CHAPTER II

LITERATURE REVIEW

Introduction

Periodontal diseases are conditions that affect the supporting structures of the teeth. Some of these diseases are caused by accumulation of plaque that extends into the subgingival areas of the periodontium. This produces an inflammatory response in adjacent tissues and these diseases may be broadly classified according to the extent of periodontal tissue involvement. The inflammatory response is confined to the gingiva in gingivitis but extends to deeper tissues in periodontitis. Progression of periodontitis results in loss of tooth support structures so the teeth become mobile and cannot function properly. In some cases if treatment is not instituted support structures degenerate to a point was repair is not possible and tooth extraction is required. The role of bacteria in the etiology of these diseases has been well established. The bacteria accumulate in the space (or pocket) that develops between the roots of affected teeth and the soft tissues. Conventional methods for the removal of sub-gingival bacteria include periodic mechanical debridement of plaque from tooth surfaces and repeated topical or systemic administration of antibacterial agents.

The periodontitis result in working of teeth. Adult periodontitis which is one type of periodontitis was presented in the majority of adults, and periodontitis was considered to be the major cause of tooth loss in adults. The bacterial flora presented in periodontal pockets play an important role in the etiology of periodontal disease. Systemic administration of antimicrobial agents has proved to be useful in the management of subgingival flora. But this type of treatment contains the risk of systemic side effects and emergence of resistant strains and superimposed infection.

For local treatment in the sub-gingival infection, various substances have been tried for example chlorhexidine, tetracycline and metronidazole alone and in combination with scaling. The carrier system, mostly are non-biodegradable and the drug-depleted polymer carriers must be removed mechanically.

Periodontal disease

Various pathological conditions are characterized by the attack of microbes on body tissues, resulting in local inflammation and the appearance of lesions characterized by tissue destruction at the infection site. The result of the accumulated tissue destruction may be a characteristic cavity or gab in the tissue, which may become enlarged as the infection and inflammation progresses. Periodontal disease, especially adult periodontitis is characterized by this study.

The human periodontal diseases were broadly classified into two major disorders, gingivitis and periodontitis. Patients were considered to have gingivitis when gingival inflammation was present without loss of alveolar bone, and to have periodontitis when alveolar bone had been lost. Gingival inflammation was through to

be present in virtually all children and adults periodontitis was believed to be present in the majority of adults periodontal disease comprise the various pathological conditions which are clinically and histologically detectable as inflammation of the periodontal tissues. The latter are collectively comprised of the gingiva, cementum, periodontal ligament and alveolar bone. Periodontal diseases are caused by bacterial colonization of the surfaces of the teeth, and may be complicated by other hosts and causative factors. The disease is common in humans as well as in animals. Untreated periodontal disease caused loss of teeth which compromises the integrity and function of the masticatory system. Periodontal disease was related to groups of microorganisms in the gingival plaque, the local and systemic host response, and systemic modifiers such as diabetes mellitus and smoking history.

The effectiveness of these conventional treatments is limited by the lack of accessibility to bacteria in the periodontal pocket. Pitcher et al (1987) showed that a plaque disclosing agent administered as a mouth rinse did not penetrate into periodontal pockets, indicating that this method of delivery is not suitable for the treatment of subgingival infections. In contrast, systemic administration has been shown to achieve therapeutic concentrations at the site of infection (Britt and pohlod, 1986). These concentrations are, however, usually maintained for short periods of time after a single dose and the doses employed are capable of producing systemic side effects. Generally, systemic administration is recommended for treatment of rapidly progressing or refractory periodontitis. Another approach has been to administer antibacterial solutions directly into the periodontal pockets using specialised irrigating devices. This method of delivery has been reviewed by Greenstein (1987). The duration of action is generally short when agents are administered in solution, and frequent application is required to maintain effective concentrations in the periodontal pocket. This makes patient compliance critical to ensure optimal clinical efficacy. Because of the shortcomings associated with the above methods of delivery, attention has focused on the development of prolonged release intrapocket delivery systems.

Ideally, these systems should deliver the antibacterial agent for prolonged periods to the affected pocket at levels in excess of the minimum inhibitory concentration for the causative organisms. Goodson et al (1989) were the first to devise an intrapocket delivery system. They suggested that "a controlled release suppository form of a drug placed within the periodontal pocket could be a highly effective method for administering antibacterial agents for periodontal therapy". Since then a number of other systems have been developed which have attempted to achieve a sustained antibacterial concentration in the periodontal pocket.

This review summarises the major biopharmaceutical considerations of drug delivery in the treatment of periodontal disease, discusses the development of intrapocket delivery systems and identifies areas where further research may lead to improvement in the clinical usefulness of this method of treatment (Steinberg et al, 1990).

Pathogenesis of and biologic basis for the periodontal diseases

Periodontal disease is defined as the pathologic destruction and loss of the tissues supporting the teeth. The association of periodontal disease with bacteria and

plaque accumulation has been firmly established. It is well documented that the progression of periodontal disease through various stages of severity begins with an accumulation of bacteria. Colonization of the gingival margin and gingival crevice begins with accumulation of bacteria that thrive in the presence of oxygen (aerobic bacteria) as well as bacteria that can tolerate low levels of oxygen (facultative anaerobes).

Gingivitis is associated with an increase in the quantity and complexity of plaque. In adult periodontitis, the total amount of plaque is further is increased. Three to four hundred species of bacteria have been identified in the oral cavity, the vast majority of these in the subgingival plaque, which characteristically contains anaerobic bacteria (those that thrive in an environment free of oxygen) and gram-negative microorganisms. The microbial flora associated with periodontal diseases is comprised of gram negative bacteria and implicated as pathogens in adult periodontitis include *Porphyromonas gingivalis, Actinobacillus Actinomycetemcomitans, Prevotella intermidia, Eikenella corrodens, Bacterodes forsythus, Fusobacterium nucleatum, Campylobacter rectus,* and *Capnocytophaga species* (Seymour and Heasman, 1995).

The important pathogen is the gram-negative rod-shaped organism, *Actinomycetemcomitans,* which was first identified in juvenile periodontitis and is through to be the major pathogen in that disorder. *Actinomycetemcomitans* has also been found in a variety of aggressive forms of periodontitis, such as rapidly progressive periodontitis and prepubertal periodontiitis; *P. gingivalis* is considered to be a pathogenic factor in adult or rapidly progressive periodontitis. However, although a gram-negative infection is a prerequisite to periodontal destruction, the host response plays an important role in determining the outcome of the process. Early inflammatory lesions of the gingival tissue are associated with a predominantly granulocyte infiltration, while chronic disease and sites with active disease exhibit a predominantly mononuclear cell infiltrate. Lymphocytes constitute the major cell type while monocytes and macrophages constitute a minor component (Soskolone and Freidman, 1996).

Once microorganisms have colonized the gingival crevice, lymphocytes derived from periodontal pathogens have the ability to penetrate the root cementum and gingival tissues. Lymphocytes derived from periodontal pathogens can stimulate the minor cell population of monocytes and macrophage to secrete cytokines. These cytokines have the ability to stimulate other cell types associated with the inflammatory infiltrate, such as lymphocytes and fibroblasts, to secrete additional inflammatory mediators, amplifying the inflammatory response. This cascade of events suggests an important role for LPS-induced inflammatory mediators in the tissue destruction, characteristic of periodontitis. High levels of pro-inflammatory mediators such as interleukin-β1and prostagladin-E_2 with the potential to induce bone resorption have been documented at periodontal disease sites. Furthermore, interleukin-β1and prostagladin-E_2 have been correlated to active phases of periodontal breakdown.

Periodontal diseases commonly affects adults over the age of 35 and the incidence increases with age. This type of presentation is termed Adult-type

Periodontitis. Young people below 35 years are rarely affected by periodontal disease but when they are it is referred to as Early Onset Periodontitis (EOP).

Increase severity of disease, the proportions of anaerobic, gram-negative, and non-motile organisms increase significantly. Overview of the subgingival microflora in periodontal health and disease. The response top this formation of microbial plaque is an inflammation of the gingiva and the resulting breakdown of tissues, which causes the formation of an opening along the tooth surface known as the "periodontal pocket". As the bacterial colonization of the pocket and the inflammatory process continue, the destruction of the periodontal tissue progresses and the tooth becomes loose. Once deprived of supporting tissues, which will not regenerate, the tooth will eventually be lost. The infection is local. The same patient may have entirely healthy teeth immediately proximal to the diseased tooth.

Periodontal bacteria can induce tissue damage by two general mechanisms: first, biologically active substances (virulence) released from the microorganisms can directly affect the periodontal tissues; second, the bacteria can be recognized by the host as foreign body, leading to tissue damage as a consequence of activation of the host's inflammatory and immune responses. Virulence factors include enzymes (such as bacteria collagenase, which degrades collagen), bacteria waste product (such as hydrogen sulfide), toxins (such as leukotoxin released by *Actinomycetemcomitans*, which causes lysis of polymorphonuclear leukocytes) and lipopolysacharide (endotoxin).

The body's response to the challenge of periodontal bacteria thus appears to be a two-edged sword. While it is generally recognized that an appropriate host response to the periodontal infection helps to control the pathogenic microorganisms, a growing body of evidence also suggests that alteration in the host response may account for tissue loss in periodontitis.

Adult periodontitis

The human periodontal diseases were broadly classified into two major disorders, gingivitis and periodontitis. The adult periodontitis is one type of periodontitis. Gingivitis inflammation was thought to be present in virtually all children and adults, periodontitis was believed to be present in the majority of adults, and periodontal disease was considered to be the major cause of tooth loss in adult. Adult periodontitis is the most common form of periodontitis; it is a localized or generalized chronic disease of the periodontium, with over 80% of the cases occurring in those over age 35. The plaque induced inflammatory process proceeds slowly, continuing to involve deeper tissues. Apical migration of the junctional epithelium cause the formation of a periodontal pocket that contains the subgingival microbiota. As the disease progresses, the periodontal ligament and alveolar bone are slowly destroyed, culminating in loss of tooth support and exfoliation. The entire process may occur with periods of inactivity over a time span of 20 or more years.

Adult periodontitis lesions contain high proportions of anaerobes (90%) gram-negative organism (75%). May cases of adult periodontitis show greatly elevated proportions of a limited number of microorganisms. However, the composition of the periodontopathic microflora can differ markedly from patient to patient and from pocket to pocket in a given patient (Seymour and Heasman, 1995).

P. gingivalis is closely related to advanced adult periodontitis and seems to be one of the numerically most important pathogens in the disease. *P. gingivalis* possess one of the highest virulence potential of any oral organism tested so far. *P. intermedia* is associated with periodontitis as well as gingivitis, complicating the exact assignment of periodontopathic significance. *A. actinomycetemcomitans* occurs in about one third of severe adult periodontitis cases and is mostly associated with juvinile periodontitis.

P. gingivalis was formally classified with *Bacteroides gingivalis* (Bacteroides group); recently, DNA analysis of *P. gingivalis* has shown it to be different from bacteroides. *P. gingivalis* is a short rod-shape 0.5x 0.8x 1.0-3.0 microgram gram negative anaerobe, nonsporing and non-motile. Generally cells from brown to black colonies on blood agar due to protoheme production using porphyrin. *P. gingivalis* is a pathogenic factor in adult or rapidly progressive periodontitis. High numbers occur at progressing and few at non progressing periodontal sites. It is the most virulent destructive bacteria in periodontal disease (Seymour and Heasman, 1995).

Virulence factors are divided into 3 groups: adherance survival and toxin factors. Slots and Rams (1990) have shown that: First, *P. gingivalis* is the most adhesive of the black-pigmented anaerobic bacteria. A cell surface protein is the dominant antigen. *P. gingivalis* can adhere to type IV collagen fiber, a structural component of the basement membrane, and to type I collagen fibers that penetrate to deeper tissues. In addition, *P. gingivalis* can adhere to red blood cell, resulting in hemagglutination. Second, *P. gingivalis* is protected by a capsule that resists attack by white blood cells. Antibodies againsts *P. gingivalis* are high in serum. Third, *P. gingivalis* releases toxins.

The periodontium consists of four distinct structures that support the teeth in the oral cavity, namely the gingiva, alveolar bone, cementum and periodontal ligament. In healthy sites a shallow gingival sulcus exists between the gingiva and the tooth. This is generally less than 3 mm deep but deepens with disease progression. The gingival sulcus is not completely free from bacteria and a scanty microflora consisting of mainly gram-positive aerobic species can be isolated from healthy sites. These bacteria are compatible with tissue health and are thought to exist in equilibrium with the host defence mechanisms so that any damage they cause can be easily repaired by the host. An increase in the number of subgingival bacteria then a shift in the composition of the microflora is observed with the development of periodontal diseases. A number of studies have attempted to determine the bacteria responsible for individual periodontal diseases but lack of agreement as to specific pathogens has been apparent. The most commonly isolated bacteria from diseased sites are gram-negative facultative or obligate anaerobes. *Bacteroides spp., Actinobacillus actinomycetemcomitans, Eikenella corrodens, Fusobacterium nucleatum, Wolinella recta, Capnocytophaga spp.* and spirochaetes have usually been associated with the various forms of periodontitis (Slots, 1990). Damage to the periodontium results from the direct toxic effects of subgingival bacteria and the destructive effects of the host inflammatory response.

A loss of attachment of the periodontal ligament from the tooth root surface and apical migration of the junctional epithelium occurs so that a periodontal pocket is formed. This progression has been shown to occur in a cyclic fashion in which

destructive phases are interspersed with periods of disease remission. The periodontal pocket, however, remains and if it continues to harbour the bacteria associated with the disease a potential for further destructive phases exists. Therefore, clearance of the subgingival infection and elimination of the periodontal pocket are considered a priority in the treatment of periodontitis.

The periodontal pocket

The periodontal pocket is lined with an epithelium on one side and tooth cementum on the other. No actual space exists as these two tissues rest against each other. Lack of attachment is demonstrated by insertion of a periodontal probe and changes in the attachment level or pocket depths have been monitored to assess disease progression. Pocket depths ranging from 4 to 12 mm are generally observed at diseased sites.

The characteristics of the gingival crevicular fluid which fills the periodontal pocket have been reviewed by Medlicott et al (1992). Healthy sites are associated with small volumes (0.04 µl) and low flow rates (0.03 µl/min) and examination of the protein concentrations show it to be similar to extracellular fluid and it is thought to represent a normal extracellular transudate. In contrast, at diseased sites there is increased fluid production and the protein composition is similar to that of serum, indicating that an exudate is formed at these sites. The volume and fluid flow rate, however, depends of the degree of inflammation at indivedual sites. Volumes of about 0.5 µl and flow rates of 0.5 µl/min and 20 µl/h (0.33 µl/min) have been reported by Hattingh et al (1980) and Goodson (1989) respectively. Goodson calculated the turnover rate of gingival fluid to be 40 times per hour and suggested that this accounts for the rapid clearance and short duration of action observed with irrigation treatment (Goodson, 1989).

Special aspects of pocket anatomy

The periodontal pocket is a result of the progression of periodontal disease. It is a space, virtual or potential, which results from the pathological detachment of the periodontal tissues from the dental tissues. It is usually confined by the pocket epithelial lining covering the soft tissue walls of the pockets, and by the cementum and dentin of the root surface. The anatomy of the pocket varies extensively and can be tortuous. At one end of the spectrum, when inflammation in the pocket wall is minimal or absent a complete epithelium lining covering the soft tissues exists. In most successful post-treatment circumstances the epithelium attaches to the root surface (long epithelial attachment) and the pocket excess as a potential space cavity. At the other end of the spectrum the pocket is a cavity accessible space, occupied by bacterial plaque, calculus and other debris with an incomplete epithelial lining. The degree of ulceration of the epithelial lining is dependent on the degree of inflammation in the pocket wall. The pocket is constantly washed by the gingival crevicular fluid.

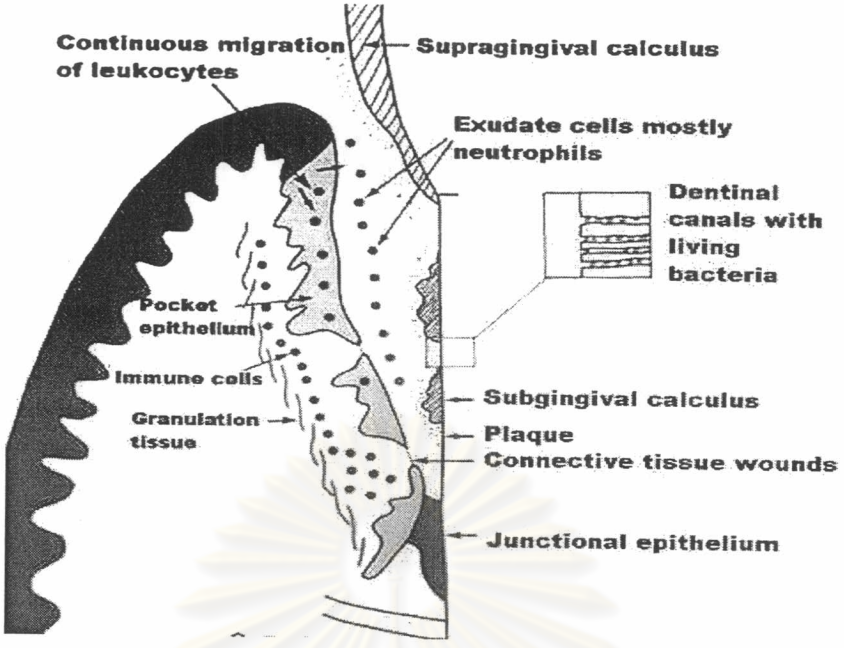


Figure 1 The physiology of periodontal pocket and tissue pathogen (from Soskolone and Freidman, 1996).

The direction of flow of the crevicular fluid is from the gingival tissues, through the gingival pocket, into the oral cavity. This flow could result in 40 folds turnover of the pocket volume per hour. The flow rate and volume of the crevicular fluid is dependent on the degree of inflammation is the pocket wall and the volume of the pocket.

Assessment of the effectiveness of periodontal disease treatment

A number of protocols have been employed to evaluate the effectiveness of periodontal therapy making comparison among studies difficult. Generally, the effects of treatments are assessed by measurement of the clinical improvement and changes in the subgingival microflora. Clinical improvement is measured with a series of indices and physical measurements that assess the degree of plaque accumulation and tissue inflammation. These include the plaque index, gingival index, degree of bleeding on probing, pocket depth, attachment level and gingival crevicular flow rate measurements. Assessment from a microbiological perspective involves determination of changes in the subgingival microflora. Bacteria are collected and identified or classified according to their morphology, gram stain uptake, motility and oxygen requirements The proportions are calculated for each species or group and shifts from pathogenic to non-pathogenic populations are recorded with treatment. Pathogenic populations comprise motile gram-negative anaerobic rods, filaments, cocci and spirachaetes whereas non-pathogenic populations consist of predominantly non-motile gram-positive aerobic cocci.

Analysis of the drug concentration in the gingival crevicular fluid is the best method to determine the effectiveness of a delivery system in maintaining drug levels. A number of drugs are effective against the bacteria associated with periodontal disease and reports of their minimum inhibitory concentrations can be found in the

literature. If concentrations in the gingival crevicular fluid are monitored, it is possible to ascertain whether effective delivery of the agent is achieved. The major problem of this approach has been the development of sensitive assays for antibacterial agents in the small volume (0.5 μl) of gingival crevicular fluid.

Bioassays have been developed for tetracycline, minocycline and clindamycin in which gingival crevicular fluid samples are collected with filter paper strips and placed onto agar plates seeded with a test strain of bacteria. These plates are incubated and the resultant zone of inhibition is compared to that produced by standard filter papers containing a known amount of durg. A similar bioassay has been used by Esposito et al (1996) for the determination of metronidazole: 1-μl capillary tubes filled with seeded agar were used instead of plates and the gingival crevicular fluid samples were collected with filter paper points which were cut and placed in the bottom of the capillary tubes. After incubation the length of the zone of inhibition within the capillary tube is measured and compared with standards (Britt and Pohlod, 1986).

Metronidazole has also been determined in gingival crevicular fluid by HPLC. Britt and Pohlod did not report the limit of detection of their assay but reported metronidazole concentrations, in gingival crevicular fluid, of about 0.5 to 6.5 μg/ml over an 18-h period after oral administration of a 200 mg dose.

Treatment of periodontal disease

All modes of periodontal prophylaxis and therapy seek to reduce or eliminate bacteria plaque and plaque-derived products and to aid in repair or regeneration of tissue. Conventional periodontal therapy encompasses oral hygiene and plaque control: every patient should be taught the correct method of tooth brushing and how to use dental floss and other devices. Using various instruments such as curettes, sickles, files, etc., the bacteria are removed from the infected areas by scraping the teeth and pockets, one by one. Such mechanical procedures are very time consuming. Each tooth takes about 5-15 minutes to treat. The discomfort and pain for the patient are considerable. Mechanical removal of bacteria is a method of therapy which requires highly skilled dental operators. The procedure may be difficult to perform particularly if the pockets are deep, and are presented with root fissures or furcations. In order to improve mechanical removal, the accessibility and visibility of the root surface may be surgical procedures such as flap operation.

Since most periodontal disease is of bacteria origin, antibacterial agents have been used for prevention and treatment. The substances using are divided into 2 groups: First, systemic; tablet, injection; Second, local; mouth rinse, dialysis tube, monolithic, film (membrane) and gel (Addy et al, 1994).

I. Systemic antobiotics

Systemic antibiotic such as tetracycline, penicillins, erythromycin, clindamycin and metronidazole tablets prescribed for periodontal infections are usually given orally. An effective concentration of antibiotic drug at the site of infection may be achieved by the systemic administration of a high dosage of the drug. In order to maintain an effective concentration over an extended period of time,

it is necessary to repeat the dosage 2-3 times daily for several weeks. Long term exposure to high dosage of antibiotics is associated with a high risk of side effects, a fact that has seriously limited the use of this treatment in periodontal therapy.

The infection is local, a better way to obtain an effective drug concentration at the site of infection is to apply the drug directly to the peridontal pocket. According to this methodology, only the amount of drug locally required is administration. The total dosage of the drug is thus reduced considerably (Seymour and Heasman, 1995).

II. Local antibiotic

1. Mouth rinses

The advantages and the disadvantages of topical antimicrobial in mouth rinses were shown. However, in order to maintain an effective concentration of drug for an extended period of time (days to week), the drug-containing system must be retained within the pocket, and the drug must be released slowly.

2. Local delivery of antibiotics

A major disadvantages of systemic antibiotics is that the concentration at the site of infection is dependent on the level achieved in the serum. Because the administered dose is distributed throughout the blood stream, the concentration at the site of infection is relatively low. This distribution exposes non-infected areas of the body to the antibiotics, sometimes upsetting the body's normal microflora. Systemic antibiotic treatment limited by adverse reaction such as toxicity, acquired bacteria resistance and drug interaction. The oral rinses and irrigation have been used as adjuncts to conventional therapy. Since oral rinses and irrigation at the gingival margin do not reach subgingival area on a predictable basis, local delivery to the subgingival compartment requires alternative approaches.

III. Intrapocket delivery systems for periodontal disease

Rational for the use of intra-pocket delivery systems

Local application is one way of targeting a drug to a desired site. However, to prolong the therapeutic levels of the drug at the site, a local sustained release device is required. The periodontal pocket is a defined site surrounded by hard and soft tissues forming a natural reservoir with easy access for inserting a delivery device. The presence of the gingival crevicular fluid bathing the device provides a leaching media for the release of a drug from a solid dosage form. These features, together with the fact that the periodontal diseases are localized to the immediate environment of the pocket make the periodontal pocket a natural site for treatment with local sustained release delivery systems (Soskolone and Freidman, 1996).

The goals to be achieved in using an intra-pocket delivery device are the release of the therapeutic agent into the pocket and the achievement and maintenance of therapeutic levels of the drug for the required period of time. Although the delivery system and drug release are an integral unit, and most discussions on the subject relate to them as such, it is important to bear in mind that they are in fact two distinct

entities. A single drug may be delivered by different delivery systems or alternatively a specific delivery system can be used to deliver different drugs. However there is a strong interdependence between the two which determines (at least in part) the release kinetics of the drug and thus its efficacy in treating the disease.

Types of intra-pocket devices

Intra-pocket devices can be divided into two broad categories depending on whether they are degradable or not. Non-degradable devices have the advantage that the therapist controls the removal of the device and therefore has greater control over the time of exposure of the pocket environment to the drug. However, a non-degradable device left in situ beyond its period of therapeutic efficacy is a potential hazard in that it could result in a foreign body response. The degradable devices have the great advantage of requiring the patient to pay only a single visit to the therapist for inserting the device. This minimizes patient visits and assures compliance in that the patient does not have to return to have the device removed.

1. Fibers

The use of fibers, or thread like devices for the sustained release of drugs into the periodontal pocket was first introduced by Goodson et al (1989) using cellulose acetate dialysis tubing to deliver tetracycline hydrochloride into the periodontal pocket. Although the system showed some clinical effect it was concluded that it was unable to sustain therapeutic levels of the drug for sufficient time to be clinically useful. Using the same delivery system studies were done on the release of chlorhexidine gluconate and metronidazole with similar conclusions. These initial studies were followed by a series of further studies by the same investigators which have led to the development of a commercially available delivery system (Actisite, Alza Corporation, Palo Alto, California) based on a monolithic ethylene vinyl acetate fiber that delivers tetracycline hydrochloride. Two multicenter studies show that the treatment of periodontal pockets with this system resulted in significant reductions in pocket probing depths and bleeding on probing and significant increases in attachment levels compared to the other treatment modalities tested.

Clinically the placement of fibers into a periodontal pocket is well within the ability of the dental practitioner being simiilar to the standard dental technique of placing retraction cord into the gingival crevice prior to taking an impression of a crown preparation. The fiber can be placed around the circumference of the tooth to the depth of the pocket and folded back on itself repeatedly to completely fill the pockets. Its disadvantages are the relatively long time it takes to place the fiber (7-10 minutes/ tooth). A fairly high risk of 23% has been reported for extrusion of these fibers from the pockets during the 10 days of treatment. There is also the possibility of lack of compliance with the patient not returning to remove the device (Goodson et al, 1989).

2. Films

A far more widely used form of intra-pocket delivery device has been in the shape of a film. This dosage form has several advantageous physical properties for intra-pocket use. The dimensions and shape of the film can be easily controlled to

correspond to the dimensions of the pocket to be treated. It can be rapidly inserted into the pocket with minimal discomfort to the patient. It can be inserted to the base of the pocket and be totally submerged. If the thickness of the film does not exceed approximately 400 μm, and its physical properties provide it with sufficient adhesiveness, it will remain submerged without any noticeable interference to the patient's eating and oral hygiene habits. Both degradable and non-degradable forms of films have been developed and therefore the discussion will relate to these two forms under separate headings (Soskolone and Freidman, 1996).

3. Non-degradable films

The first descriptions of film intra-pocket delivery devices appeared in 1982. Addy and coworkers (1982) described the use of films of methylmethacrylate for the intra-pocket delivery of tetracycline, metronidazole and chlorhexidine. A self polymerizing mixture of the polymer, monomer, and the appropriate drug were cured, as sheets, under high pressure and then cut into suitable sized films. Studies showed that the release of drugs from acrylic films measuring 10 x1 x 0.5 mm was dependent on the nature of the drug and its concentration in the delivery device. They described formulations which delivered, in-vitro, therapeutic levels of all three drugs over a 14 day period. In later studies they showed various degrees of clinical efficacy but this system has not been developed for clinical use.

In the same year an ethylcellulose film for intra-pocket drug delivery was described. These films were made by casting ethanol or chloroform solutions of the polymer into molds and allowing the solvent to evaporate. The appropriate drug and plasticizing agent were incorporated into the solution prior to casting. The dried films (200-300 μm thick) were then cut into the required shapes. Films containing chlorhexidine, metronidazole, minocycline and tetracycline have been developed and tested to varying degrees. The release of the therapeutic agent from these films is dependent on the solvent used, the presence of a plasticizer, the nature and concentration of the drug in the film and on the physical dimensions of the film. The most extensive, published, clinical studies have been carried out using films that release 80% of their chlorhexidine content over a three day period (Steinberg et al, 1990).

4. Degradable devices

Many degradable devices in the form of a film have been tested experimentally. Resorbable hydroxypropylcellulose based devices for the delivery of tetracycline and chlorhexidine and ofloxacin have been tested clinically. The first report on a degradable intra-pocket sustained release delivery system was the study of Higashi et al (1990) using hydroxypropylcellulose films. In this study a rapid release of the drugs from the film within 2 hours was demonstrated *in vitro* with maximum dissolution of the film occurring after 3 hours. *In vivo*, retention of tetracycline in the pockets could be detected 24 hours after insertion of the device and significant clinical and microbiological advantages over the control group were described. Although this was a pioneering study in the development of a degradable system, the rapid degradation of the device and the short duration of drug exposure were distinct disadvantages. Using a modification of this system by incorporating slowly soluble methacrylic acid copolymer particles into the hydroxypropylcellulose films, the

release of the drug ofloxacin from the device was prolonged such that 70% was released in the first 8 hours in vitro. In vivo levels above 2 μg/mL of ofloxacin were maintained for 7 days after treatment with the device. Two applications of this device one week apart resulted in significant reductions in the number of spirochetes and motile organisms in the pockets as identified by dark field microscopy.

The biodegradable polyester poly (ε-caprolactone) has been tested in vitro as a matrix for sustained release delivery both as a fiber for the delivery of tetracycline and as a film for the delivery of chlorhexidine. Clinically the fibers released their tetracycline content very rapidly with a halflife of 11 hours. No clinical studies could be found in which the films containing chlorhexidine had been tested in periodontal pockets (Higashi et al, 1990).

5. Injectable devices

The possibility of injecting a delivery system into the pocket has a number of advantages. It is a relatively simple procedure with little or no discomfort associated with the insertion of the dose form. The initial fluid nature of the formulations, which is necessary for its use with a syringe, would theoretically allow the formulation to gain access to the entire pocket. In order to be retained in the pocket the formulation would need to undergo a change into a sticky semi-solid or solid phase so as to prevent it from being washed out of the pocket by the gingival crevicular fluid.

Two different systems have been described in the literature, both of which are commercially available. The first, a 2% minocycline containing ointment (Dentomycin, Cyanamid International, Lederle Divesion, Wayne, NJ. and Sunstar, Osaka, Japan)., does not appear to have any sustained release properties. In a study using this ointment as an adjunct to scaling and root planing, 4 applications of the ointment into the pocket were made at two weekly intervals starting immediately after completion of the scaling and root planing. This resulted in a significant, 0.3 mm greater improvement in pocket depth than did the placebo gel at 12 weeks post scaling and root planing and 6 weeks after the last application of the gel. The changes in the bleeding index and probing attachment levels were not significantly different. In the minocycline treated pockets there was a significant increase in the number of pockets with undetectable levels of *Porphyromonas gingivalis* and *Porphyromonas intermedius* compared to the controls, throughout the study. The minocycline effect on *Actinobacillus actinomycetemcommitans* only became significant after the 3rd application of the ointment and remained significant 6 weeks after the last application.

The second system (Elyzol, Dumex, Copenhagen, Denmark) is a benefit controlled release delivery system. The liquid phase of this formulation consists of a water free mixture of melted glycerol mono-oleate and metronidazole benzoate to which a triglyceride, sesame oil, has been added to lower the melting point in order to improve the flow properties of the gel in the syringe. When the mixture comes into contact with water it sets in a liquid crystalline state. The formulation contains 25% metronidazole as 40% w/w metronidazole benzoate. The solubility of the drug and its concentration in the formulation influences its release profile. The matrix is degraded by neutrophil and bacterial lipases present in the GCF. Concentrations of 103-1297 μ g/mL of metronidazole were recorded in inflamed pockets treated with this device with effective doses being maintained for 24-36 hours. Systemic levels of

metronidazole between 0.2-1.3 μg/mL were measured after the administration of 29-103 mg of the gel (Norling et al, 1992).

The recommended therapy is two separate applications into each pocket, one week apart. The results of clinical studies comparing this therapeutic approach alone, to scaling and root planing, indicate that the metronidazole gel results in a reduction in probing pocket depth and bleeding on probing which is not significantly different from the results obtained with scaling and root planing. The intra-pocket drug delivery system can be classified into two major group; reservoir delivery systems and matrix delivery systems.

Intrapocket delivery systems for periodontal disease

I. Reservoir delivery systems

The first prolonged release intrapcket delivery system was a reservoir device in which solid tetracycline hydrochloride was enclosed in cellulose acetate dialysis tubing (diameter = 250 μm). An appropriate length of tubing was administered by placement at the opening of the periodontal pocket and application of gentle pressure to insert it below the gingival margin. Bromberg et al (2001) showed that drug release occurred rapidly with 95% of the drug load depleted in vivo in the first 2 hours. They also reported use in a patient with generalised gingivitis resulted in elimination of spirochaetes from treated sites. Further evaluation by Goodson et al (1989) in patients with periodontitis showed that treatment of deeper pockets resulted in a reduction but not complete elimination in pathogenic subgingival microorganisms. Effects were comparable, but slightly lower in magnitude than those obtained by scaling and root planing.

These results were explained in a further study by Goodson et al (1989). when the duration of treatment was assessed by analysis of the gingival crevicular fluid. The tetracycline concentration after administration of tubing containing approximately 1 mg tetracycline per tooth rose rapidly to a saturated solution (230,000 μg/ml) minutes after placement of the device then declined exponentially over 24 h to a level of 15 μ g/ml. At this time all the drug was depleted, and it was concluded that this delivery system did not provide an effective drug concentration for sufficiently long periods to achieve complete elimination of bacteria. Andrews et al (1984) have shown that a tetracycline concentration of 32 μg/ml inhibits the growth of 345 subgingival bacterial isolates. The clinical improvements that resulted from use of the dialysis tubing delivery system may therefore be attributed to the high initial drug concentration. Drug was present at effective levels for 24 h, so placement of a single tubing does not provide sufficient treatment to prevent pocket recolonisation.

Drug solutions have also been incorporated into dialysis tubing. Clinical use of fibers containing 20% v/v chlorhexidine gluconate and 0.5% w/v metronidazole has led to reduction in the signs and symptoms of periodontal disease. The concentration of drug in the gingival crevicular fluid and the kinetics of drug release were not however investigated, but chlorhexidine fibers removed after 1 week demonstrated an antibacterial effect against a test strain of bacteria in vitro, indicating some drug remained in the reservoir (Medlicott et al, 1992).

II. Matrix delivery systems

In matrix delivery systems the drug is distributed throughout the polymer and release occurs by drug diffusion and/or matrix dissolution or erosion. A summary of the matrix systems under investigation for use as intrapocket delivery systems in periodontal disease is given in Table 1 and Table 2. Those that release by diffusion alone are prepared using water insoluble non-degradable polymers, whereas those that release by diffusion and matrix erosion or dissolution use soluble or biodegradable polymers in the matrix. The major limitation of the non-degradable systems is that they must be removed after the treatment period and a number of problems have been associated with this procedure. Usually, technical competence is required to ensure complete recovery of the depleted device as retention of all or fragments of non-degrading systems may lead to a foreign-body reaction and tissue inflammation. Also, device removal is accompanied by a rapid decline in the antibacterial concentration which is often associated with recolonisation of the periodontal pocket. In contrast, the biodegradable systems (in most cases) do not need removal and may overcome the above problems provided drug release and polymer degradation occur over compatable periods.

(a) Non-degradable matrix systems

Table 1 Non-degradable matrix systems under investigation for use as intra-pocket devices in periodontal disease.

Matrix material	Fabrication technique	Form	Drug incorporated
Polyethylmethacrylate films (Orthoresin®)	Moulding and compression	Film	Chlorhexidine, tetracyclin, metronidazole
Ethylcellulose films	Casting from ethanol or chloroform solution	Films	Chlorhexidine, tetracyclin, metronidazole, minocyclin
Ethylene vinyl acetate	Heat extrusion	Fibers	Tetracyclin
Eudragit RL®	Cast from ethanol water mixture	Films	Clindamycin

(from Soskolone and Freidman, 1996).

(b) Degradable matrix systems

In contrast from non-degradable systems above, the degradable delivery systems erode or dissolve in the gingival crevice so that removal after treatment is not required. Drug release occurs by device erosion or dissolution and drug diffusion through the matrix, the contribution of each of these mechanisms to the overall release process being dependent on their relative rates. Prolonged release can generally be achieved by manipulation of dissolution or erosion characteristics of the delivery system. These degradable systems are shown in Table 2;

Hydroxypropylcellulose films, bioadsorbable, cross-linked collagen and protein films, biodegradable polyester formulations and glycerol mono-oleate/sesame oil gel are given detail of each system in that table.

The interesting system, glyceryl mono-oleate/sesame oil gel has been developed recently to deliver metronidazole using a glycerol mono-oleate/sesame oil carrier and perform as biodegradable gel. The glyceryl mono-oleate/sesame oil preparation is fluid enough to allow placement into peridontal pockets using a syringes but hydrated on contact with water to give a viscous gel with liquid crystalline structure. Drug is released from the gel as it slowly erodes, degradation occurring by the action of neutrophil lysosomal enzymes which break down the glycerol mono-oleate into oleic and glycerol. These have a considerably lower viscosity and are flushed from the pocket by flow of gingival crevicular fluid. In vitro metronidazole release was found to depend on the drug salt used and the amount of gel applied.

Use of metronidazole benzoate produced a slower release than metronidazole, and the penetration of water into the gel was affected by the amount of gel applied. Stolze (1992) monitored the systemic absorption and gingival crevicular fluid concentrations of metronidazole after gel application. Peak metronidazole blood level of 0.2-1.3 μg/ml were reported 2-8 hours after doses of 29 to 103 mg were administered to inflamed periodontal pockets. This compared to 5 μg/ml after oral administration in the gingival crevicular fluid, after administration of 25-108 mg metronidazole to a pair of teeth, were 103-1297 μg/ml and effective concentrations were maintained for 24-36 hours. It was suggested that this formulation maintained effective gingival crevicular fluid levels of metronidazole while absorption produced lower systemic levels than a 250 mg oral dose. Subsequent clinical use showed the optimal dose to be two weekly applications of the 25%w/w metronidazole benzoate gel, and this was as effective as scaling in the treatment of periodontitis.

Biopharmaceutical considerations in drug delivery to the periodontium

The pathophysiology of periodontal diseases influences the subgingival environment and therefore must be considered in order to determine the characteristics of an ideal delivery system. The size of periodontal pockets limits the dimensions of intrapocket delivery systems and as a consequence also limits the amount of drug that may be administered. The host's inflammatory response leads to production of gingival crevicular fluid which fills the periodontal pocket and comprises the release medium for intrapocket delivery systems. Characteristics of this fluid may influence the release of drug from delivery systems. And as it flows through the pocket into the oral cavity it constitutes a potential route of elimination for drug from the site of infection (Medlicott et al, 1994).

Development of a pharmacokinetic model for the periodontal pocket

One-compartment model for the periodontal pocket based on the release of methylene blue, acid red and clindamycin from a non-degradable film and clindamycin from two degradable polymer film formulations was developed by Higashi et al (1990). The polymers used were ethyl methacrylate/chlorotrimethyl

ammonium methylmethacrylate copolylmer (Eudragit RL) which is not soluble in water and two methacrylic acid/methylmethacrylate copolymers (Eudragit L and Eudragit S) which are slowly soluble in alkaline conditions. Films were prepared by casting a solution of the drug and polymer in a mixture of ethanol and water (95:5). Examination of the in vitro release profiles showed that drug was released by diffusion (release rate was proportional to the square root of time) from the water insoluble matrix and zero-order from the slowly soluble films. The in vivo release, although slower than that observed in vitro, followed the same type of release (i.e. diffusional release for non-degradable films and zero-order release for degradable films.

A significant correlation (r = 0.998) was found between the in vitro and in vivo release rates for the non-degradable films containing methylene blue, acid red and clindamycin. It was suggested that this would allow estimation of the in vivo release rate from in vitro release studies for films that release by diffusion. This creates a non-sink environment in some areas of the surface of the preparation, and it was suggested that the decrease in release rate seen in vivo was due to "a reduction in the effective area of drug release, caused by the close contact of the preparation with the body surface". They also measured the clindamycin concentration in the periodontal pocket after administration of these delivery systems and found that it was dependent on the pattern of drug release. The in vivo clindamycin concentration was initially high (700 μg/ml) after insertion of the non-degradable delivery system and decreased over 16 h to about 100 μg/ml. In contrast, concentrations were maintained with the degradable systems at about 500-400 μg/ml for 24 h (Eudragit L films) or at about 200 μg/ml for at least 72 h (Eudragit S films). From this data Higashi et al (1990) developed a one-compartment model with a pseudo first-order elimination process.

The elimination process was studied by administration of a clindamycin solution into the periodontal pockets and analysis of the changes in concentration in the gingival crevicular fluid with time. Elimination was attributed to both absorption from the periodontal pocket epithelium and outflow of gingival crevicular fluid into the oral cavity. Eqs. (1) and (2) were used to simulate the in vivo data for degradable and non-degradable films respectively:

$$C = \frac{k}{E_t * V}\,(1 - \exp(-E_T * t)) \qquad \text{(Eq.1)}$$

where C = drug concentration in the periodontal pocket fluid, (μg/ml), k = *in vivo* drug release rate constant (μg/h/film), t = time after administration (h), V = periodontal pocket fluid (ml), and E_T = drug elimination rate constant in the periodontal pocket (h^{-1});

$$C = \frac{Kt^{1/2}}{V}\left(\sum_{n=1}^{\alpha} \frac{(E_T * t)^n}{n!(2n+1)} + 1\right)\exp(-E_T * t) \qquad \text{(Eq.2)}$$

where K = *in vivo* drug release rate constant($\mu g/h^{1/2}$/film), V= periodontal pocket fluid

The periodontal pocket concentrations generated from these simulations were shown to be in close agreement with those measured in vivo, so that it was suggested that this one-compartment model was a suitable model for the periodontal pocket. However, since only two degradable formulations were tested, these authors were not able to establish an in vitro/in vivo correlation for these systems. They did, however, suggest that once correlation was established between the in vitro and in vivo release rate (as was done for the non-degradable systems), it may be possible to predict the in vivo drug concentrations from the results of *in vitro* release studies.

Despite the number of delivery systems investigated for use in periodontal disease, the ideal system has yet to be developed. Clinical and microbiological evaluations generally shown improvements achieved by single administration of any one system to be of short duration so that reapplication is necessary to produce prolonged improveints in periodontal health. Also the magnitude of improvements has not been, in most cases, significantly greater than those obtained by conventional mechanical scaling and root planing.

The greatest advantage associated with the use of intrapocket delivery systems has been that administration is less time-consuming than mechanical debridement, and treatment does not rely as heavily on patient compliance as is the case with conventional topical delivery systems such as subgingival irrigation. Also, the amount of drug required to achieve effective concentrations in the gingival crevicular fluid is considerably less than that required if the drug is delivered systemically. The ethylene vinyl acetate fibre system achieved effective concentrations in the gingival crevicular fluid for the longest period, with tetracycline levels of 1590 μg/ml maintained for 10 days, but this involved securing the pocket opening so that the delivery system was retained and consequently altering the flow of gingival crevicular fluid. Biodegradable devices have mostly produced a short duration of effective drug levels (generally 1 to3 days and up to 7 days with the collagen films) or degradation occurred too slowly for the system to be regarded as truly degradable and removal after drug delivery was required (e.g., polyhydroxybutyric acid compacts). Device degradation and drug release occur over similar periods for the hydroxypropylcellulose films and glyceryl mono-oleate gel formulations, but since these formulations offered relatively short duration of effective therapy (24-36 hours), repeated application is needed if effective drug concentration are to be maintained. Other degradable systems, for which degradation occurs over longer periods, appeared to release their drug load more rapidly than the device degrades and in many cases the device degradation rate is not reported. The cross-linked protein film released drug over 15 to 90 hours in vitro but degraded over 60 to 140 hours. This means that if a sustained antibacterial concentration is required the device must be removed and replaced after drug depletion, but if intermittent antibacterial concentrations are required, time may be allowed for device degradation prior to repeated treatment.

To improve the usefulness of intrapocket delivery systems, the aims of treatment with antibacterial agents must be clearly defined. At present, delivery systems may be used to administer antibacterial agents directly to the site of infection within the periodontal pocket for a range of time periods. Treatment for 1 to 3 days appears to be sufficient to alleviate the signs and symptoms of periodontal disease, but not to prevent recolonisation and reoccurrence of the condition.

Table 2. Degradable matrix systems under investigation for use as intrapocket devices in periodontal disease.

Matrix material	Fabrication technique	Form	Drug incorporated
Hydroxypropylcellulose films	Cast from ethanol solution	Films	Tetracycline, chlorhexidine
Bioabsorbable dental material	Equillibration in drug solution	Gauze, wound dressing fibrin sealant	Doxycycline
A telocollagen	Cast from aqueous solution cross-linked with glutaraldehyde	Films, sponge, gel	Tetracycline
Byco® protien	Cast from water/ethanol mixtures cross-linked with glutaraldehyde	Films	Chlorhexidine, Tetracycline
Hydroxy propylcellulose metracrylic acid copolymer	Direct compression	Compacts	Tetracycline, metronidazole
Poly hydroxypropyl cellulose	Heat extrusion	Fibers	Tetracycline
Lactic acid/glycolic acid copolymer	Solvent evaporation	Microcapsules	Tetracycline
Poly(glycolide-co-DL-lactude)	Phase separation	Microcapsules	Minocycline
Poly-caprolactone	Casting from dichloromethane solutions	Films	Chlorhexidine
Glycerol mono-oleate/sesame oil gel	Mixing	Gel	Metronidazole
Methacrylic acid/methyl methacrylate coplymer (Eudragit L® and Eudragit S®)	Cast from ethanol water mixture	Films	Clindamycin

(from Medlicott et al, 1994

Longer periods of treatment (9 days to 1 month) have been associated with longer periods of reduced disease activity, but recolonisation and reoccurrence of inflammation have generally occurred. Also it is important to distinguish whether the prolonged clinical and microbiological improvement achieved with the administration of antibacterial agents is due to the long-term effects of these treatments in the priodontal pocket, or whether other improved oral hygiene measures undertaken during the in vivo evaluation influenced pocket recolonisation and disease progression by suppressing marginal plaque. It may be that the most effective treatment is achieved with a combination of delivery systems. Initial treatment with a stort-acting biodegradable system may be useful to provide a bactericidal concentration of the antibacterial agent within the periodontal pocket.

The carrier material would then be cleared from the pocket and would not interfere with the regenerating tissues. Subsequent prolonged delivery of antibacterial agents to the area surrounding the pocket opening may then prevent pocket recolonisation from the oral cavity by suppression of marginal plaque. With the development of sensitive analytical procedures it is becoming possible to measure the drug concentration at different sites and from this ascertain whether effective delivery is achieved with prolonged delivery systems. Models such as that developed by Higashi et al (1990) will also aid the development of effective delivery systems, as it may be possible to estimate the levels of drug achieved in the periodontal pocket from the results of in vitro release studies. This will allow the release profile of delivery systems to be optimised from a knowledge of the in vivo conditions and in vitro release characteristics, so that a formulation may be modified to provide the desired concentration of drug at required sites to eliminate the subgingival infection and create an environment which favours healing of the periodontal tissues, and ultimately elimination of the periodontal pocket.

Microemulsion gel and liquid crystal system

A pharmaceutical composition containing microemulsion made up of a hydrophilic component, lipophilic component, surfactant and drug, when examined on a macroscopic scale, a one-phase gel were obtained. The hydrophilic component is dispersed as colloidal droplets in the lipophilic component, or the lipophilic component is dispersed as colloidal droplets in the hydrophilic component. The hydrophilic and the lipophilic components form microemulsion gel with bicontinuous structure where the components form elongated adjacent channels. The drug is dissolved in the dispersed component or, in the case of a microemulsion with bicontinuous structure, in the hydrophilic or the lipophilic component. The microemulsion is stabilized by means of the surfactant. It is characteristic that a gelatinizer and water are added to the microemulsion thereby bringing the microemulsion into a gel form (U.S. Patent No. 5,143,934; 1992).

Due to their excellent dissolution properties microemulsions and liquid crystal have been increasingly used in various technological fields. They are used, for instance as detergents, in oil recovery and as the reaction environment of enzyme catalysts and used as dosage forms of drugs. Microemulsion gel denotes a thermodynamically stable and optically isotropic which consists of water (or more generally hydrophilic component), oil (or more generally lipophilic component) and surfactant which denotes a surface active substance with an amphiphillic character.

Microemulsions are macroscopically, e.g. when observed visually, homogeneous one-phase. Microemulsions are composed of microscopic continuous domains of water or oil which are separated from one another by a monomolecular layer of the surfactant. The role of surfactant is stabilization of the microemulsion, for instance through decreasing interfacial tension. The microemulsion is of an oil-in-water type (o/w, "oil in water"); a water-in-oil type (w/o, "water in oil") or has a bicontinuous structure.

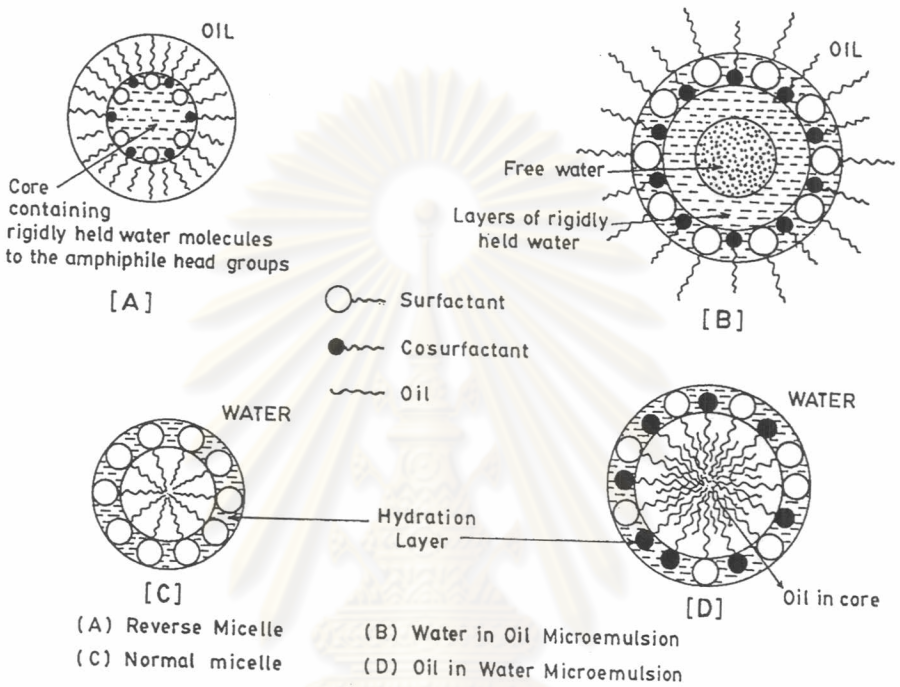


Figure 2 Pictorial representation possible microemulsion and reverse micelles structure (from Moulik and Paul, 1998).

The experiences of workers have revealed that the phase forming behaviors of ternary and quaternary microemulsion forming combinations depend on a number of factors, i.e. the types of polar medium water, glycol, glycerol etc., oils, amphiphiles, the presence of additive especially electrolytes , the temperature, the pressure, etc.The extent of the phases and their internal structure are obviously influenced by the intrinsic and extrinsic factors mentioned earlier. Both spherical and non-spherical forms of the dispersed state may aggregate forming chains, lamellae, mesophases, liquid crystalline states etc. Often gels of varying consistency are also formed. There higher states of aggregation are distinct departures from the genuine state of microemulsion. Pioneering works in this direction have been done by Kantaria et al (1999). The mixed water, amphiphile, oil systems can have complex phase intricacies, their identifications are painstaking but rewarding. In Figure 3.phase manifestations on a triangular and tetrahedral representations are illustrated for two typical mixed systems.

The monomeric solubility of the surfactant both in water (hydrophilic component) and in oil (lipophilic component) must be low so that the surfactant could

form a microemulsion with the highest possible stability. The surfactant may be ionic or non-ionic. If the surfactant is ionic, it must have two hydrocarbon chains to form a microemulsion. If the ionic surfactant does not have two hydrocarbon chains, a neutral inorganic salt and a co-surfactant must be added. A short-chain alcohol is often used as the co-surfactant. When a two-chained ionic or a non-ionic surfactant is used, the microemulsion may be formed without any additives. In case the surfactant is too lipophilic to form spontaneously microemulsions in water, a component like ethanol may be added which reduces hydrophilicity of water. Due to its high solubility in water, ethanol is not considered to take part in the formation of a stabilizing interface but to remain in water. Hence it is often called as co-solvent.

Gelatination and gels

By gelatinizing or gel formation is traditionally meant formation of a colloidal suspension in which the dispersed particles (lyophilic sol) partly coagulate so that a gel is formed. The particles in a lyophilic sol are stable because they are solvated. The gel formed is a threadlike mass containing a major part of the solvent. The final result is a pseudosolid or jelly-like product. One gelatination agent generally used is gelatin which is a naturally occurring polypeptide. Gelatin is insoluble in cold water but in warm water it dissolves and gelatinizes upon cooling the solution. When the solution is warmed, the gel reverts into a sol, i.e. the gel formation is reversible.

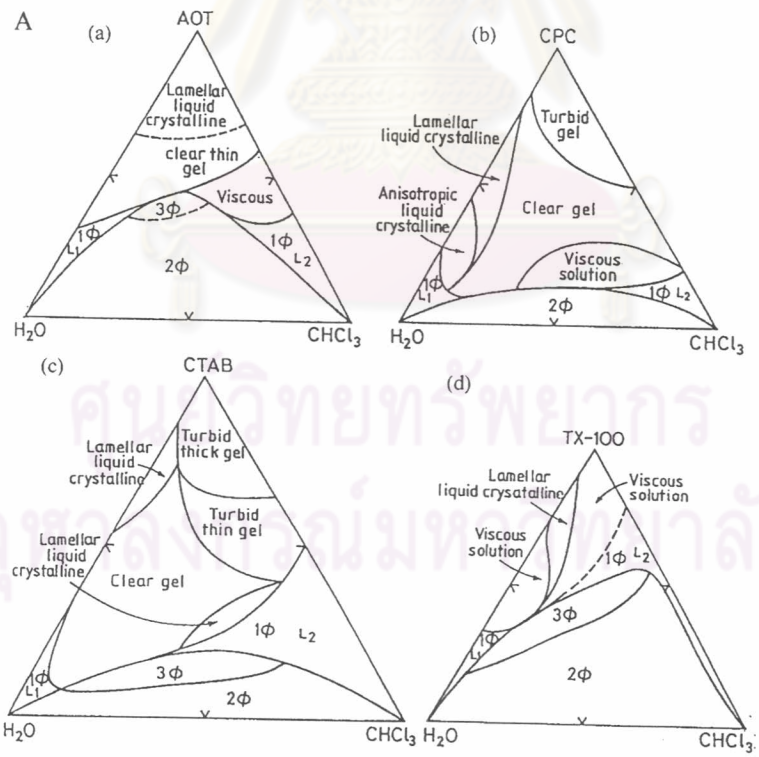


Figure 3 Phase manifestations on a triangular and tetrahedral representations are illustrated for two typical mixed systems (from Moulik and Paul, 1998).

Microemulsion-based gels

Gels denote soft, solid or pseudo solid systems comprising of two or more components one of which is a liquid constituting a major part (Murden et al, 1999). The mutual gel formation of gelatin and microemulsion was first described in the literature (Peter et al, 1988). The microemulsion in question was a w/o type microemulsion of water, isooctane and a sodium 1,4-bis (2-ethylhexyl) sulfosuccinate, which is generally called AOT. When small amounts of gelatin is added to this w/o microemulsion, the gelatin chains are enclosed into colloidal water droplets while part of the hydrophobic side chains of the gelatin chains adsorbs on the interface of the water droplet. Here gelatin acts as a co-surfactant. These gelatin-water droplets are 20-80 mm in size. At this stage the system forms a sol in which droplets appear as single colloidal particles.

The literature further discloses other types of gels, i.e. lecithin gels (Scartazzini and Luisi, 1998), which are called organogels. These gels are not, however, formed by gelatination of microemulsions by a separate gelatinizer. These gels are formed simply by adding a very small amount of water into a solution of an organic solvent and lecithin.

The above gels based on w/o type microemulsions are suitable for various technical applications like immobilization of enzymes and as membranes in separation processes. Due to the toxicity of the AOT surfactants, the microemulsion-based gels described in the literature cannot be used as carriers for drugs, especially not for oral and other systemic pharmaceutical preparations. In patent publication, the inventor suggests an idea that w/o type microemulsions brought into a gel form could be applied as a pharmaceutical carrier and mentions that the AOT surfactant could be replaced with another surfactant (U.S. Patent no. 6,004,58, 1999).

However, there are presented no examples of microemulsion-based gels based on other surfactants than AOT. In his article several years later (Luisi et al, 1990), the authors stated at the end of the article that application of microemulsion-based gels in the field of cosmetics and pharmaceutics is problematic because AOT and organic solvents used would have to be substituted with pharmaceutically acceptable alternatives. It is further emphasized that it is not yet clear to what extend substitution of AOT is possible. Therefore, microemulsion relates to a pharmaceutical composition derived from a microemulsion-based gel with an o/w-, w/o-, or bicontinuous structure and a method for its preparation. The microemulsion gel further relates to gels as such that are based on o/w-type microemulsions or microemulsions of bicontinuous structure. These novel microemulsion-based gels can be used for immobilization of any substance provided that said substance dissolves in the hydrophilic or lipophilic part of the microemulsion.

The pharmaceutical composition comprises a microemulsion made up of a hydrophilic component, a lipophilic component, a surfactant and a drug, wherein the hydrophilic component, lipophilic component and surfactant form, when examined on a macroscopic scale, a one-phase. The drug is dissolved in the dispersed component or in the hydrophilic or the lipophilic component of the bicontinuous microemulsion. The microemulsion is stabilized by means of the surfactant. It is characteristic that a gelatinizer and water is added to the microemulsion thereby bringing the

microemulsion into a gel form. The droplet size is typically in the size range 1-100 nm. Particularly preferred is such a composition in which the hydrophilic component is water or a mixture of water and a pharmaceutically acceptable alcohol, and the lipophilic component is a pharmaceutically acceptable hydrocarbon, fatty acid, a mono-, di- or triglyceride of a fatty acid or mixtures.

As suitable lipophilic components that are pharmaceutically acceptable can be mentioned paraffin oil which is a mixture of hydrocarbons or animal or vegetable oils with one or more C_{8-20} fatty acid or a mono-, di- or triglyceride of a fatty acid. Examples of suitable fatty acid based oils are fish oil, cod-liver oil, castor oil, soybean oil, maize oil, olive oil, almond oil, peanut oil, peach oil, palm oil, coconut oil, rape oil or sunflower oil. These kinds of oils are mixtures of several unsaturated and saturated esterified fatty acids. Castor oil has been proved to be particularly suitable, the main component (87%) being castor oil acid, a hydroxy substituted acid with the formula. Other fatty acids of castor oil are oleic acid (7%), linoleic acid (3%), palmitinic acid (2%) and stearic acid (1%). The excellent microemulsion and solvent properties of the castor oil are probably due to the high hydroxy acid concentration. This leads to the assumption that also other natural or synthetic, pharmaceutically acceptable hydroxy substituted fatty acids with intermediate chain length are suitable as the lipophilic component or as a part (U.S.Patent No. 6,004,580, 1999).

Pharmaceutically acceptable surfactants are ionic or non-ionic surface active materials. Particularly suitable surfactants are e.g. phospholipids, especially naturally occuring egg and soybean lecithins. Besides these any other pharmaceutically acceptable surfactant can be naturally used like the surfactants known under the trade names tween, Cremophor, Nikkol, Myrj, Cetiol etc.

Phospholipids, especially naturally occurring egg or soybean lecithins represent important surfactants. Lecithin is highly lipophilic due to two hydrocarbon chains but at the same time it is also hydrophilic due to polar zwitterionic head group. In water lecithins have a strong tendency to form lamellar liquid crystals at high lecithin contents. In water-oil systems, lecithin is slightly too lipophilic to form a stable microemulsion. By adding a lower alcohol to the water, the hydrophilic component is made less hydrophilic and in a system like this lecithin gives stable microemulsions. Alcohol acts as an amphilic co-solvent (U.S.Patent No. 5,143,934, 1992).

Suitable gelatinizers are e.g. gelatin or polysaccharides like agarose, agar, pectin or carrageenan. The pharmaceutical compositions are suitable as carriers for both water soluble and lipo-soluble drugs. If the microemulsion is of the w/o type, the composition suits as carrier for water soluble drugs whereas gels based on o/w type microemulsions suit for immobilization of lipo-soluble drugs. If the microemulsion has a bicontinuous structure, the composition suits as carrier for both water soluble and lipo-soluble drugs.

In particular the gels based on o/w microemulsions suit as carriers for cyclosporin, lipo-soluble vitamins or a steroid like e.g. hydrocortisone. Cyclosporins are cyclic polypeptides with a strong immunosuppressive effect. The body is formed by a ring structure of 11 amino acids in which the substituent R may vary. An important commercial drug is cyclosporin or cyclosporin A in which R is ethyl. Other

cyclosporins are cyclosporin B (R =--CH(OH)—CH_3), cyclosporin D (R = isopropyl), and cyclosporin G (R = 1-propyl) (Backlund et al, 1999).

The obtained microemulsion-based gel is moulded into pieces of desired size and shape. The gel can be used as such for oral, rectal, intravaginal, transdermal or topical pharmaceutical preparations without any additives besides possible preserving agents. A further prerequisite for a successful pharmaceutical combination is that the drug can be incorporated into the microemulsion and does not break the structure of the microemulsion. Through testing only it is possible to definitely find out which component combinations yield the desired final result. Method for the preparation of a pharmaceutical composition based on a microemulsion based gel. It is characteristic that a hydrophilic component, a lipophilic component and a surfactant are mixed into a one-phase microemulsion and a drug is added to the microemulsion and a gelatinizer is dissolved in water at a raised temperature and the microemulsion containing the drug is added. The pharmaceutical compositions derived from microemulsion-based gels are manufactured essentially in the following way. When a certain surfactant and a lipophilic component have been selected, at least a partial phase diagram should be created in order to show the liquid phases. A phase diagram for a particular system is usually created by weighing each component separately in a test tube. Different compositions of the system can then be analyzed visually after they have stabilized at 25°C for twenty-four hours.

The most important compositions with respect to gelatinizing are one-phase solutions (microemulsions). As stated above they are either water/oil, oil/water or bicontinuous microemulsions. Such a specific composition is then warmed to 45°C. at which temperature the drug is added. After this the microemulsion containing the drug is mixed with a 40% gelatin-water mixture at about 50-55°C. Mixing is continued until the solution is homogeneous. Upon lowering the temperature the solution changes pseudo solid. If lecithin is used as the surfactant, it does not, however, become quite transparent but has a yellow thick color due to lecithin. The structure of microemulsions (water/oil, oil/water or bicontinuous) can be determined by means of published methods e.g. TEM, electron microscope, or electrical conductivity (Park and Kim, 1999).

Microemulsion Gel Formulations

The microemulsion generally includes one or more lipids, one or more surfactants, optionally more humectants and water as a diluent (Backlund et al, 1999).

Lipid

Suitable lipids include those generally know to be useful for creating oil-in-water microemulsions. Preferred examples include fatty acid glyceride eters, preferably medium chain C_6-C_{12} fatty acid glyceride esters, and the like. Preferred C_6-C_{12} fatty acid glyceride esters include medium chain C_6-C_{12} monoglycerides and triglycerides, with the triglycerides being more preferred. Triglycerides of caprylic/capric acid are particularly suitable for use as the lipid component in the composition. Suitable triglycerides of caprylic/capric acid include Captex 300®, Captex®355, Captex®350 and Captex®200, which are commercially available. The most preferred being Captex 300®. Mixtures of suitable lipids can be used.

Surfactant

Suitable surfactants include those generally know to be useful for creating oil-in-water microemulsions wherein lipids are used as the oil component in the microemulsion, and preferably are well suited to aid in emulsifying the particular lipid being used. Non-ionic surfactants are generally preferred. Examples of suitable surfactants include ethoxylated castor oil and phospholipids. One suitable ethoxylated castor oil is Cremophor EL® commercially availalble from BASF Corp. Preferred phospholipids include purified soybean phospholipid or lecithins such as phosphatidylcholine. One suitable purified soybean phospholipid or lecithins is Phospholipon®90G. Other suitable non-ionic surfactants include block copolymers of ethylene oxide and propylene oxide. Suitable commercially available block copolymers of ethylene oxide and propylene oxide include; Pluronic F-68. Pluronic F-77, Pluronic F-87, and pluronic F-88, commercially available from BASF Corp. Mixtures of suitable surfactants can be used both ethoxylated caster oil and phospholipids are used as surfactants.

Humectants

The microemulsion also optionally includes one or more humectants. Preferred humectants include propylene glycol such as 1,2-propanediol, and polyethylene glycol (PEG) with an average molecular weight in the range of 100 to 500, preferably in the range of 150 to 300. Mixtures of suitable humectants can be used. Preferably both propylene glycol and polyethylene glycol are used as humectants. Suitable polyethylene glycol includes Carbowax, polyethylene glycol.

Water

Water is used as the diluent, and preferably purified or distilled water is used.

Thickening agents

Gel-microemulsion formulation also includes one or more thickening agents, such as a polymeric hydrogel. Generally, the hydrogel is a hydrophilic natural or synthetic gel-forming polymer, a natural gel-forming polymer could be preferably including carrageenan, xanthan gum, gum karaya, gum acacia, locust bean gum, guar gum. Mixtures of suitable humectants can be used. Suitable carrageenans include Seaspen® carrageenan and Viscarin® carrageenan commercially available.

Additives

The formulation can also optionally include one or more additives such as preservatives or antioxidants to help maintain and prolong the useful life of the microemulsion gel. Preservatives and antioxidants that are generally known, and do not detract significantly from the usefulness of the microemulsion gel for the particular purpose it is being used, can be incorporated into the gel-microemulsion formulation. Particularly suitable preservatives include sodium benzoate, methyl parabens, propyl parabens, sorbic acid, and the like. Sodium benzoate is most preferred. The prevention of the action of microorganisms in the formulation can be brought about by various antibacterial and antifungal agents, for example, parabens,

chlorobutanol, phenol, sorbic acid, thimerosal. If desired, other additives, such as colorants, scents, isotonic agents, for example, sugars, buffers or sodium chloride, can be added to the microemulsion gel to the extent desired.

The composition is formulated to provide a gel-microemulsion with a submicron particle size, preferably in the range of 30-80 nm. Additionally, the viscosity of the gel-microemulsion is in the range of about 100 to 1100 centipoises. Those of skill in the art will recognize that the amounts of each of the individual components used to produce a suitable gel-microemulsion are dependent upon the amounts and type of other components used. Therefore, the amounts and types of components are interdependent and can be identified through systematic mapping of ternary phase diagrams. The concentration of the components can be selected from within this region Suitable gel-polymer suspensions can then be selected as additives to the microemulsion-based system to obtain a gel of desirable viscosity with high thickening capability and compatibility with the microemulsion. It is preferable that the gel-microemulsion be stable at ambient temperature.

Representative examples of constituent concentration ranges for base components of some gel-microemulsion formulations can be found in Table 1, wherein the values are given in wt. % of the ingredients in reference to the total weight of the formulation.

Table 3. Example of constituent to form microemulsion gel.

constituent	example excipient	ranges (percentage)	preferred ranges (percentage)
Lipid	medium chain triglyceride	2 to 25	6 to 23
Surfactant	ethoxylated castor oil	3 to 30	4 to 17
	phospholipid	1.5 to 6	3 to 6
Humectant	propylene glycol	2 to 24	3 to 12
	PEG-200	1.5 to 6	3 to 6
Polymer Gel	natural polymer gel	0.5 to 4	1 to 2
Additives	preservative	0 to 0.5	0.1 to 0.3
Water	water	balance	balance

(from U.S. Patent no. 5,143,934, 1992).

Preparation of Gel-microemulsions

A simple procedure allows for the preparation of a gel-microemulsion at even a one-milliliter scale. The following generally describes such a simple procedure: Combine surfactants, hydrophilic components, and the lipids in an appropriate container. Mix the components using a stir bar with mild heat until a clear and homogeneous microemulsion is formed. Remove the composition from the heat, and

wait until it reaches room temperature. Add two parts of a pre-prepared polymer dispersion to each part of microemulsion with continued mixing. The resulting gel-microemulsion is dispersion with a viscosity in the range of 200-1000 centipoise, and a submicron particle size, preferably in the range of 30-80 nm.

Another aspect is the use of gel-microemulsion formulations as formulation bases for incorporating therapeutically active agents. The base gel-microemulsion formulations generally include the components and concentrations discussed above. The therapeutically active agents can include any generally known therapeutic agent where it would be desirable to administer such an agent with a gel-microemulsion formulation.

Structure Determination of Liquid crystalline and MEG

The elucidation of the internal structure of microemulsion, although important, can be very complex, and sophisticated physical techniques are required for this purpose. Small angle X-ray scattering (SAXS) , small angle neutron scattering (SANS) , dynamic or laser light scattering (DLS) , transmission electron microscopy (TEM) , nuclear magnetic resonance (NMR) , time resolved fluorescence quenching (TRFQ) methods have been in growing use over the last two decades. Other methods, i.e. conductance, viscosity, ultrasonic interferometry, ultrasonic absorption, dielectric permittivity, thermal conductivity, transient electrical birefringence, infrared spectroscopy, calorimetry, etc., are also in frequent use for the understanding of the internal physicochemical states of microemulsions (Swarbick and Boyland, 1994; Esposito, 1996; Constantinides, 1997 and Moulik et al, 1997).

1. SANS and SAXS methods

In scattering techniques, the waves scattered at a given angle by all points in the sample interfere with each other to produce one point in an interference pattern, which is then transformed to reconstruct an image of all correlations within the sample. For structural information, experiments must be designed which can measure distances on a scale comparable with the dimensions of the aggregates. For micelles and microemulsions, this can be profitably achieved by the use of neutron and X-ray radiations wavelength-1.0 nm where measurements at low angles produce enough interference to derive information about the scattering species. In small angle scattering method the measured intensity is correlated with the number density of scatterers, the interparticle form factor, and interparticle structure factor to derive information on the particle size and structural correlation. Thus, SANS and SAXS are powerful experimental techniques to study systems, their static structures, interactions. Analysis of SANS data on microemulsions is capable of showing polydispersity of the droplet size and shape and their fluctuations (Cordobes et al, 1997). Information on the correlation length of density fluctuations and isothermal compressibility can also be obtained from SANS measurements (Radler et al, 1989). The water induced structural changes within the *L* -phase of the same microemulsion system have also been reported from SANS measurements by Nina et al (1999).

2. Small angle X-ray diffraction

Small angle X-ray diffraction technique has been particularly valuable in determining the type of liquid crystal phase (Peter et al, 1988). The ratio between the

characteristic distances from the film are different among the phases. The ratio is 1: 1/2:1/4 in the lamellar phase, 1: $(1/3)^{1/2}$: $(1/4)^{1/2}$ in the hexagonal phase and 1: $(3/4)^{1/2}$:1/ $(3/8)^{1/2}$ in the cubic phase. The interlayer spacing of the liquid crystalline phase can be estimated by small angle X-ray diffraction technique (Lang et al, 2000). The interlayer spacing was determined by the standard equation ($n\lambda = 2d \sin \theta$) where n is the order of diffraction, λ is wavelength of the incident X-ray beam, d is the interlayer spacing, and θ is the diffraction angle. The diffraction angle is calculated by $\tan (2\theta) = DS / 2 (1+X)$, where D is the distance between the peak obtained from X-ray measurements, S is calibration factor associated with the detector, l is the length (mm) between sample and detector, and X is the calculated length (mm) from the calibration curves.

3. TEM

TEM technique has been limited but potentially used in the understanding of the microstructure of microemulsions under varied conditions of dispersant composition and concentration. In the TEM technique, a direct imaging method was successfully employed for the first time for glass forming microemulsions (Gao et al, 1998). The freeze-fracture electron microscopy (FFEM) employed by Hasse and Keipert (1997) can be of special mention.

4. Polarized light microscopy

Among all the available techniques, polarized light microscopy provides the easiest way to qualitatively identify the different phases of liquid crystals by their textures. The lamellar and the hexagonal phases are optically anisotropic, and thus can be directly observed under an optical microscope with polarized light. The sample of these phases will look radiant when viewed against a light source placed between crossed polarizers whereas the cubic phase is optically isotropic and consequently not visible in polarized light. Therefore, the characterization of cubic phase requires other technique. Under the microscope with polarized light, the two phases, the lamellar and the hexagonal, also display different patterns (Malcomson and Lawrence, 1993). These patterns are very useful for primary identification. This technique was used by several reseachers who previously studied liquid crystals (Kale and Allen, 1989; Constantinides and Scalart, 1997).

5. Others Evaluation Method For MEG and LC for periodontal used

In addition to the methods described earlier, other methods, i.e. viscosity, conductance, thermal conductivity, dielectric permittivity, electrophoretic birefringence, ultrasonic interferometry, ultrasonic absorption etc. The following method are widely use due to its useful and rapid way to characterized the primary structure of MEG and LC (Nema et al, 1997).

5.1 Electrical conductivity

This technique has been widely used to determined the nature of the continuous phase and to detect phase inversion phenomena. The distinction between o/w and w/o microemulsion which had high and low conductivity, respectively.

Dilutability by the excess of the dispersed phase are also employed to identified the structure of microemulsion. Oil in water microemulsion are dilutable with water whereas water in oil systems are not undergo a phase inversion into oil in water microemulsion (Swarbick and Boyland, 1994).

5.2 Viscosity

Viscosity describes the resistance to flow with applied stress for a Particular system; a more viscous system requires greater force or stress to make it flow at the same rate as less viscous system. This parameter is directly used to describe the syringeability and injectability of emulsion for parenteral administration. Many techniques could be used to measure this parameter. Capillary viscometers and the falling ball viscometers are simple instruments for measuring viscosity but only for newtonian liquids. Rotational viscometers including to coaxial cylinder sensor systems (cup and bob viscometer) and cone-and-plate sensor systems are instruments may be used with both newtonian and non-newtonian liquids (Martin, 1993).

5.3 Syringeability

Syringeability describes the ability of microemulsion to pass easily through a hypodermic needle or transfer from the vial or container prior to injection. It includes characteristics such as the ease of withdrawal, clogging and foaming tendencies, accuracy of dose measurements. Increasing the viscosity, density and particle size hinder the syringeability of the emulsion. A suitable test is to ensure that the entire emulsion pass through a 25-gauge needle of internal diameter 0.3 mm.

5.4 Injectability

Injectability refers to the performance of micromulsion during injection and includes factors such as pressure or force required to injection, evenness of flow, aspiration quality and freedom from clogging. The syringeability and Injectability are closely related to the viscosity and particle characteristics of the microemulsion (Hatefi and Amsden, 2002).

5.5 pH

The pH of the micromulsion is usually adjusted to approximately 8.0 prior to sterilization . This is preferred because the pH of the micromulsion falls on autoclaving and also a function of time during storage, as the result of glyceride and phosphatide hydrolysis liberating free fatty acid (FFA). The rate of FAA production is minimal if the pH of the microemulsion is between 6 and 7 after sterilization (Floyd and Jain, 1996). This parameter affects to physical and chemical stability. The pH meter is generally instrument to be used for determining the pH of preparation.

5.6 Particle size

Particle size of lipid globules has been direct effect on both toxicityand stability. Particle size greater than 4 to 6 nm are known to increase the incidence of emboli and blood changes. For intravenous injection, particle should be less than 1

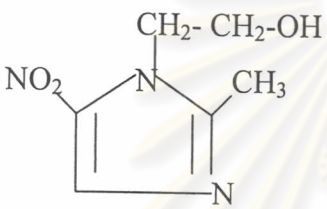
nm in diameter. For subcutaneous or intramuscular injections, the particle should preferably be less than 250 nm in diameter. Larger particle size can be used for oral formulations. The microemulsion has particle sizes in the range 10-140 nm. Hence, the microemulsion has the advantage of very small disperse phase diameter, which may impart thermodynamic stability (Paul and Moulik, 1997).

Physicochemical property of metronidazole

Physical property of metronidazole (Macdonald, 1972).

Metronidazole is a white to pale yellow crystalline powder; odorless or almost odorless. Melting point range between 159°C-163 °C.

Structure formular :



Empirical formula : $C_6H_9N_3O_3$
Chemical name : 2-metyl-5-nitroimidazole-1-ethanol
Molecular weight : 171.16
Dissociation constant, pKa : 2.5
Solubility : Metronidazole is soluble 1 in 100 of water, 1 in 200 of ethanol and 1 in 250 of chloroform; very slightly soluble in ether; slightly soluble in acetone and in dichloromethane; sparingly soluble in dimethylformamide; soluble in dilute acids. The solubility of metronidazole at 37 °C in an ascending homologous series of triglycerides decreased in order; tricaprin > trilaurin > trimyristin > tripalmitin > tritearin.

Stability of metronidazole :

The degradation kinetics of metronidazole in aqueous solutions of pH 3.1 to 9.9 at 90±0.2°C were studied by Lund et al (1994). The stability of metronidazole in solutions containing propylene glycol or polyethylene glycol 400 was also investigated. The reaction order for metronidazole in these aqueous and solvent systems followed pseudo-first order degradation kinetics. The degradation rate of metronidazole was invariant under various total buffer concentrations at each specific pH within the investigated pH range. These results indicated that no general acid/base catalysis imposed by acetate, phosphate and borate buffer species was responsible for the degradation of metronidazole. The pH rate profile shows a pH-independent region of pH 3.9-6.6. Maximum stability of metronidazole was at pH 5.6 under zero total buffer species conditions.

Metronidazole has been reported to be sensitive to light (Moore et al, 2000). Previous work (Reynold et al, 1996) has indicated that the photodecomposition

reaction of metronidazole appeared to followed pseudo-first order kinetic. The rate of decomposition was found to increase with pH and intensity of radiation and to decrese with drug concentration. The presence or absence of oxygen was found to exert very little effect on the photodecomposition rate of metronidazole. The degradation of metronidazole in different solvent decreased in the order: chloroform > isopropanol > methanol > water.

Aqueous solution of metronidazole 0.5% in citrate: phosphate buffer at pH 5 became bright yellow after exposure to daylight for 18 months but faded to colorless after further exposure to daylight for 21 months. These products have been characterized by UV, IR, proton NMR, mass spectroscopy and melting point determination. It is proposed that the initial yellow degradation product is an "excimer ion-pair" formed by the stabilization of metronidazole in its first electronic excited state by the citrate molecule (Stoltze et al, 1992).

Reynolds et al (1996) has pointed out that photodegradation of metronidazole gel followed first order reaction. After exposure to accelerated light throughout 24 weeks, the color of metronidazole gels changed to yellow whereas that of metronidazole gels wrapped in aluminium foil showed no physical change and no degradation occurred. The formulations composing of poloxamer 407 as gelling agent were less stable than the formulations composing of hydroxyethyl cellulose. Acetate buffer, D-glucose and sodium showed no effect on the chemical stability of metronidazole in the formulation containing hydroxyethyl cellulose except for the formulations containing poloxamer 407 which acetate buffer retarded degradation rate constants.