended to be slightly better than scaling during the study period (Ainamo et al., 1992; Pedrazzoli, Kilian and Karring; 1992). Local metronidazole in combination with scaling and root planing seems to be more effective in both clinical and microbiological improvements (Noyan et al., 1997). Another study showed that local metronidazole in combination with scaling and root planing was statistical significantly better ($P<0.001$) than scaling and root planing alone, and these improvements were maintained for 9 months of the study (Griffiths et al., 2000).

Figure 10 Elyzol® (metronidazole) Dentalgel

Two different semi-solid formulations based on poly(oxyethylene) poly(oxypropylene) block copolymer (poloxamer 407) and monoglycerides were designed for administration of tetracycline. These two formulations were characterized by sol-gel transition, becoming semi-solid once in the periodontal pocket. In the case of monoglycerides gel, tetracycline release was slower than poloxamer gel. After 7 hr, the released tetracycline was 18% and 65% of the entrapped drug for monoglycerides and poloxamer gels, respectively. *In vivo* retention results reported that the persistence of monoglycerides gel was more prolonged than poloxamer gel. Poloxamer gel disappeared after 1 hr while monoglycerides gel was still retained after 8 hr. However, clinical study indicated that both gels in conjunction with scaling and root planing produced a significantly improvement outcome in moderate to deep periodontal pockets (Esposito et al., 1996).

Another injectable biodegradable delivery system (Atrigel®) containing 10% doxycycline hyclate in poly(DL-lactide) dissolved in a biocompatible solvent *N*-methyl-2-pyrrolidone was widely studied. In this system, a water insoluble biodegradable polymer is dissolved in a biocompatible water soluble solvent. Upon injection into an aqueous environment, the solvent diffuses into the surrounding aqueous environment while water diffuses into the polymer matrix. This leads to precipitation or coagulation of polymer to form an *in situ* implant (Hatefi and

Amsden, 2002). Pharmacokinetic data of controlled delivery system were obtained from GCF, saliva and serum of adult periodontitis patients. Results showed the high concentration of drug available at the treated sites coupled with the relatively low levels in the saliva and almost non existent levels in the serum indicating that this biodegradable controlled release delivery system displayed an appropriate pharmacokinetic profile for the delivery of doxycycline into periodontal pockets (Stoller et al., 1998). The clinical efficacy and safety of doxycycline hyclate delivered subgingivally in a biodegradable polymer was compared to placebo control, oral hygiene and scaling and root planing in 2 multi-center studies. Results demonstrated that treatment of periodontitis with subgingivally delivered doxycycline was equally effective as scaling and root planing and superior in effect to placebo control and oral hygiene in reducing the clinical signs of adult periodontitis over a 9-month period. Safety data demonstrate a benign safety profile with use of this product (Garrett et al., 1999). Another study showed that local delivery of doxycycline hyclate without concomitant mechanical scaling and root planing was equally effective in periodontal maintenance patients over a 9-month study period (Garrett et al., 2000).

Recently, the Atrigel® delivery system for controlled release of 10% doxycycline hyclate has been approved by the U.S. Food and Drug Administration for commercialization under the trademark Atridox®. This product composed of 2 syringe mixing system. Syringe A contains 450 mg of Atrigel® delivery system. Syringe B contains doxycycline hyclate which is equivalent to 42.5 mg doxycycline (Figure 11). The constituted product is a viscous liquid; upon contact with the GCF, the liquid product solidifies and then allows for controlled release of drug for a period of 7 days. However, this product has some disadvantage; Atridox® has to be maintained in the pocket by cover the pocket with either Coe-Pak® periodontal dressing or Octyldent® dental adhesive which may suffer the patients.
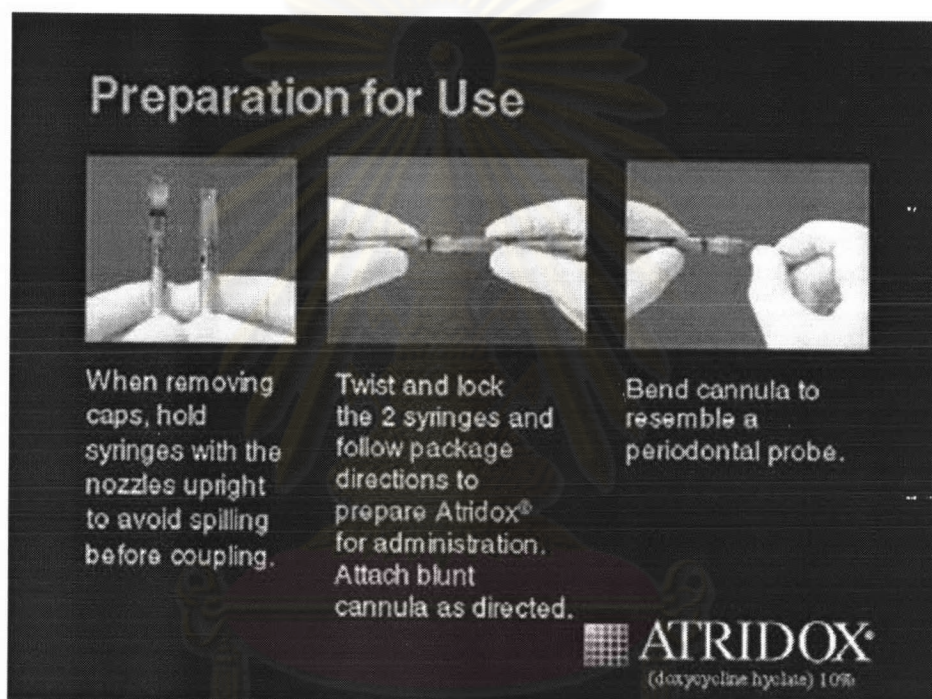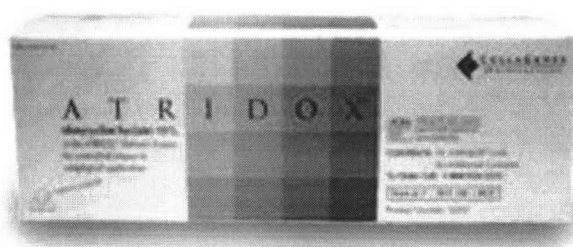
Figure 11 Atridox® (doxycycline hyclate)

In conclusion, the publications dealing with efficacy studies suggest that the controlled delivery devices are a useful adjunct to conventional treatments. Despite the large number of studies, there are insufficient comparative data to support any one of the local delivery systems as superior to another. Variability from site to site has been repeatedly noted that the same system could not work equally in all sites and in all patients. Answer to this question should allow an optimal treatment for each case of periodontal disease in the future.

D. Liquid Crystalline Phases as Drug Delivery Systems

Liquid crystals, also called mesomorphs or mesophases, are states of matter intermediate between crystalline solids and isotropic liquids (Figure 12). It may be prepared either by heating the crystalline solid (thermotropic mesomorphism) or by addition of controlled amounts of polar solvents, generally water, to certain organic compounds (lyotropic mesomorphism). Many amphiphilic compounds, such as soaps and surfactants, exhibit a tendency to form lyotropic mesophases on the addition of water. These structures result from amphiphilic nature of the molecule with both a hydrophobic and a hydrophilic end. There are many types of liquid crystalline phases such as lamellar, hexagonal and cubic phases.

Solid Crystal   Liquid crystal   Liquid

Figure 12 Arrangement of molecules in solid crystal, liquid crystal and liquid

Liquid crystalline phases offer a number of useful properties for drug delivery. First, they allow drug solubilization and with a proper choice of self-association structure both water soluble and oil soluble drugs may be incorporated also in rather high concentrations. This offers possibilities to increase the drug solubility, decrease drug degradation and control and sustain the drug release rate. Second, liquid crystalline phases frequently display a rather high viscosity, which may also offer opportunities when the drug formulation needs to be localized such as intramuscular injection or application in the oral cavity (Malmsten, 2002).

Due to their frequently high viscosity and stiffness, liquid crystalline phases are often difficult to prepare and handle from a practical perspective. For example, mixing is difficult and administration is complicated, of limited patient compliance or inefficient. Therefore, the *in situ* transition from a low-viscous state to the required high-viscous liquid crystalline phase after administration is of major importance for the use of liquid crystalline phases for drug delivery. There are several parameters which may be used for triggering such a transition *in situ* after administration (Malmsten, 2002), including

1. Temperature (The body temperature is higher than the storage temperature)
2. Dilution (The formulation is often in contact with excess water after administration)
3. Salt (The physiological electrolyte concentration may be used to screen electrostatic interactions in the formulation)
4. pH (The physiological pH at the administration site may be used to either reduce or increase electrostatic interactions in the formulation)
5. Calcium ion concentration (Strong binding of Ca^{2+} to carboxyl groups may be used to change the electrostatic interactions in the formulation after administration)

Polar amphiphilic lipids such as glyceryl monooleate (GMO) or monoolein is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate. The acyl chain (oleic acid) is attached to the glycerol backbone by an ester bond (Figure 13). The two remaining carbons of the glycerol have active hydroxyl groups, giving polar characteristics to this portion of the molecule. The glycerol moiety may form hydrogen bonds with water in an aqueous environment and is commonly referred to as the head group. The hydrocarbon chain gives hydrophobic characteristics to glyceryl monooleate and is often termed the tail (Ganem-Quintanar, Quintanar-Guerrero and Buri, 2000).
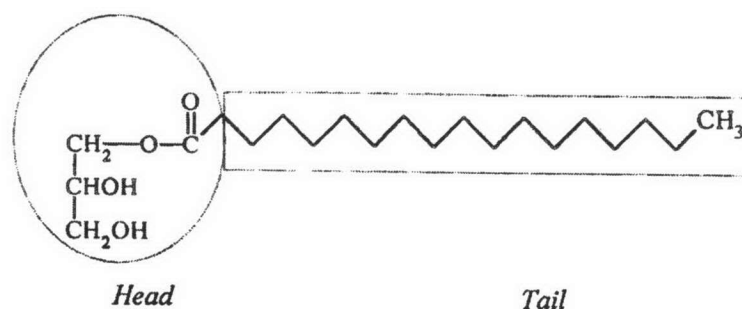
Figure 13 Structural formula of glyceryl monooleate

Glyceryl monooleate when placed in water reorganizes into lipid bilayers forming a reversed micellar phase (L_2) and three types of liquid crystalline phases (lamellar, reversed hexagonal and cubic phase) depend upon the temperature and water content. The lamellar phase (L_α) has a long-range order in one dimension. Its structure consists of a linear arrangement of alternating lipid bilayers and water channels. The reversed hexagonal phase (H_{II}) consists of infinite water rods arranged in a two-dimensional lattice and separated by lipid bilayers. The cubic phase (C) is usually observed between the lamellar and the reversed hexagonal phases as the water content is increased. The cubic phase consists primarily of two phases with similar structures, the cubic phase of the type G, also known as gyroid and the cubic phase D or diamond, although the structural similarity of these phases is such that in practice the two are usually considered to be equivalent. As seen from phase diagram of glyceryl monooleate-water system (Figure 14), with increased hydrocarbon chain disorder, obtained either by heating or increasing the water content, there is a transition from the L_α phase to the cubic phase and finally into the H_{II} phase (Shah, Sadhale and Chilukuri, 2001).
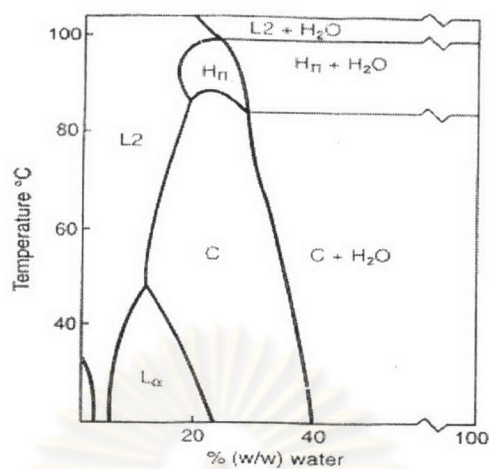
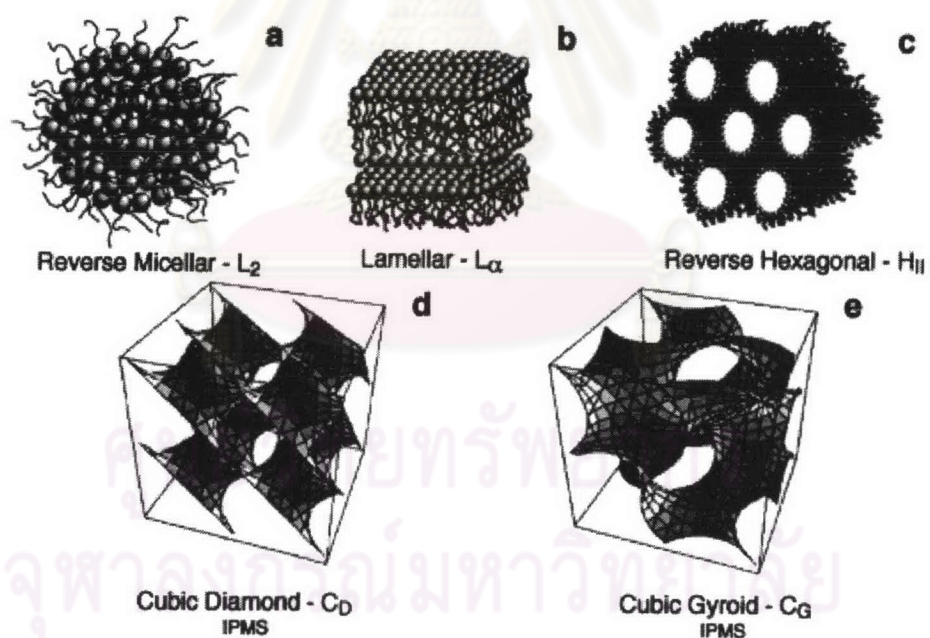Figure 14 Binary phase diagram of glyceryl monooleate-water system

Figure 15 Schematic illustrations of a reversed micellar phase and liquid crystalline phases of glyceryl monooleate

The liquid crystalline phases formed by glyceryl monooleate in excess water have the potential of acting as an *in situ* forming biodegradable drug delivery system. The biodegradability arises from the fact that glyceryl monooleate is subject to lipolysis due to different kinds of esterase activity in different tissues. Moreover, glyceryl monooleate is a nontoxic and biocompatible material classified as GRAS (generally recognized as safe) and it is included in the FDA Inactive Ingredients Guide and in nonparenteral medicines licensed in the United Kingdom (Ganem-Quintanar, Quintanar-Guerrero and Buri, 2000).

The glyceryl monooleate-water system have been described its use as a sustained release carrier for both conventional and peptide or protein drugs (Leslie et al., 1996; Chang and Bodmeier, 1997, 1998). For oral delivery, the model drugs were dispersed in monoglycerides and filled into capsules and then transformed *in situ* into the cubic phase upon contact with gastrointestinal body fluids (Wyatt and Dorschel, 1992; Sallam et al., 2002). For buccal delivery, Lee and Kellaway (2000) suggested that the cubic and lamellar liquid crystalline phases can be considered as promising drug carriers for the buccal delivery of peptide drugs as well as acting as permeation enhancers. For vaginal delivery, glyceryl monooleate-water system was studied for vaginal delivery of antimuscarinic drugs to treat urinary incontinence. Geraghty et al. (1996) found that the release of the drugs *in vitro* was sustained over a period of 18 hr and followed square root of time kinetics, indicating that the rate of release was diffusion controlled. For parenteral delivery, the high viscosity and stiffness of the cubic phase gel limits its potential use as the delivery system by itself. However, the ability of the less viscous lamellar phase to form cubic phase gel upon absorbing more water has resulted in novel drug delivery. The studies have shown that cubic phase can be used intramuscularly and subcutaneously as a delivery system for peptide drugs such as somatostatin, desmopressin and insulin (Sadhale and Shah, 1999; Shah et al., 2001). Another interesting application of an *in situ* forming was for periodontal delivery of antibiotics for the treatment of infections. Norling et al. (1992) suggested that addition of triglyceride (sesame oil) into glyceryl monooleate could be lowering the melting point which was improved the flow properties of glyceryl monooleate and

could be administered with a syringe into a periodontal pocket. Moreover, the liquid crystal structure of the gel would be the reversed hexagonal phase instead of the cubic phase. The *in vitro* release data showed that the reversed hexagonal form gave slower released of metronidazole benzoate when compared with the cubic form. Since the diffusion pathway is more obstructed in the reversed hexagonal form than in the cubic one, which has connected water channels. The closed water channels of the reversed hexagonal phase slow down the diffusion of dissolved drug through the matrix. Komwatchara (1996) developed the mixtures of glyceryl monooleate and various vegetable oils containing the *Andrographis paniculata* extract for treatment of adult periodontitis. Upon contact with the gingival crevicular fluid, the reversed hexagonal matrix were formed and showed desirable release of the extract. The results suggested that soybean oil was the best formulation since it showed the most drug release and antimicrobial activity. In addition, soybean oil was not expensive, easily available and stable. In another study, viscous solutions prepared with either poloxamer or glyceryl monooleate were delivered by a syringe and needle into a periodontal pocket. Both formulations undergo a transformation to gel upon administration resulting in local drug delivery. While poloxamer undergoes thermoreversible gelling, glyceryl monooleate formed the viscous cubic phase *in situ* upon absorption of water. The results indicated that glyceryl monooleate showed slower release of the drug when compared with poloxamer (Esposito et al., 1996). The successful results of the above studies demonstrate an interesting application of liquid crystalline phases for periodontal drug delivery.

E. Characterization of Liquid Crystalline Phases

There are several ways to investigate liquid crystalline systems in order to identify the phases and to map the phase diagram, and for investigating the microstructure of the phases formed. Below, only a couple of these methods are discussed in brief.

1. Small Angle X-Ray Diffraction

Small angle X-ray diffraction is a useful method in studying liquid crystalline phases. The crystal structure is built up of unit cells which are the smallest building units of a crystal, describing the three-dimensional relationship between the molecules in the crystal lattice. Both the symmetry and the dimensions of the unit cell can be studied by X-ray diffraction. Since liquid crystals possess long-range structural order, interaction with electromagnetic radiation of a suitable wavelength may result in the generation of diffraction patterns. The sample is fixed and sealed between two mica windows and placed in the beam of monochromatic X-rays. The scattered radiation satisfying Bragg's law:

$$n\lambda = 2d \sin \theta$$

where n is the order of the diffraction pattern, λ is the radiation wavelength, d is the distance between planes in the crystal and θ is the scattering angle, giving a diffraction pattern. This technique has been extensively used for investigating the structure of liquid crystalline phases. When the composition of sample is known together with the nature of the liquid crystalline phase, X-ray diffraction data can be used to extract information about the characteristic dimensions in the liquid crystalline structure (Scherlund, 2000; Malmsten, 2002).

Table 5 Relation between the first reflections for different liquid crystalline phases

Liquid crystalline phases	Orders
Cubic	$1:1/\sqrt{2}:1/\sqrt{3}:1/\sqrt{4}:1/\sqrt{5}:1/\sqrt{6}:1/\sqrt{8}:\ldots$
Lamellar	$1:1/2:1/3:1/4:\ldots$
Hexagonal	$1:1/\sqrt{3}:1/\sqrt{4}:1/\sqrt{7}:\ldots$

2. Nuclear Magnetic Resonance (NMR)

NMR offers a range of possibilities for investigating liquid crystalline phases. Deuterium (^{2}H) NMR spectroscopy offers a powerful technique for investigating phase equilibria, since the occurrence of different phases can be straightforwardly identified with this method. The basis for this method is that isotropic phases yield a narrow singlet resonance signal of quadrupolar nuclei such as ^{2}H, where as anisotropic phases result in so-called quadrupolar splitting, thereby yielding a doublet resonance signal. Furthermore, anisotropic phases may be separated through the magnitude of the splitting. Moreover, mixtures of several phases yield ^{2}H-NMR signals which are made up through superposition of signals of the individual phases. From this, not only the nature of the phase in the case of a single-phase system, but also the composition of multiphase systems may be determined (Malmsten, 2002).
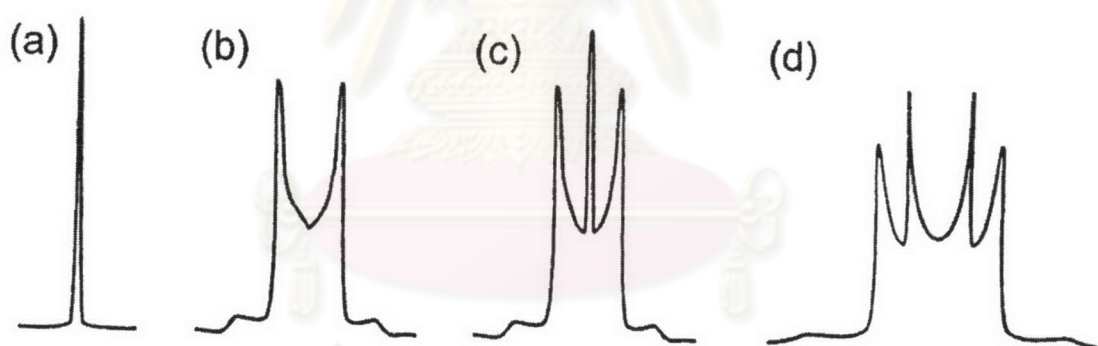

Figure 16 Typical ^{2}H-NMR spectra of liquid crystalline phases; (a) isotropic phases (e.g., micellar and cubic phases); (b) an anisotropic liquid crystalline phase, where the magnitude of the splitting increases with the degree of anisotropy, and therefore being larger for a lamellar phase than for a hexagonal one; (c) a two-phase sample consisting of the phases present in samples (a) and (b); and (d) a two-phase system containing two anisotropic phases (e.g., a lamellar phase and a hexagonal phase) (Malmsten, 2002)

3. Polarizing Microscopy

As a result of their molecular ordering, anisotropic liquid crystalline phases, such as the hexagonal, the lamellar, and the reversed hexagonal phases, are optically birefringent (Figure 17). This property can be used for studying such phases with polarizing microscopy. The lamellar phase usually yields mosaic patterns under the polarizing microscope, whereas the hexagonal phases normally show nongeometric textures. The occurrence of crystals may also be identified by this method. On the other hand, isotropic phases (e.g., micellar and reversed micellar solutions and cubic phases) are nonbirefringent and generate a dark background when investigated under the polarizing microscope (Malmsten, 2002).

4. Differential Scanning Calorimetry (DSC)

DSC is a thermal analysis method for detecting changes in physical or chemical properties of materials as a function of temperature by measuring the heat changes associated with such processes. The sample and reference are placed in a temperature controlled chamber and the heat flow required to maintain the sample and the reference at the same temperature is measured. This results in either absorption of heat (endothermic reaction) or release of heat (exothermic reaction). DSC has been described for studying a phase transition which requires the input of additional thermal energy show up as endothermic peaks on heating. However, DSC cannot identify the type of a liquid crystalline phase, only that a phase transition has occurred (Scherlund, 2000).
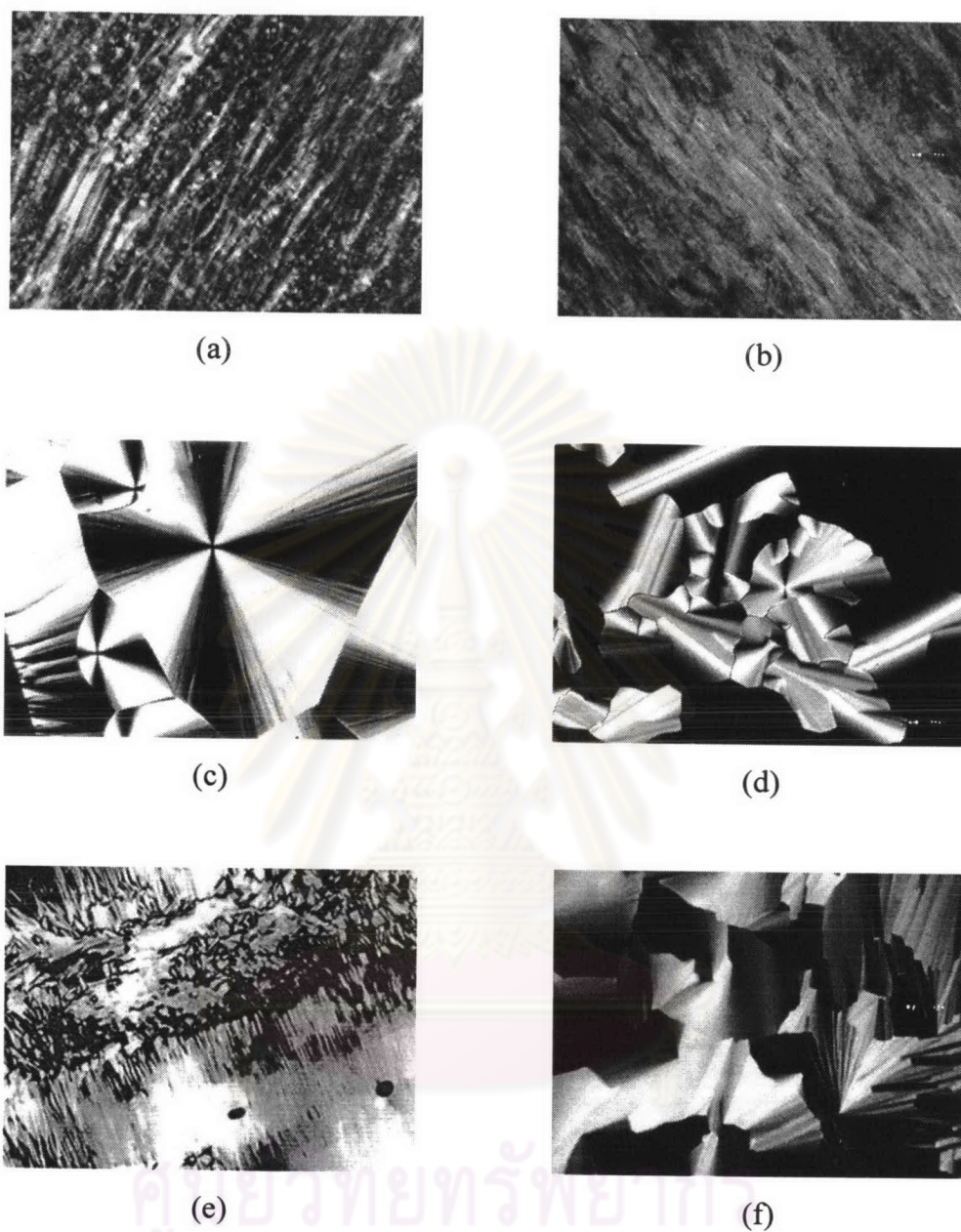
(a)

(b)

(c)

(d)

(e)

(f)

Figure 17 Polarizing microscopic textures of anisotropic liquid crystalline phases; (a) and (b) lamellar phases; (c) and (d) hexagonal phases; and (e) and (f) reversed hexagonal phase (Geraghty et al., 1996, Schwarzwälder and Meier, 1997; Makai et al., 2003; Goldmann et al., 2004; Barauskas et al., 2005)