Artificial Caries-Like Lesions Analysed by the Optical Caries Monitor

- An In Vitro Investigation -

Dr. med. dent. Nikolas Wilhelm

Zahnarzt

(Univ. of Mainz/Rheinland-Pfalz, Fed. Rep. of Germany)

Thesis submitted in fulfilment of the requirements for the degree of

M. Sc. (Med. Sci.) by Research in the Faculty of Medicine,

University of Glasgow.

Dental School (Orthodontics),

University of Glasgow.

September, 1996.

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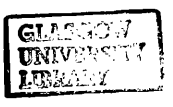

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ACKNOWLEDGEMENTS

I would like to express my gratitude to both my supervisors Professor W.J.S. Kerr and Dr. S.L. Creanor for their continous support, help and patience during this project. I wish to acknowledge their valuable help by giving attention to detail in reading the manuscript and encouraging me to continue my efforts to complete this thesis.

I owe special gratitude to Mr. Richard Foye, for his never ending patience and kindness during the last two years in which he introduced me to the numerous technical appliances of the laboratory and always gave me valuable advice when I seemed to be "at the end of my Latin".

Statistical advice for this thesis was provided by Mr. J.H. Mc Coll for whose time, advice and patience to provide simple explanations for the statistical analysis of the results, I am very greatful.

I would also like to thank Dr. A.F. Hall for providing me with valuable photographic material and information during my work and the Department Of Dental Illustration, for their assistance in developing and providing all the photographs presented in this thesis.

On a more personal note I would like to thank my parents for their continous love and support which gave me a lasting source of motivation and strength throughout my life.

With regard to the tragic event in the recent past I want to express my gratitude to Klaus Schmider, Holger Schmidt, Thomas Lehrer, Peter Anterist and Rhona Lawson for having supported me with all their friendship in difficult times.

This thesis is dedicated to the loving memory of my mother

Dr. Margot Wilhelm

geb. Lenz

* 05.01.1934 † 25.07.1996

SUMMARY

Optical methods are one of many non-destructive methods investigated at the current time for the early detection of decalcification around orthodontic brackets.

The visual method of von der Fehr was used firstly to assess decalcification. The results of the optical method were then compared with those from microradiography and microdensitometry.

This study investigates the use of an optical probe *in vitro* (SENSOPTIC optical caries monitor) which works on the principle of light scattering by enamel crystals in relation to their surrounding environment. The optical caries monitor employs a narrow beam of white light illuminating a circular spot of ~1mm diameter. The light which emerges from the same spot is recollected and measured.

The optical caries monitor was investigated with regard to its capability to produce repeatable and reproducible measurements of mineralised and demineralised tooth surfaces. For this purpose, 60 bovine incisors were obtained and prepared. All teeth were exposed to a demineralisation solution to create enamel surface lesions in specially designed areas. The lesions, exhibiting varying grades of white spot intensity, were assessed visually under a microscope and photographed for later visual assessment. Optical caries monitor readings were taken in the lesion areas which formed two bands on the mid labial surface of the crown aligned in two horizontal bands being ~ 5-6 mm long 1 mm wide and running ~ 1 mm parallel

apart from each other. In each of the two lesion bands described as upper and lower carious lesion bands, four measurements were taken in each band at different locations. These measurements were each repeated four times and one average value was calculated for each of the spots. This value represented 16 measurents in total for each of the two carious lesion bands. The collection of the data for the sound enamel surface followed the same technique by using the area above the upper lesion band and below the lower lesion band as a control area. A total of 64 measurements were collected for each tooth grid provided data which consisted of 3776 measurements. One tooth was excluded from the sample because of excessive decalcification.

To assess the reliability of the optical caries measurements, all 59 samples were subjected to further laboratory investigations. To assess the mineral changes which had occurred due to the demineralisation procedure the samples underwent examination by microradiography and microdensitometry. Von der Fehr Index assessment was made using photographic slides of the samples after demineralisation. Visual scoring was carried out on two different occasions, by two examiners, giving a time interval of one week before the second assessment. The slides were projected onto a screen for each scoring session. The slides were precoded prior to the scoring session and randomly arranged by the author for each of the sessions. At the end of the scoring sessions the code was broken for analysis of the scores.

The results of the study demonstrated the capability of the caries monitor to differentiate differences between sound enamel areas and decalcified lesion areas. The caries lesion bands and non-caries lesion areas did not show any significant difference.

The visual assessment scoring, based on the von der Fehr Index, showed reproducible results only for one examiner who had a clinical background, whereas for the non-clinical examiner they were not strong enough to show a consistent assessment during both scoring sessions.

The results of this investigation, indicate that microdensitometry / microradioraphy was considered to be the most accurate method of lesion assessment. Comparisons between optical caries monitor scores and microdensitometry showed good correlations, although the prediction interval had a wide range. Furthermore, Delta Z and Lesion Depth showed good correlation values when compared to the caries monitor readings. When Surface Zone and Lesion Body were compared to the optical caries monitor readings, there was a poor correlation.

There is great potential for future use of the optical caries monitor if some of the handling procedures, such as calibration, could be more simplified and the design of the apparatus more orientated towards future clinical use.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction.

One of the primary aims of modern orthodontics is seen in the creation of the best possible dental relationship within the framework of acceptable facial aesthetics and stability of the occlusion on a long term basis. Dento-facial aesthetics is one of the major concerns for patients seeking orthodontic treatment. As soon as these goals are achieved, through a period of fixed or removable appliance treatment, the longevity of the dentition is the primary concern of the parents, the orthodontist and the dentist, who have been working together throughout this period to achieve this goal. If good oral hygiene standards have been maintained through constant awareness of the importance of having clean teeth before and during orthodontic treatment, especially if fixed appliances have been used, the risk of jeopardizing the treatment result through caries is normally quite low. Unfortunately, this goal is not always achieved during a treatment period, which can last up to two years. To maintain the achievements of treatment, awareness of the dangers that can be caused by the caries process is mandatory. Prevention and early detection of surface changes in dental enamel is vital to prevent the process from becoming more advanced and irreversible, leading to breakdown of the enamel tissue. In the case of detection of white spot

lesions, prophylactic measures must be taken to preserve the tooth from being affected by the occurence of white spot lesions (Figure 1.1) and even cavitation.

1.2 The Incidence Of White Spot Lesions In Orthodontics.

White spot lesions or areas of decalcification can be described as carious lesions to a varying degree. The incidence and severity of white spot lesions often becomes apparent after orthodontic treatment, when the fixed appliances are being debonded (Figure 1.1) and the underlying tooth surface is exposed. The presence of fixed appliances in the oral cavity facilitate an increase in plaque accumulation over a longer period of time and an increase in a retentive surface for it (Zachrisson and Zachrisson, 1971). White spot lesion formation on the labial surface of bonded or banded teeth during orthodontic treatment has long been recognized as a problem (Gorelick *et al.*, 1982; Mizrahi, 1983). Clinical observations and quantitative studies of the incidence, areas of susceptibility and prevention of decalcification have been reported (Zachrisson and Zachrisson, 1971; Gorelick *et al.*, 1982; Mizrahi, 1983; Geiger *et al.*, 1988). In several investigations the retention of plaque (Ceen, 1980), oral hygiene efficiency, and varied caries resistance levels of patients have been identified as being related to the incidence of white spot lesions during orthodontic treatment (Zachrisson and Zachrisson, 1971; Ceen, 1980). The cervical parts of enamel seem to be most at risk by the occurence of white spot lesions (Artun and Brobakken, 1986; Artun and Thylstup, 1986). Plaque accumulation takes place underneath orthodontic bands, where the bonding cement has been lost due to seal breakdown, inadequate structural and bonding strength of the cements and their solubility in oral fluids

(Sadowsky, 1976; Mizrahi, 1982). Demineralised surface enamel is considered to be the precursor of early enamel caries and is due primarily to the action of acids, which during orthodontic treatment may come from the cement used for retaining the bands and breakdown products of food debris by plaque (Darling, 1956). New and often extensive patterns of plaque accumulation can develop in association with resin-bonded orthodontic brackets in addition to a subject's already established plaque pattern (Gwinnett *et al.*, 1978). The surface of the resin is, due to its roughness, predisposed to the rapid attachment and growth of oral organisms which can be found 24 hours after the placement of the bracket on its surface. The presence of plaque can be confirmed histologically both supragingivally and subgingivally on acrylic and other restorative materials. Therefore, rough surfaces are likely to encourage rapid attachment of bacteria and the ultimate promotion of not just caries, but also periodontal desease. Since filled and unfilled resins are used to bond orthodontic brackets, it has been postulated that they may be among the factors contributing to the plaque patterns causing white spot lesions (Gwinnett *et al.*, 1978). Plaque accumulation on brackets and some of the resins used to bond has been found, even in subjects with good oral hygiene (Gwinnett *et al.*, 1979). Clinical observations indicate that the junction between the resin and the enamel is one of the most common sites for demineralisation (Gwinnett *et al.*, 1979) which are usually found peripheral and gingival to the bracket base. When mesh brackets are bonded with highly filled resins, the plaque index scores are especially high (Zachrisson *et al.*, 1978). Some demineralisation is seen as been mild and hardly noticeable unless the teeth are dried and examined closely (Zachrisson *et al.*, 1971). In the initial stages the lesion is a

surface softening of the enamel rather than a subsurface lesion with a surface layer (Arends, 1986).

Measurable demineralisation can occur adjacent to orthodontic bands or brackets *in vivo* within one mouth (Holmen *et al.*, 1984; Glatz, 1985). Even within four weeks, white spot lesions with a depth of about 100 μm may develop under orthodontic bands (O´Reilly *et al.*, 1985, 1987; Ogaard, 1989). Scanning electron microscopy (SEM) has demonstrated that bacterial accumulation around modified orthodontic bands leads to a marked and localized direct etching of the tooth under the plaque at the junction of the tooth and band after only one week (O´Reilly *et al.*, 1987). With longer periods of exposure to the carious environment (Figure 1.2), a gradual worsening of the direct surface dissolution was observed (Holmen *et al.*, 1984). After a period of twelve months banded patients developed less demineralisation than bonded patients (Zachrisson, 1977). The introduction of fixed orthodontic appliances were seen to enhance plaque accumulation and have brought about a slight increase in the clinical signs of gingival inflamation after three months (Zachrisson, 1977). During the time taken to complete orthodontic treatment, which can be up to two years, a significant increase in white spot lesions can be found compared to earlier stages (Geiger *et al.*, 1992). Orthodontic bands and brackets can change the physical, chemical and biological conditions in the oral cavity. Due to those changes in the microbiological environment, there is an increase in the accumulation of plaque of up to 35 times for specific types of bacteria (Balenseifen and Madonia, 1970). The additional increase in the volume of dental plaque (Gwinnett and Ceen, 1979; O´Reilly and Featherstone, 1987) is due to a larger proportion of carbohydrates and bacteria

which can, therefore, produce a greater amount of acid on the tooth surface. Not only in the early stages of treatment, when initial archwires and additional ligatures, like lacebacks are used during the phase of levelling and aligning, are surfaces for plaque accumulation increased. Food debris and plaque can be trapped between different parts of the appliance. Even in the later stages of treatment, when teeth are moved bodily along archwires with the help of pushcoils, elastics or closing loops, even more plaque can accumulate on those parts of the appliance. As a consequence, the natural self-cleansing process from mastication, salvary flow and tooth brushing is impeded and a very versatile oral hygiene technique is required to keep oral hygiene at an acceptable level. Most orthodontic appliances cover the lingual surface of the teeth and many studies have shown an increase in the incidence of carious lesions during fixed orthodontic appliance treatment (Zachrisson and Zachrisson, 1971; Gorelick *et al.*, 1982; Ogaard, 1983; Artun *et al.*, 1986). Zachrisson and Zachrisson (1971) found the majority of white spot lesions in the vestibulo-gingival enamel areas (Figure 1.1). The partly covered vestibular and lingual surfaces of the anterior teeth are subject to an increased caries susceptibility and especially the upper lateral incisors showed the highest percentage of new white spot lesions (Meyers, 1952; Ingervall, 1962). In Meyer´s study, 34 out of 76 banded (Table 2) lateral incisors showed signs of white spot lesions of which 10 were on the vestibular and 24 on the lingual side, whereas the upper canines had only 1 out of 12 teeth affected by caries. In the lower arch, the first molars showed the greatest amount of caries with 5 out of 11 teeth affected. In a study of 19-year old patients either subjected to or not subjected to orthodontic treatment, a significantly higher median white spot score on the vestibular surfaces was found (Ogaard, 1989). The median of the white spot score was significantly

higher in the orthodontic group when compared to the untreated group. A high prevalence of white spot lesions was also observed on the mandibular premolars and canines and the maxillary lateral incisors.

There is no general consensus for the distribution of opacities within the dentition following orthodontic treatment. Many authors have found that in the mandibular arch the first molars showed a significant higher prevalence of of enamel opacities (Meyers, 1952; Gorelick *et al.*, 1982; Mizrahi, 1983). The area of enamel covered by the cemented band generally covers the middle and cervical third of the clinical crown. The cervical third of the crown is the area which is most likely to attract plaque and, therefore, is at risk to the development of white spot lesions. The development of enamel opacities beneath the surface of the orthodontic band will occur only following breakdown of the luting cement layer. The use of ultrasonic instruments for the removal of excess cement, at the time of placement, contributes to the breakdown of the cement layer between the band and the tooth when the instrument is placed on the band surface; forces produced during mastication may also induce stresses on the band surface great enough to lead to the physical breakdown of the luting cement layer.

Most of the lesions recorded in the literature covered less than one third of the vestibular enamel surface along the gingival margin (Ogaard, 1989). Especially on mandibular premolars and canines and on maxillary lateral incisors (Dolce, 1950; Meyers, 1952; Bach, 1953, 1954; Ingervall, 1962; Zachrisson and Zachrisson, 1971). The prescribed bracket position related to the incisal edge places the brackets very

close to the gingival margin (Artun and Brobakken, 1986; Ogaard, 1989). If plaque retention occurs and the gingiva responds with inflammation, plaque will soon cover the gingival third of the crown. In these areas, pockets may develop and plaque retention will increase. The complete removal of plaque is usually difficult to achieve for the patient unless his/her oral hygiene is of a high standard (Lundström and Hamp, 1980). Glatz *et al.*, (1985) found no relationship between mineral loss and Oral Hygiene Index or the initial fluoride content of the enamel. Other authors found that the introduction of fixed orthodontic appliances enhanced plaque accumulation and brought about a slight increase in the clinical signs of gingival inflammation after the first three months of treatment. Lundström and Hamp (1980) found that it was possible to maintain a minimal level of caries activity using an intense period of increased oral hygiene education in fixed appliance-treated patients. They found only minor differences in the number of new restorations (Table I) occuring on those surfaces where orthodontic bands have been placed when compared to the untreated control group. These findings are strongly supported by Zachrisson (1977) where only 15 per cent of the treated children experienced white spot lesion formation. He stressed the fact that in this investigation the findings were low because his patients were not fully banded (Zachrisson and Zachrisson, 1971). In their opinion, caries incidence had improved due to the use of sealant coating during bonding and regular fluoride supplementation combined with intense oral hygiene instruction. Consequently, a very low incidence of new interproximal caries (Table V) and buccal demineralisation was found.

The use of fluoride to prevent acid attack on enamel can considerably slow down rates of decay (ten Cate and Duijsters, 1983). If a combination of daily brushing with a fluoridated dentifrice, coupled with daily rinsing with a fluoride mouthrinse is used, this combined therapy could provide not only complete protection against denineralisation but also remineralisation of surfaces at risk (O´Reilly and Featherstone, 1987).

1.3 Prevention Of White Spot Lesions After Orthodontic Treatment.

The prevalence of enamel opacities with primarily fixed, but also removable appliances (Mizrahi, 1982, 1983; Bjerklin *et al.*, 1983), is still a serious problem and an unwanted side effect of orthodontic treatment. Not only does it jeopardize the quality of the treatment result as a whole, but it can also lead to the discontinuation of the orthodontic therapy in the middle of the treatment period which may be up to 18-24 months. This decision is left with the orthodontist and fortunately only in very few cases and, therefore, is very important to be able to assess whether patient cooperation and compliance is sufficient enough to justify the cost, time and effort which are invested in orthodontic treatment.

The cooperation is largely dependent on how the patient manages to control his/her dietary habits, plaque control and appliance management. On the other side, the orthodontist must check all these aspects regularly and document the course of their development. This can become a very important issue if the patient fails to cooperate

and may wish to claim compensation for the occurence of white spot lesions (Machem, 1991).

Finally, the orthodontic practitioner bears the responsibility for the supervision of the overall oral care of the patient throughout orthodontic treatment. However, the use of fluoride mouthrinses, fluoridated dentifrices and direct application with fluoride varnish have been proven to reduce the occurence of white spot lesions. Recent developments in orthodontic materials have been encouraging in their role to improve certain areas which are associated with the development of white spot lesions. Also, the introduction of mini brackets with a reduced bracket base has lead to a reduction of the plaque retention surfaces and has also enlarged the distance between bracket base and gingival margin. Fluoride incorporated into orthodontic adhesives and resins is another supportive step into the role and function of fluoride as an anticaries agent during orthodontic treatment. The changes from the use of more bonded than banded appliances in the early 80´s has been another major step forward in reducing the white spot lesion problem.

1.3.1 Historical Account Of Fluoride.

It is now well established that tooth structure can be altered by dental fluorosis if excessive fluoride exposure occurs during the formation of dental tissue formation. McKay and Black (1916) described for the first time a syndrome which they called `mottled enamel´. They related their findings to changes in tooth substance influenced

by increased fluoride uptake. They demonstrated the endemic nature of the enamel lesions and suggested that the cause of mottled enamel was related to trace elements.

In 1931 several independent laboratories compared the amount of trace elements in water from areas with high levels of enamel mottling and determined that the water had relatively high concentration of fluoride (Churchill, 1932; Dean, 1934). The conclusion reached in each of these independent investigations was that fluoride was the aetiologic factor of mottled enamel. Ainsworth (1933) was another of those researchers who associated dental fluorosis and mottled enamel with high levels of fluoride in the water. He stated that if fluoride levels were to exceed 5 ppm, the population would show signs of fluorosis at a 90% level.

Following these studies, Dean (1934) developed an index of fluorosis and used it to relate the severity of flourosis to the level of fluoride exposure. In a series of epidemiological studies, Dean *et al.,* (1942) empirically determined the optimal level of fluoride in drinking water by plotting both dental caries experience and prevalence of dental flourosis against the level of fluoride in drinking water. The intersection of the two curves occured at approximately 1.0 ppm fluoride, representing the minimal level of both dental caries and dental fluorosis.

Later, the recommendation for optimal levels of fluoride was modified to include levels from 0.7 to 1.2 ppm fluoride in drinking water relative to the environmental degree for a known level of fluoride in the water supply (Galagan *et al.*, 1953). Forrest (1956) related the level of fluoride in the water directly to the incidence of

mottling (Table III). Furthermore, he described pitting of the enamel surface which sometimes occurs in the more severe types of mottling. He found that premolars were more affected by mottling than any other tooth in the dentition.

The optimal level of fluoride should be regarded as one where the maximum reduction of caries is combined with a minimum amount of mottling. Forrest (1956) regarded values between 1.0 and 1.5 ppm as an optimum level for fluoridation. In some investigations it was assumed that malnutrition in combination with high fluoride levels could increase the degree of fluorosis, but no scientific evidence so far has been verified.

1.3.2 Role Of Fluoride Prevention Of Caries.

The influence of fluoride upon the remineralisation of artificially formed early lesions makes it one of the most investigated and examined anticaries agents currently available (Nikiforuk, 1985). There have been experimental models developed to investigate and assess its effectiveness and the functional processes involved, but the exact mode of action needs further investigation Creanor (1987) and Damato (1990) have reported the following suggestions.

(1) Fluoride incorporated into enamel structure reduces its solubility in dilute acids:

The loss of mineral in carious lesions is believed to result from dissolution by an acid attack on its surface. The solubility of the mineral is a major factor in the degree of

cariogenicity of teeth. The solubility of the calcium-phosphate phase can be transformed by fluoride uptake into the apatite lattice to the more stable and resistant hydroxyapatite and fluorapatite phase (Brown *et al.*, 1977; Moreno *et al.*, 1977). Fluoride incorporated into crystals decreases solubility by eliminating impurities and defects. In fact, the higher the concentration of fluoride during apatite formation, the greater is the incoporation into the apatitic lattice (Margolis and Moreno, 1990).

The inhibition of dental caries resulting from the topical application of relatively high concentration of fluoride ions is also related to a surface coating of calcium fluoride which protects the enamel phase (Gray *et al.*, 1958). A fluoride ion concentration as low as $1x10^{-5}$ M. in a calcium phosphate solution can enhance the rate of remineralisation of dental enamel *in vitro* (Amjad and Nancollas, 1979). In contrast to their findings, some researchers found no significant relationship could be demonstrated between caries experience of the individual and fluoride content of enamel (Fejerskov *et al.*, 1981; Weatherell *et al.*, 1984). Fejerskov *et al.* (1981) found that the fluoride content in surface enamel between teeth developed in optimal and low fluoride areas is too small to explain any significant effect on dissolution rate of the enamel. They concluded that the local effect on the oral environment had served as a major explanation for the cariostatic effect of fluoride. Weatherell *et al.* (1984) found no indicators that fluoride accumulates in non-mineralising tissues but it can be traced in soft tissues (aorta, placenta, dental plaque) which can accumulate minerals. Furthermore, they found that most of the fluoride uptake was acquired prior to eruption of the tooth, probably during the preeruptive maturation period, when the surface seems to be porous allowing a relatively easy absorption. Damato (1990)

states that relatively little fluorapatite exists in enamel even if the teeth developed in fluoridated communities. Fluorapatite, with its hydroxyl groups replaced by fluoride, contains 38,000 ppm. fluoride, whereas the fluoride content of enamel is usually only 500-1500 ppm (Weatherell *et al.*, 1977) in the outer 2 μm of the surface (Arends and Christoffersen, 1990). Damato (1990) acknowledged that the substitution of fluoride at the enamel surface may reach a level that would have the potential to reduce its solubility, but accepted it only on the basis of a partial explanation of the anticarious mechanism of fluoride.

(2) Fluoride present in the aquaeous phase around the tooth during a cariogenic challenge inhibits demineralisation and enhances remineralisation:

Systemic and topical exposure to fluoride results in increased enamel fluoride content. A number of studies have shown that low concentrations of fluoride present in acidic solutions greatly reduce the rate of enamel demineralisation (Brown *et al.*, 1977). Margolis and Moreno (1990) showed that increasing the fluoride concentration in solution greatly reduced the rate of demineralisation; at low concentrations, cavitations were predominantely observed, whereas, at a fluoride concentration of 1 ppm, no mineral loss could be detected even after 10 days of continuous exposure to a solution which was undersaturated with respect to enamel mineral. When the rate of deposition of fluorapatite exceeds the rate of transport of ions out of the enamel surface, enamel demineralisation will be effectively inhibited. The two processes occur competitively and consequently, the amount of fluoride required to inhibit enamel

demineralisation is completely dependent upon the driving force for enamel demineralisation.

Margolis and Moreno (1990), as well as ten Cate and Duijsters (1983), found that using a partially saturated acetate buffer, the demineralisation of bovine enamel could be completely inhibited in the presence of 2 ppm fluoride. Arends *et al.* (1983) used a 0.1 mol/L lactic acid solution (pH 4,5) containing no pre-dissolved mineral ion constituents, estimated that a concentration of 30 ppm would be sufficient to prevent bovine enamel demineralisation. Fejerskov *et al.* (1981) suggested that the cariostatic effect provided through water fluoridation results predominantely from a topical effect.

Despite possible differences in enamel solubility, the overwhelming factor responsible for the observed reduction in caries formation is the enhanced rate of deposition of fluoridated phases within the enamel surface induced by the presence of fluoride in solution (Brudevold and McCann, 1968; Amjad and Nancollas, 1971; Larson *et al.*, 1976; Kidd *et al.*, 1980;). Incorporated amounts of fluoride did provide measurable caries protection but it is lesser affected by the concentrations of incorporated fluoride. The driving force is the reprecipitation of fluoridated apatites of the solid-solution interference in solution, which will be greatly enhanced even by relatively low concentrations of fluoride.

(3) Fluoride´s inhibition of the acid production by microorganisms:

Fluoride accumulates in plaque in much higher concentrations than in saliva (Jenkins *et al.*, 1969; Hamilton, 1977). It is, therefore, able to assist in early enamel lesion remineralisation. An alteration in plaque metabolism, particulary related to the acidogenicity, may account for the observation that white spot incidence was significantly reduced in those patients with poor oral hygiene, yet were compliant with fluoride mouthwash rinsing of 10 ml every other day (Geiger *et al.*, 1992). The effectiveness of fluoride as a cariostatic agent depends on the availability of ionic fluoride in plaque during a cariogenic challenge i.e. during acid production (Margolis and Moreno, 1990). Several researchers have found that fluoride has the potential to inhibit microbial growth and metabolic activity in dental plaque *in vivo* (Jenkins *et al.*, 1967, 1969; de Stoppelaar *et al.*, 1969; Wolley and Rickles, 1971; Bowen, 1972; Loesche *et al.*, 1973, 1975). Compared to a low fluoride level in saliva of 0.02 ppm, concentrations can range from 0-60 ppm in plaque (Hardwick and Leach, 1962) although only 2-3% of this fluoride appears to be ionized (Jenkins *et al.*, 1969). Despite these findings, a recent study by Ophaug *et al.* (1987) found that the quantity of tightly bound fluoride is much smaller than previously believed and constitutes less than 3% of the total plaque fluoride. The rest of this fluoride is thought to be bound either to inorganic components (Singer *et al.*, 1970; Birkeland and Rölla, 1972) or to bacteria (Jenkins *et al.*, 1969). A decrease in plaque pH, resulting from microbiological metabolism resulted in increased fluoride ion activity in the fluid phase (Birkeland and Charlton, 1976).

Levels of fluoride can inhibit carbohydrate metabolism by oral microorganisms by inhibiting one of the following processes alone, in combination with others or all of them together (Hamiton, 1977): (i) enolase and consequently the transport of glucose into cells, (ii) sugar translocations in membranes, (iii) cation transport and accumulation in cells and (iv) cellular phosphatase with dephosphorylate sugar-phosphates resulting from transport. Despite these findings, Carlsson *et al.*, (1969) found that fluoride (0.52-520 mM) did not change the activity of the enzyme dextransucrase which synthesizes dextran from sucrose. Dextran is regarded as an important factor for the accumulation of bacteria on the teeth (Carlsson *et al.*, 1969).

(4) Fluoride effects on tooth morphology:

Ockerse (1949), Forrest (1956) and Cooper and Ludwig (1965) have all investigated the influence of fluoride on tooth morphology. Their conclusions suggested that children who grew up in areas with a high level of fluoride in the drinking water showed significantly smaller teeth and presented shallower pits and fissures than their counterparts in non-fluoridated areas. Alteration of tooth morphology alone is not sufficient to account for the anti-caries mechanism of fluoride.

1.4 Enamel Structure.

Mature enamel is the most highly mineralised of all the tissues of the body; it covers the anatomical crown of the tooth and is the only visible part of the tooth in the oral cavity under normal physiological circumstances. The mineral component of enamel is

hydroxyapatite-like, which constitutes 95-97% of the tissue by weight (approximately 80-85% by volume). The remaining volume is taken up by a small amount of residual protein, originally from the protein matrix, and water, which fills micropores throughout the tissue. It's gross dimensions vary depending upon species and position of the tooth, in humans varying from approximately 2.5 mm at the cusp to a knife edge at the cervical margin of the crown, where it adjoins the cementum covering the root. Deciduous teeth have considerably thinner enamel covering, where the maximum is 1-1.3 mm (Huszar, 1971). Visually, enamel is described as white, although the true colour varies between deciduous and permanent teeth and also with age. The whiteness depends on a lack of translucency of the tissue, i.e. its light scattering properties (Boyde, 1989). With age, enamel becomes more translucent and thinner, thereby permitting the yellow colour of the underlying dentine to show through. The true translucency of the enamel may be seen at the incisal edge where no dentine intervenes.

The enamel, although hard and wear-resistant, is a brittle material and without the resilience of the underlying dentine, would fracture easily and disintegrate under normal occlusal loads. Surface enamel is harder and more mineralised than deeper enamel layers. The prism is the basic fundamental structural unit of enamel - all enamels, except for the very thinnest enamels. There is also varying amounts of interprismatic materials. The main inorganic components of mature dental enamel are crystals of hydroxyapatite. They average 50 nm in width and 25 nm in thickness and are extremely long, perhaps extending from dentine to the enamel surface. Most crystals have a flattened hexagonal profile. This is in great contrast to dentine,

cementum and bone where crystals are of similar stoichiometry, but are much smaller, assuming the form of flattened plates 50 by 5 nm.

Apatite itself is capable of acquiring a range of other ions by heteroionic substitution. In addition, the calcium phosphate system itself is complex and a variety of species may be present, which may include minor inorganic components. Many other ions substitute into the apatite lattice such as Pb, Zn, Sr and Al. Calcium, phosphorous, fluoride, lead and chloride concentrations all generally decrease from the surface of the enamel towards the enamel dentine junction. Conversely protein, carbonate, magnesium and sodium all tend to increase towards the dentine. All those ions have some effect on crystal properties. The amounts are often so small, however, that little observable effect has been recorded. Unlike developing enamel, the mature tissue contains very little organic matrix. In addition, the organic material in mature enamel is profoundly different from that of developing enamel, in both composition and molecular distribution.

1.5 The Caries Process.

Dental caries is commonly considered as an oral desease in which the dental tissues are attacked and may be destroyed by acid from bacteria. The desease is most prevalent in the young and can affect enamel, dentine and cementum. It develops invariably beneath the bacteria-rich dental plaque that accumulates preferrably at sites where the turnover of saliva is low or where the oral surfaces are cleaned less effectively. The most common site for an enamel lesion to occur is the molar fissure

region, which is less accessible to the cleaning action of saliva. Contact points of adjacent teeth are also areas where interproximal lesions develop beneath plaque. The existence of orthodontic or prosthetic appliances, as well as dental restorations, can attract even more plaque accumulation.

The primary cause of caries is acid, which is formed by bacteria in the plaque, as a result of carbohydrate metabolism from the diet (Figure 1.2). As a result of the production of acid, the pH of the plaque will reduce and the mineral content of the underlying dental hard tissue starts to demineralise. As soon as individual crystals start to diminish, the intercrystalline spaces are enlarged and the porosity of the enamel is enlarged. These changes are a very sensitive indicator of the initial development of the formation of a caries lesion. The porosity of the enamel can develop so far that it extends into the underlying dentine by following the tissue's microstructure (Darling, 1958; Darling, 1961; Crabb, 1966; Scott *et al.,* 1974). Removal of plaque will allow increased access for salivary fluids and prevent further destruction of the underlying enamel. The porosity of the sites that had demineralised can now be decreased by the uptake of minerals contained in the oral fluids. Normally these periods of demineralisation and remineralisation follow each other several times a day. The development of dental caries is based on the progressive dissolution of the mineral component of the dental hard tissues, resulting in structural breakdown and eventual cavitation (Creanor, 1987) if a state of imbalence exists favouring demineralisation rather than remineralisation.

1.5.1 Classification Of Dental Caries.

Dental caries does not attack any surface of a tooth randomly. Furthermore, there is a specific pattern in the way lesions are predilected to specific anatomical sites. Although dental caries has been subjected to intensive research investigations, no systematic classification scheme of international recognition has been introduced so far, apart from Black´s classification of cavitation (Black, 1914). Nevertheless, there is a commonly used classification which operates by using the three following factors:

(i) morphology

(ii) dynamics

(iii) chronology

(i) Morphology is concerned with the anatomical site of the lesion and is divided into occlusal and smooth surface caries. The first one refers to pit, fissure and occlusal surfaces and the second one to root, interproximal, cervical and gingival surfaces.

(ii) Dynamics gives an account of the severity and rate of progression of the lesion (Klein and Palmer, 1941). The severity of a lesion can be described ranging from mild, moderate, severe, very severe, rampant, incipient or arrested caries. In milder cases, usually the most early erupted, and vulnerable teeth and enamel surfaces are attacked as for example the occlusal surfaces of the first permanent molars. In moderate types, other teeth that erupted at a later stage can be involved as well as a shift to a lesion site at interproximal surfaces. Rampant cases exhibit defects on more anterior located teeth which under normal circumstances are usually less frequently attacked.

(iii) Chronology of caries is primarily concerned with age groups and patterns in which caries occurs. Children and young adults seem to be more affected by caries than other age groups.

The earliest form is infancy, soother or nursing, caries. It has to be seen in a wider context with children that present an unusual dietary history, such as a diet which is rich in sweets and sweet- or sugar-enriched teas, syrups or honey. Adolescent caries presents as problems in both the 4-8 and 11-18 age groups. Caused primarily by poor diet, poor oral hygiene and a steadily increasing consumption of carbonated, acidic drinks this age group is more affected than the rest of the population. Despite the different locations and types of caries, it is always the same aetiology which leads to the occurence of the caries lesion. The basic mechanism may be influenced by several different factors at different sites, but it usually leads to the same clinical outcome.

1.5.2 The Aetiology Of Dental Caries.

Dental caries can be described as a disease which is based on the interplay of three main factors. The basis for development is given (Figure 1.2) if the host (tooth), the agent (flora) and an appropriate environment (substrate) are combined together (Nikiforuk, 1985). As a forth factor (König, 1971; Newbrun, 1983) the dimension of time was introduced during which the other three factors work together. If one of these four factors in the model is missing, carious lesions will not develop.

- microorganisms (spec. acid producers)
- host organism (teeth with acid soluble hard tissues and retentive morphology)
- time (a long lasting decalcification time; if it is compared to the relatively short time for remineralisation of dental hard tissue)

Apart from the primary factors without which a disease process cannot develop, there are secondary or predisposing factors which control the rate of progress of a disease. Secondary factors include, for example salivary composition and flow rate, oral hygiene and diet. Each can affect the tooth´s (host) resistance to dental caries and the cariogenicity of local substrate by either increasing or reducing each of them. The interplay between the secondary factors is diagrammatically depicted in figure 1.2. Some other factors, in addition to saliva, influence the rate of caries by significantly affecting one of the primary factors. Fluoride is an example of an important trace element that affects the susceptibility resistance of mineral in enamel to the caries process and enhances remineralisation of incipient lesions. Oral hygiene and dental plaque control are other important secondary factors.

1.5.3 The Incipient Caries Lesion In Enamel.

The first discernible clinical sign of dental caries is the white spot lesion (Figure 1.1). The white colour results from the loss of translucency of the affected area. Clinically, it is observed more clearly if the surface area of the enamel is dried thoroughly. At this stage the surface of the enamel is still intact but the porosity is increased. In general, an early caries lesion in enamel is observed clinically as a white opaque spot. The

lesion area is slightly softer than the surrounding sound enamel and increases in whiteness when dried with air. The major characteristics of carious enamel can, however, be observed if a cross section of the whitish area is made. It becomes obvious then that a carious lesion is an enamel defect with a relatively intact surface layer and varying degrees of subsurface damage. It is usually not detectable on routine bitewing radiographs (Silverstone, 1977). Appelbaum (1940) found that the persistence of a radio-opaque white line on the surface of some incipient caries lesions could not be dissmissed as an artifact, but had to be considered as a significant clinical phenomenon.

Presently, there is a substantial amount of information available from various experimental techniques:

- microradiography and microdensitometry (Groeneveld *et al.*, 1974)
- polarized light experiments (Darling, 1958; Silverstone, 1968)
- microhardness data (Featherstone *et al.*, 1983)

  electron microscopy (Poole *et al.*, 1969; Haikel *et al.*, 1983; Palmara *et al.*, 1986).

These types of investigations have led to the following conclusions:

1) The surface layer covering an enamel lesion is a porous but mineral rich area being 30-40 μm deep in permanent and 20 μm in decidous teeth
2) The surface area (body of the lesion) is low in mineral (10-70 vol. %).

3) The surface morphology of the mineral lesion is slightly different for that of sound enamel.

1.5.4 The Morphological And Chemical Events Of The Caries Process.

The carious lesion in enamel has been well documented, both with clinical studies and by histological examinations of sections cut through lesions in extracted teeth. It has a number of characteristic histological features where distinct zones of change are visible (Darling, 1958; Silverstone, 1977).

The first visible sign of attack in sections of enamel is the translucent zone, so called because it becomes translucent when the lesion section is immersed in a fluid, such as chloronaphthalene which has a refractive index of 1.62 (similar to that of enamel itself). It cannot be detected by the naked eye or an x-ray (Silverstone, 1982).

The second stage is known as the positively birefringent or dark zone. This is due to the fact that whilst the lesion has lost more mineral than the translucent zone (5% as opposed to 1%), it's structure includes many extremely small pores. These are much smaller than those of the translucent zone. Such pores will not admit chloronaphtalene and therefore contain air. When viewed by transillumination, the light is refracted and reflected by this zone, resulting in the dark appearance.

The final zone is the body of the lesion. This may have lost 20 to 50% of it's mineral and it's structure contains many large pores that will admit chloronavphthalene, once more giving the enamel a translucent appearance.

The progress of enamel destruction through these zones also follows a microscopical pathway. At the earliest visible attack (i.e., the translucent zone) dissolution seems first to occur at the prism boundaries and then progress to the prism interior via the cross striations. Both microscopic and macroscopic changes in enamel associated with the carious lesion also appear to be linked to specific chemical changes as described in the following section.

Hallsworth *et al.* (1972, 1973) and Robinson *et al.* (1981, 1983) dissected out and chemically analyzed the different histological zones of the enamel lesion described in the previous section. They found that in the translucent zone, the enamel had lost 1 to 2% of its mineral content, a figure that agrees well with previous estimates of mineral loss based on birefringence measurements (Darling, 1958). These had indicated that in the translucent zone the 0.1% per unit volume porosity of the sound enamel had increased to about 1% per unit volume. Birefringence measurements are not the most reliable guide to mineral content, however, and give no definitive information about the chemical nature of the material removed (Theuns *et al.*, 1993). However, since fully mineralised enamel consists of at least 97% mineral, a 1% increase in porosity probably closely approximates to a 1% loss of mineral. Calculations based on direct chemical analysis of both sound and carious tissue showed that the 1% of mineral lost from the translucent zone contains about 28% carbonate and 2% magnesium

(Robinson, 1983) (Table 1, Fig.3). Since sound enamel contains only 2 to 4% carbonate and approximately 0.1 to 0.4% magnesium, this fraction of mineral removed is not representative of bulk enamel and is certainly not apatitic.

It probably corresponds to either:

a) a fraction of enamel residing at a new crystal surface, or

b) a selective dissolution of magnesium- and carbonate-rich crystals, or

c) a preferential dissolution of a seperate, perhaps non-crystalline universal phase-

such as that suggested by Bachara *et al.* (1964). The identity and precise location of this acid soluble fraction has still not been established. However, according to histological evidence, the first enamel removed during caries attack comes from the prism periphery. It seems likely therefore that this magnesium- and carbonate rich, acid-soluble fraction of enamel is largely located in this region.

1.6 Detection And Quantification Of White Spot Lesions.

The importance of detecting caries lesions at a very early stage is important to allow the use of preventive measures rather than restorative ones. It is in the best interest of the patient to preserve the tooth substance in its natural composition rather than use restorative materials which can never match exactely the natural tooth substance in quality and longevity.

Dental caries exists around the world, but the prevalence and severity varies in different populations and fluctuates with time (World Health Organisation, 1979; van de Rijke *et al.*, 1990).

The prevalence of dental caries has declined substantially in several countries of the western world, some of which had no organised preventive programs of water flouridation (Truin *et* al., 1981; Glass, 1981, 1982; Kalsbeek *et al.*, 1982; Thylstrup *et al.*, 1982). This has consequently influenced both clinical research and investigations (Alman, 1982; Koenig, 1982). A substantial need arises, therefore, to develop methods which are highly effective in early caries prevention and detection. The new methods should ideally give better reproducibility in caries detection. Repeatability is becoming more important also with the ever decreasing number of carious lesions. Additionally, training and calibration of examiners may be facilitated. An easier and more reliable method of detecting incipient lesions which have not yet developed into cavities is essential (Fishman, 1984). A permanent form of record keeping of the pathology at a given point in time would make documentation easier for the comparison of the status at different examination times. Radiographs/photographs give a good example of record keeping of this kind. For future caries studies, the measurement of small changes in tooth mineral content will be required.

Quantitative analysis of changes in mineral content in a single caries lesion would be desirable (ten Bosch and Manson, 1991). The amount of mineral loss with the severity of the lesion and its associated changes. *In vitro* and *in vivo* investigations face the same problem that different parts of the same tooth have varying predispositions to

carious attack (Groot *et al.*, 1986). The intra- and inter-tooth variations are important considerations when analysing statistical data (ten Bosch and Boersboom, 1984; Creanor *et al.*, 1990; MacPherson *et al.*, 1991). The area and site under investigation should be registered on specially marked points that allow a second reading to be taken at any later point of time in the same location as the previous ones.

1.7 Quantitative Techniques.

Most clinical methods which are currently in use cannot give a definite diagnosis of a caries lesion until it has reached an advanced state of demineralisation. The procedure should be reliable, allowing the detection the carious lesion at its earliest stage, permit the separation of reversible from irreversible lesion, and permit documentation by photographs, radiographs, or similar recording methods (Fishman, 1989).

Quantitative measuring methods for surface enamel mineralisation changes are either destructive or non-destructive. The problem with most of the techniques used at present is that they are destructive and allow only one measurement at a single time point. Once they have been performed the sample cannot be reanalysed using a different method.

Some of the most commonly used methods for assessment are conventional microradiography (Angmar *et al.*, 1963; Groeneveld, 1974; Theuns *et al.*, 1980; de Josslin de Jong *et al.*, 1987), microhardness tests (Davidson *et al.*, 1974; Koulourides *et al.*, 1974; Arends *et al.*, 1979; Featherstone *et al.*, 1983), polarising light

microscopy (Carlstrom, 1964; Gwinnett, 1966; Shellis and Poole, 1985) chemical analysis (Hallsworth *et al.*, 1972; Sakkab *et al.*, 1984; Weatherell *et al.*, 1985) and scanning electron microscopy/electron probe (Haikel *et al.*, 1983; Fejerskov *et al.*, 1984; Holmen *et al.*, 1985).

Featherstone and Silverstone (1982) developed the single section technique which used acceptable caries-like lesions in undemineralised sections of teeth instead of whole teeth. Test sites were selected on 80 μm thick sections of sound human teeth and microradiographs taken. The technique can be applied by using either sections of up to 200-300 μm cut perpendicular (transversally) to the tooth surface (Buskes *et al.*, 1987) or longitudinal (parallel) cut. The longitudinal sections have been used by Creanor *et al.* (1986) for caries de- and remineralisation investigartions with an *´in situ´* appliance. The advantage of this technique, in addition to the short exposure times of caries attack, is that the sound enamel can be examined in detail prior to creating a lesion over that same site. Also, after initiation of a lesion, its progression can be followed at short intervals of time (Featherstone and Silverstone, 1982). On the other hand it gives only observation over very small tooth volumes which can influence the results of destructive methods because of the existing variations inside an enamel lesion (ten Bosch *et al.*, 1984). Non-destructive methods will improve this situation by being applicable not only *in vitro* but also *in vivo* which will also provide a wider range of diagnostic tools for the clinician as well as the researcher. Research will benefit from the possibility of using longitudinal studies allowing samples to be examined by different methods which then could be compared directly to each other on the basis of having used the same research material. For this purpose optical

methods seem to be most appropriate (Spitzer *et al.*, 1975; Borsboom *et al.*, 1982; Brinkman *et al.* 1983). The changes in the normal enamel surface leading to different stages of demineralisation and later on cavitation can be assessed by visual inspection (Gorelick *et al*, 1982) or by the use of a dissecting microscope at 20 times magnification (von der Fehr, 1961).

The extensive range of different methods with all their accompanying advantages and disadvantages precludes exclusive recommendation of any one particular method. Unfortunately, only very small numbers, of usually not more than two, are being used in one and the same research institute according to ten Bosch and Angmar-Mansson (1991). Another great obstacle is that most of these Institutes are employing different types of caries models, making it more difficult to compare the results of their work with others. To achieve a more standardised research program, where the results are exchangable with other institutes has been suggested by Featherstone (1986). That program should envolve comparable studies, between different methods in a single institute and between different institutes with the same method.

1.7.1 Visual Assessment Scoring Systems.

Subjective indices can be useful and measure the severity of a disease state when more objective methods are either unavailable or inappropriate (von der Fehr, 1961). They are relatively inexpensive, rapid and easy to use although a disadvantage would be greater inter-examiner variability. However, with appropriate criteria, this can be a valuable and rapid technique. In the von der Fehr Caries Index (Appendix B),

variations in the enamel appearance were given a numerical score. These grades have already been shown to be related to the extent of subsurface demineralisation in an *in vitro* study (von der Fehr, 1966). When the enamel surface appears intact, the score is `0´. When the enamel surface shows limited greyish tinge, with or without accentuated perikymata, the score is `1´.When the perikymata is well accentuated, with some areas confluencing into greyish-white spots, a score of `2´ is given. Pronounced white decalcification is given score `3´. Lesions that did not fit definitely into the previously described categories can be given intermediate scores (0.5; 1.5, or 2.5). The von der Fehr Caries Index will be employed in this study as a means of comparison of various methods. This technique will be described in more detail in Chapter 2.

Gorelick *et al.* (1982) proposed another scoring system which was a modification of the Caries Index of von der Fehr for the assessment of white spot lesions. In this index, the vestibular enamel surface area outside the area covered by the bracket was divided into thirds. When the white spot lesion involved less than one third of the investigated area, a score of `1´ was given. A score of `2´ involved more than one third but less than two thirds of the assessed area. Score `3´ was selected when more than two thirds of the area was covered. When no sign of a white spot lesion was detectable, the score is `0´. This scoring system can be used if the investigation is related solely to orthodontic fixed appliance therapy.

1.7.2 Quantitative Microradiography/Microdensitometry.

The estimation of tissue porosity in the laboratory, as well as in the clinic, is a very important method of obtaining relevant information on limited loss of mineral. Of immence importance for the assessment of mineral loss, is the use of x-rays. In the laboratory they are used on the basis of a microradiographic technique which was developed by Angmar *et al.* (1963) as transverse microradiography. The technique had been originally introduced by Thewlis (1940) who was the first to use x-rays to assess tooth structure. Microradiography relies on the principle that monochromatic x-rays (Cu Kα x-ray radiation) are absorbed in a longitudinal tooth section of 100-300 μm thickness. The degree of x-ray absorption in the tooth sample is calibrated against an aluminium step wedge (Figure 2.25) of known thickness, which are both mounted on a photographic film or plate (de Josselin de Jong *et al.*, 1988). The optical film transmission is measured with a circular microdensitometer window. The calcium content of the tooth is measured as the volume percent mineral using the equation of Angmar *et al.* (1963) or a modification to this calculation proposed by de Josselin de Jong *et al.* (1987).

Although quantitative microradiography has improved considerably over the years and is widely used (Creanor and Strang, 1989) the method is considered less efficient for measuring early mineral loss in enamel as compared to techniques aimed at measuring the corresponding changes in size of the intercrystallite spaces, which can be estimated using, for example, polarized light microscopy. Although microradiography gives only a quantitative measure of the amount mineral in a specimen,

microradiography does not differentiate between the quantitative forms of mineral within the enamel lesion. It is, however, a simple, accurate and reproducible technique (Featherstone *et al.*, 1983; Creanor, 1987).

1.7.3 Microhardness.

The visible evidence of histological heterogenety is also reflected in differences in the mineral content from site to site. Microhardness is one of several ways to prove that mineral content increases from dentine towards the enamel surface in an approximately incremental fashion, suggesting an intrinsic developmental pattern. The technique involves a `Knoop´ or `Vickers´ diamond indenter on a `Leitz´ miniload tester which is used under a given load of 15-20 gm or more over a given period of time which is usually in the range of 10 sec. The diamond indentation covers between 5 and 20 enamel prisms. For the length of each of the diamond indentations, the `Knoop´ hardness values are calculated at regular intervals throughout the lesion and into the underlying `normal´ enamel.

Two different types of measurement technique are possible. In one type the indenter load is perpendicular to the polished enamel surface (type I) giving greater accuracy; in the second, the indenter load is parallel to the anatomical enamel surface (type II). The distribution of the measurement sites over the sample is a matter of intense discussion. There are divided opinions as to whether the application of the technique over the whole sample or only on cross sections should be used for assessment of microhardness (Featherstone *et al.*, 1983; Creanor, 1987). The microhardness

technique does not give, however, a quantitative assessment of the mineral content in an investigated area, but the difference in penetration between the initial and final thickness of the enamel layer that has been affected by de- or remineralisation (Koulourides, 1968; Feagin *et al.*, 1969; Purdell-Lewis *et al.*, 1976; Creanor, 1987). This type of technique is influenced greatly by operator variation and is both labour and time intensive. It belongs to a group of destructive investigation methods (Arends *et al.*, 1980).

1.7.4 Polarising Microscopy.

In 1861, Valentin was the first to use polarised microscopy for investigations on dental enamel (Carlström, 1964). Polarised microscopy is based on the construction principle of an ordinary light microscope. Due to their close relationship, polarising light microscopes cannot achieve a greater resolution than ordinary light microscopes. The ultimate limit of a light microscope when using a sodium light (λ=5893 Å), central illumination, and oil-immersion objective having a numerical aperture of 1.30 is around 0.5 μ. (Carlström, 1964). To study minute details, the sections must be extremely thin, preferably not thicker than the diameter of a single prism. This is usually very difficult to achieve under practical circumstances. Polarising microscopy is used, in addition to plane transmitted light, to differentiate the various zones in an enamel lesion. The method can be used for a qualitative, as well as quantitative assessment, giving information about the submicroscopic structure.

Polarised light demonstrates three components of birefringence in dental enamel. The first is the intrinsic birefringence of the apatite crystallites which is negative when related to prism length. The second is intrinsic birefringence which is made up by the organic matrix. Lastly, there is a birefringence which is caused by liquids or gases with a refractive index different from that one of the enamel itself (Darling, 1956).

The term birefringence comes from the phenomenon which is typical of biological structures and non-cubic crystal enamel. Such specimens can split a beam of plane polarised light into two rays of different velocities. Birefringent structures have two different refractive indices, related to the two planes of transmission within the crystal. A negative intrinsic birefringence in enamel is due to its orientated crystal component and a positive value (value=1.62) is caused by small orientated pores (Damato, 1990). The differences between two planes of light in enamel can be related to differences in pore volume.

Polarised light microscopy can prove an increase in pore volume during the carious process (Creanor, 1987). In non-cubic crystals, a sign of birefringence is given to the structure, determined by the path taken by the slower (+) and faster (-) rays in relation to the morphology of the crystal. In an enamel prism, the slower ray is found to vibrate at right angles to the length of the prism, and therfore the sign of birefringence of the organic component is positive with respect to prism length. Apart from the mineral and organic constituents of enamel, there are minute spaces in the tissue. In the case of a carious lesion, the volume of spaces in enamel increases. Adding these spaces or pores to the volume an additional type of birefringence rises.

Polarised light microscopy is used widely as a quick and non-destructive technique for the quantitative study of mineral distribution in carious enamel giving useful ultrastructural details (Silverstone, 1966, 1968; Kidd, 1983). It does ignore, however, possible effects of recipitated mineral. Other methods are required for interpretetation to permit pore volume to be assessed accurately (Creanor, 1987; Shellis and Poole, 1987).

1.7.5 Chemical Analysis.

This evaluation is based on direct chemical analysis of both sound and carious tissue showing that 1% of mineral loss from the translucent zone contains about 28% carbonate and 2% magnesium (Gray and Francis, 1963). Since sound enamel contains 2-4 % carbonate and approximately 0.1-0.4% magnesium, this first fraction of mineral removed is not representative of bulk enamel and is not apatite. It probably corresponds to either (a) a fraction of enamel residing at or near crystal surfaces, or (b) a selective dissolution of magnesium - and carbonate - rich crystals, or (c) a preferential dissolution of a seperate, perhaps non crystalline mineral phase such as that suggested by Bachra *et al.* (1964).

Enamel biopsies or microsamples may be collected by the techniques of acid etching (Aasenden, 1972; Retief *et al.*, 1980, 1983), microdissection (Hallsworth, 1972), microdrilling (Sakkab *et al.*, 1984) and abrasion (Weatherell, 1985). The evaluation of these methods is directly linked with chemical analysis. Its purpose can be either the

assessment of fluoride, or the evaluation of the concentration of other elements such as minerals like calcium, phosphate and magnesium (Hallsworth, 1972).

1.7.6 Scanning Electron Microscopy/Electroprobe.

The scanning electron microscopy (SEM) can be applied with or without the use of an electroprobe. The SEM is used for a more precise assessment of the ultrastructural detail of a lesion especially with regard to the surface layers of incipient white and brown spots, with no evidence of cavitation (Haikel *et al.*, 1983; Fejerskov *et al.*, 1984; Holmen *et al.*, 1985).

The preparation of samples is carried out by coating the specimen with a ~90 Å thick layer of coated gold and examined in a stable vacuum at 10^{-6}Torr (Holmen *et al.*, 1985). As a result of the coating process, SEM is considered a destructive method and the technique is restricted as a quantitative method. The advantage of SEM over techniques which are based on normal light microscopy is that it can work beyond the limits of the restriction of visible light (Creanor, 1987) at a much higher rate of magnification. This allows an assessment in areas of apparently intact enamel that exhibit focal holes and a prismatic pattern of destruction or irregular type of destruction or irregular type of destruction that lies beyond the resolution of conventional microscopy (Haikel, 1983).

Ingram and Fejerskov (1986) used scanning electron microradiographs to investigate early stuctural changes in human enamel during an artificial carious attack in an *in*

vitro study. Their results showed no significant difference between the experimental and control areas of the same teeth. The conclusion that the surface morphology of carious and sound enamel had no measurable differences was influenced greatly by the large variation in surface structures between the specimens.

The use of SEM in combination with an electroprobe operates works by bombarding the sample with a particle beam, to induce the release of radiation or particles. These are used for an assessment by describing the function of the energy of the bombarding articles. Electrons, light ions (H^+, He^{2+}), or heavy ions may be used for bombarding; measured quantities can be the energy and/or flux of emitted x-rays, secondary electrons, or ions released from the sample. For those investigations the sample has always to be examined under vacuum conditions (Lindh and Treit, 1980; Frank and Leach, 1982; Crawford and de Bruin, 1983; Halls *et al.*, 1988; Rentsch *et al.*, 1990). These techniques are based on the same principle. They are very sensitive and use particle counting. Some of them have a resolution of up to 1μm or less, but as a quantitative method, they produce concentration ratios only, because their ability to penetrate the material is dependent on its density.

The method is used, in general, for the measurement of concentrations of trace elements. The relationship of calcium to phosphate in a lesion can be evaluated by an electron probe but due to its inability to assess the amount of hydrogen or oxygen, absolute concentrations of Ca or P cannot be established (ten Bosch and Angmar Mansson, 1991).

1.7.7 Iodine Absorptiometry.

Henrijkson and Linden (1974) introduced this method, which is based on the absorption of monochromatic radiation from a radioactive source. The method can be applied to longitudinal studies of enamel demineralisation. A colliminated beam of Sn-filtered radiation from an ^{125}I source is set through a ground or cut tooth section, perpendicularly to the surface where demineralisation has occured. The flux of transmitted radiation is measured as the number of quanta transmitted in a certain time. The transmission is then calculated as the quotient of this number, and the number of quanta is recorded without any absorbing sample. The mass per unit area of the mineral in the section is calculated according to the attenuation formula for collimated radiation. The method has recently been applied to *in vitro* studies of enamel mineral loss (ten Bosch *et al.*, 1988) and studies of *in vitro* root surface caries (Almqvist *et al.*, 1988). It can also measure loss due to etching. This method is of great help if accurate volumes are needed because of its almost complete monochromatic radiation and accurately known absorption coefficients.

1.7.8 Iodide Penetration/Permeability.

The iodide penetration method evalutes the corresponding increase in enamel porosity of an intra-oral sample and was developed by Brudevold *et al.* (1984) and improved by others (Zero *et al.*, 1990). It involves the penetration of iodide into the carious lesion. The iodide is then recollected by a backward diffusion into distilled water where it is mesured with an iodide-specific electrode. Several studies by Brudevold

(1984) and Khasket (1989) have demonstrated that the method is suitable for studying the effects of different kinds of foods on enamel porosity in very short experiments. Pilot studies showed that the iodide did not bind to enamel. A moderate correlation between a modified form of the iodine permeability test, surface microhardness and mineral dissolution following an acid challenge was demonstrated by Zero *et al.* (1990). Further study on the time-dependence of the iodide uptake and/or the back-diffusion of iodide into the water droplet may shed light on the relation of Δ Iodide penetration to enamel or lesion parameters and may extent the applicability of the method. Zero *et al.* (1990) showed a moderate correlation between a modified form of the iodide permeability test, surface microhardness and mineral dissolution following acid challenge. the iodine permeability test has recently been used by Zero *et al.* (1992) as a rapid intra-oral model to assess plaque/hard tissue interactions. Arends and ten Bosch (1992) concluded that the test may be affected by surface zone pore blockage, especially if used *in vivo* or *in situ*.

1.7.9 Longitudinal Microadiography.

This technique measures the x-ray absorption of a single section of tooth material with ~0.3 mm thickness (de Josselin de Jong *et al.*, 1987). Assessments of the material are made before and after exposure to the experimental environment (Hall, 1994). The x-ray beam is in a perpendicular position to the de- or remineralisation surface, and the film is parallel to the surface. After film development, two dimensional microdensitometer scans are made of the respective images of the same slice and step wedge at the de- and remineralisation stages. The x-ray images, obtained from pre-

and post-experimental stages, show changes in mineral content. In the case of demineralisation, the loss of mineral concentration can be given in kg/cm^3 and the location of mineral change within the lesion can be described by integration (ten Bosch and Angmar Mansson, 1991). According to de Josselin de Jong (1988) the method is suitable for the detection of unhomogenous tooth mineral changes and can follow local intra-tooth variations of de- and remineralising tooth samples. It does not, however, distinguish qualitatively between different types of lesions, measuring cavities with the same accuracy as porous lesions. A systematic error of 20% was found when compared directly with chemical analysis (de Josslin de Jong *et al.*, 1988).

1.7.10 Optical Methods.

There have been numerous attempts to improve the traditional methods of caries lesion detection (Bibby and Shern, 1978; Rawls and Owen, 1978; Marthaler, 1984). New methods of detection which have been introduced recently and require visual observation of an optical signal. Marthaler (1984) recommended that there should be continuation of a new method development and evaluation so that ultimately a procedure should be:

(1) reliable,

(2) permiting detection of caries lesion at its earliest stage,

(3) permiting the separation of reversible from irreversible lesions, and

(4) able to be documented by photographs, radiographs, or similar recording methods.

The physical properties of the carious lesion are the fundamental basis for the detection and quantification of caries by means of optical methods.

The currently used optical methods in research investigations are as follows:

(1) Fibre Optic Transillumination, FOTI: This technique works on the basis that caries shows a lower index of light transmission than sound tooth structure, and the area of decay is seen as a dark spot when compared with the surrounding sound stucture.

(2) Ultraviolet Light: This is used to increase the optical contrast between the carious region and the surrounding sound tissue. The natural flourescence of tooth enamel, as seen under UV illumination is decreased in areas where the mineral content has been reduced, such as caries lesions, artificial demineralisation, or developmental defects (Naleway *et al.*, 1979; Alfano and Yao, 1981).

(3) Fluorescent Dye Uptake: fluorescent and non-fluorescent dyes have been used to stain porous carious lesions to enhance the contrast between the carious region and the surrounding sound enamel (Rawls *et al.*, 1978; Marthaler, 1984; van de Rijke *et al.*, 1991).

(4) Laser Fluorescence Method: distinguishes areas of sound tissue from carious tooth material by contrast in the fluorescence of the blue-green region of the visible light spectrum (Bjelhagen and Sundström, 1982).

(5) Light Scattering Method: is based on the scattering of a narrow pencil light beam when it is incident on a thick layer of a turbid material (enamel). Photons entering the material are scattered (Figure 1.3) and absorbed, according to the relationship between enamel crystals and their surrounding environment (Borsboom and ten Bosch, 1982). In this study the `SENSOPTIC´ light scattering monitor was used.

1.8 The Light Scattering Caries Monitor.

The oldest and simplest model in the history of light scattering is the two flux model (Kubelka, 1948). This is described as only a forward and backward light flux and from where developed the basic assumption that incident radiation is diffuse. A general theory describing scattering and absorption in turbid materials is the radiative transfer theory formulated by Chandrasekhar (1960). Later, Klier (1972) showed that the two flux equations were formally identical, with a solution of the general Chandrasekhar equation for isotropically highly scattering media with relatively low absorption. He found that the absorption and scattering corfficients, as obtained by the two theories, were related through numerical coefficients, which depended upon the absorption -

scattering ratio. Spitzer and ten Bosch (1975) used the technique in a comparison of bovine and human enamel with thin slabs in sound and white spot areas.

Ten Bosch *et al.* (1980) introduced the use of a microscope to relate the amount of optical scattering by the lesion to assess the changes in mineral content. Groenhuis *et al.* (1981) developed a method for the quantitative measurement of scattering and absorption coefficients of bulk material by determining the backscattered light (Figure 1.3) as a function of distance from an incident narrow pencil beam of light. Based on this, Borsboom and ten Bosch (1982) developed a minituarised caries monitor for the investigation of artificial acries lesion using a polychromatic (white) light source. Later, a green (λ=0,56 μm) light-emitting diode (LED) was used successfully and applied in this project. (SENSOPTIC, Stedum, The Netherlands). The Sensoptic probe works by scattering of light in the sample material.

Incipient lesions appear whiter than the surrounding sound enamel because of the strong scattering of light within the lesion. Presumably, this is due primarily to the fact that the remaining small universal particles in the lesion are embedded in water rather than mineral rich sound enamel (Angmar Mansson and ten Bosch, 1987). This stronger scattering can be quantified with methods based on optical fibre technology (Figure 1.4). Angmar Mansson and ten Bosch (1987) found a positive correlation between the readings of a caries monitor and the determination of mineral content by microradiography and microdensitometry using bovine enamel. Comparisons for chemical and microradiographical determination of calcium loss in early lesions showed good correlation with the optical monitor (ten Bosch and Angmar Mansson,

1984). The method is non-destructive, allowing repeatable measurements in the same area, and can be used theoretically both *in vitro* as well as *in vivo*. The instrument is not yet developed for routine clinical application, but can be used intraorally, if adequate electrical isolation between the optical probe and the main cabinet of the instument is provided (Brinkman *et al.*, 1988).

The basic principle of the method is shown in Figure 1.3 and 1.4 The head of the probe is simply brought into maximum contact with the tooth surface and held in a 90° position, perpendicular to the controlled area (Figure 2.11). In this position,it is held until a reading value has been registered. Photons that are emitted into the investigated area enter the material and are either scattered or absorbed. If the photons are scattered they spread sideways to a distance which is proportional to the mean free proton path length. Carious lesion areas contain more water than sound areas, making differences between particles and their environment larger in the sound one. This results in a shorter path length for lesion areas with less probability of absorption. Therefore, sound enamel posseses a higher probability of unscattered transmission than to carious sites.

1.9 Comparison Of Bovine And Human Enamel.

Sound human teeth, especially upper anteriors, are increasingly difficult to obtain. Criteria for reproducibility in experimental models have become more stringent, leading to larger sample numbers being required at a time when fewer teeth are being extracted. Consequently, investigators have turned to teeth from other mammalian

species to provide quantities of standardized material for their studies. Bovine, ovine or equine enamel specimens, for example, have been used in studies of incipient carious lesions (Pearse, 1983), rates of progress of artificial carious lesions (Featherstone and Mellberg, 1981), microhardness and lesion depth studies (Arends and Schuthof, 1980), and in studies of adhesion of resins to enamel surfaces (Nakabayashi *et al.*, 1982; Nakamichi *et al.*, 1983). In addition, a number of studies on the organic and inorganic components of these animals have been carried out (Shearer *et al.*, 1980; Finchham *et al.*, 1983), and physical properties following etching (Groenhuis *et al.*, 1980) or abrasion (Putt *et al.*, 1980; Roulet and Roulet-Mehrens, 1982) have been investigated.

Boyde (1965) highlighted that the arrangement of prisms was different in bovine and human enamel, and Arends and Jongelbloed (1978) reported that crystallites in the former were thicker. Whittaker *et al.* (1982) noted that prism dimensions, width of the interprismatic zone, and prism arrangement were different in bovine enamel compared to human enamel, but detailed measurements were not recorded. Gantt *et al.* (1985), using their microdissection technique, showed that bovine enamel had marked decussation of prisms in their inner third, narrower prisms, thicker and longer crystallites, and was more porous than human enamel. Spitzer and ten Bosch (1975) found no important differences between the refractive indices or anisotropy of human and bovine enamel samples. A more important difference was found, however, when absorption coefficients were compared. Bovine enamel absorption coefficients were about three time larger, in the region of 250-300 nm, as in human enamel. They suggested, therefore, a substantial difference in the composition of those two calcified

tissues in the investigated part of the enamel layer. Ten Cate and Duijsters (1983) found that bovine enamel demineralisation could be completely inhibited in the presence of 2 ppm fluoride.

No detailed studies appear to have been carried out on equine or ovine enamel. It is known that the rates of progression of gelatin/lactic acid lesions in bovine and ovine enamels are about three times those of human teeth (Featherstone and Mellberg, 1981), and although it seems probable that ultrastructural differences may account for these findings, the results of such studies on artificial lesions in enamel from different species are not available.

1.10 Objectives Of The Study.

1. To determine the degree of reproducibility and repeatability of the optical caries monitor in measuring the amount of tooth surface mineralisation, if normal mineralised tissue is compared to demineralised tissue *in vitro*.

2. To correlate the results produced by the optical caries monitor with conventionally used methods such as microradiography and microdensitometry.

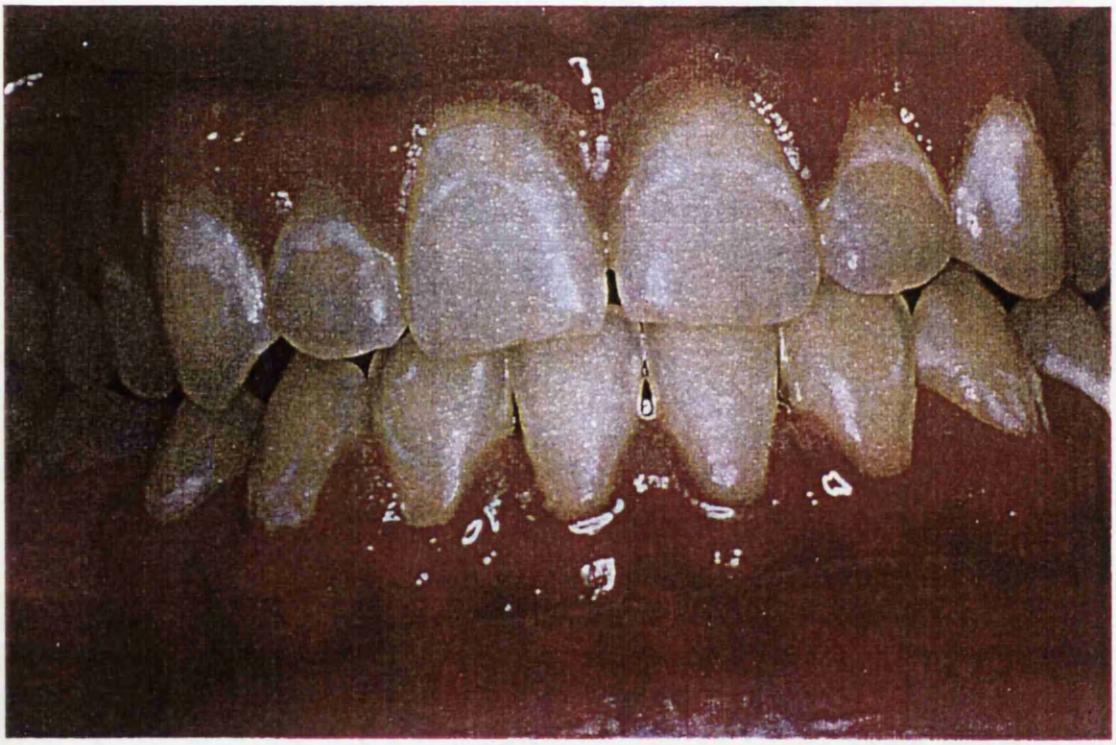


Figure 1.1 Intra-oral view of a patient after orthodontic treatment with fixed appliances, representing areas of white spot lesions especially around the base of the brackets towards the gingival margin.

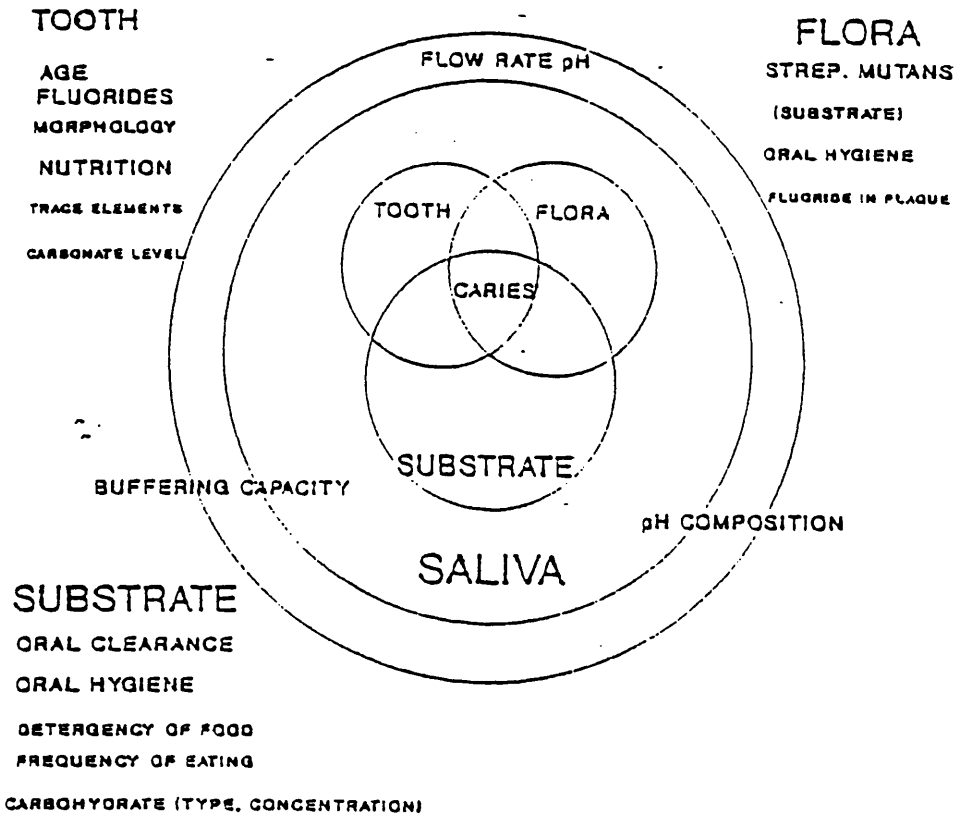
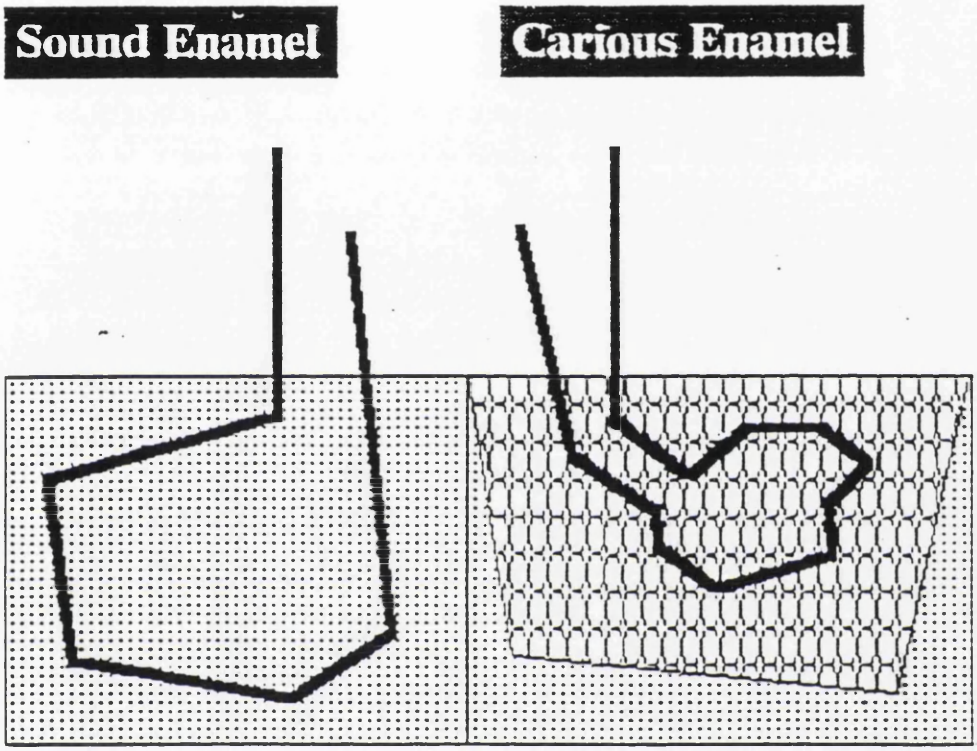


Figure 1.2 Graphic representation of the factors and processes involved in the interactions of different stages of the caries process.

Light Scattering in Enamel



There is more water in a lesion than in sound enamel. The differences between particles and their environment are much greater than in sound enamel. Therefore, in a lesion, the average free path length of a photon is shorter, the total path length is shorter, the probability of absorption is less, and the probability of unscattered transmission (transparency) is less, compared with those of sound enamel.

Figure 1.3 Physical principles of the light scattering optical caries monitor.

Fibre Optic Measuring Head

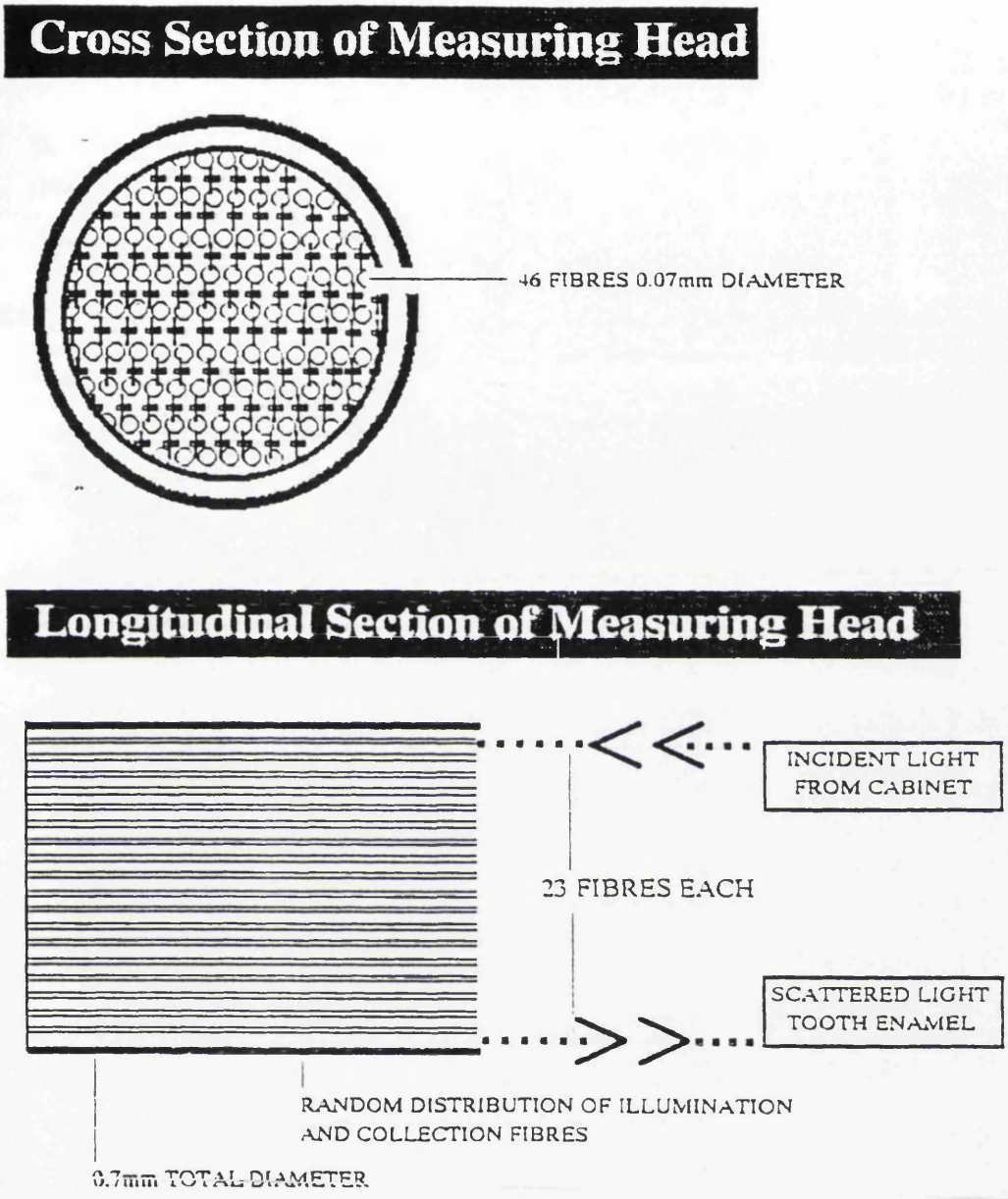


Figure 1.4 Internal view of the structure of the fibre optic measuring head. Each bundle of glass fibres is divided into two further bundles in a random distribution. The first of the two bundles serves for illumination of the investigated surface of the tooth, the second recollects the intensity of the reflected light, which is measured by a photo diode in the head.

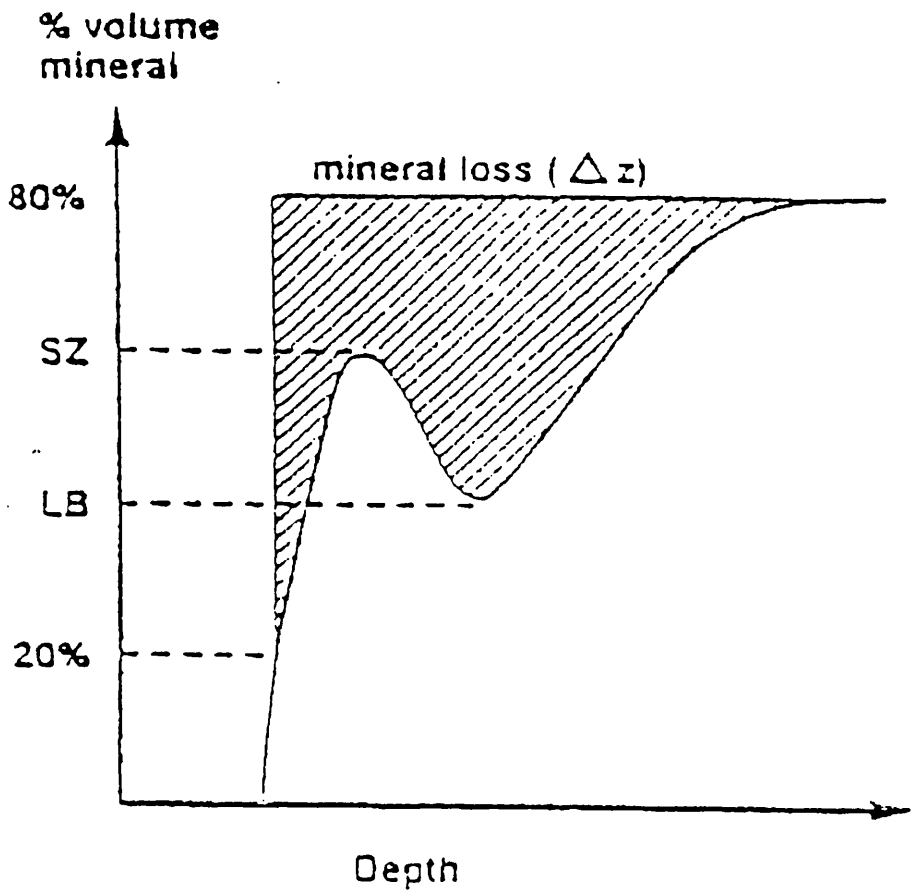


Figure 1.5 An idealised microdensitometric reading, representing the standard parameters given for evaluation of the lesion.

SZ = per cent volume mineral content of the surface zone

LB = per cent volume mineral content of the lesion body

Δz = represents the area which is affected by mineral loss

CHAPTER 2

MATERIALS AND METHODS

2.1 Introduction.

In this Chapter a detailed description of the materials and techniques used during the study will be given. The main appliance which was used to carry out the primary technique in this study was the `SENSOPTIC´optical caries monitor (Figure 2.9). Its reliability and reproducibility for an assessment of changes in enamel surface demineralisation in white spot areas was investigated. The *in vitro* sample of bovine incisors showed white spot lesions of different severity and grades within the same tooth and examination band. After the measurements with the caries monitor had been performed, the samples were subjected to a visual assessment scoring system established by von der Fehr (1961). The changes in the mineral tissue will be evaluated by the method of microradiography and microdensitometry.

2.2 The Optical Caries Monitor.

The `Sensoptic´ optical caries monitor (Sensoptic B.V., Stendum, Netherlands) consists of a larger table based electronic unit that is connected via an optical cable to an optical measuring head (Figure 2.9). This measuring head consists of an optical and an electronic component. The hand held probe is equipped with an `optical needle´ (Figure 2.11) with a flat end. The optical needle is contained within a stainless steel tube in which a bundle of around 40 optical glass fibres (Figure

1.4), with a diameter of 50 μm, is contained (Angmar Mansson and ten Bosch, 1987). The light produced by an LED (560 nm) is coupled with these optical illumination fibres, type `FIBREFLEX´, TBL. The illumination fibres are mixed with detection fibres. The assembled fibre bunch is such that there is a random distribution of illumination and detection fibres at the measuring pin end. The average centre distance from the illumination fibre to the detection fiber is 80 μm. The detection fibres are coupled to a highly sensitive light detector.

The detector is coupled to an isolating capacitor and next the measuring signal is amplified by an adjustable Op-Amp amplifier. The amplification is trimmed by the potentiometer positioned on the back of the measuring head. The amplification to be set depends on the amplification and the calibration procedure. Trimming of the amplifier will only be required, if the luminous intensity of the LED decreases sharply. The end of the needle is ground flat and optically polished. The one metre long optical cable at the other end of the measuring head is illuminated without filters, by a halogen incandescent lamp (12V, 50W) in a lamp cabinet. The radiative power emerging from the cable is in the order of a few tens of milliwatts. The light is mechanically interupted at 1000Hz to distinguish instrument light from ambient light. The other bundle terminates inside the handpiece on the sensitive surface of a photodiode with a preamplifier. This is electrically connected to the main amplifier and supply unit in the lamp cabinet. Output voltages are amplified synchronously to the interuption frequency and recorded on a strip chart recorder. These voltages are proportional to the intensity of the backscattered light.

2.2.1 Calibration.

Every time the optical caries monitor is switched on a new calibration has to be performed. This procedure is needed to receive standardized results that can be compared to results of previous trials, because fluctuations in lamp output, detector efficiency, fibre loss, current aberations, etc. can influence and bias results.

The caries monitor is calibrated by wet measuring four optically stable plastics (Figure 2.10) with known scattering coefficients. For the purpose of calibration the measuring pin surface is enlarged with the help of a perspex attachment with a locating pin. The measuring surface is placed perpendicularly on the wet calibration plastics. In this investigation the procedure was modified. It was detected that on different areas within one stable plastic area, different readings could be made at different points of the same field. Therefore, a grid (Figure 2.10) was developed with nine predetermined measuring sites for each calibration field. One mean value for each field was calculated out of the nine measurements for each of the four plastics(Appendix H). Using this grid at each calibration it was clear that for each calibration procedure the same reference points were being used. The backward scattering was measured at 560 nm. Plastics used for calibration were type ABS, (Borg and Warner Chemicals) acryl-butyl-sulphonamide, with TiO_2 added, 0; 0.1; 0.3; 1% respectively.

The scattering coefficients which are related to these four values at 560 nm are 1.3; 3.3; 4.8; 16.5 mm^{-1} respectively. The mean values out of the calibration for each of

the four fields where put into a BBC-computer (Figure 2.13) which plotted them on the Y-axis of a log-log scale graph against the four constant values plotted along the X-axis. The BBC-computer programme evaluated the most suitable straight line through these points (Appendix A). During the entire duration of the experiments, the voltage readings given by the readouts on the digital panel meter window were plotted on the graph related to their individual calibration. This procedure allowed the conversion of the readings into their back scattered intensity allowing a comparison between all the readings (Table 3.1).

2.2.2 Optical Coupling Between Probe And Tooth.

The wet probe is simply brought into contact with the tooth or lesion, perpendicular to the surface (Figure 2.11), and held in that position until a recording of sufficient duration is made. Water is used to obtain a good and reproducible optical coupling. Pellicle and small amounts of plaque appear to have no influence on the reading. Therefore, tooth brushing before the measurement is adequate for tooth cleansing.

2.3 Microradiography/Microdensitometry.

2.3.1 Introduction.

Transverse Microradiography, originating from Thewlis (1940), is based on the principle of measuring the absorption of monochromatic x-rays by a tooth section,

compared with absorption by a simultaneously exposed standard. The amount of absorption of x-rays by dental hard tissues and enamel in particular, depends on the mineral content. Mineralised enamel will absorb more x-rays, leading to a white image on the x-ray film, whereas demineralised areas of enamel will absorb a lesser amount of x-ray radiation, leaving a darker image on the x-ray film compared to the mineral rich normal areas (Figure 2.24). This relationship will be represented by the optical density of a radiograph plate after development. The technique used in this study has been widely used by several investigators (Groeneveld *et al.*, 1975; Featherstone *et al.*, 1983; ten Cate & Duijsters, 1983; Theuns *et al.*, 1985; Creanor, 1987; Damato, 1990).

Microdensitometry uses the data and values of optical film transmission (OFT) in percentage to calculate the concentration in the radiographed enamel sections, using Angmar's equation (Angmar *et al.*, 1963). At first. the OFT values of the six different steps of the step wedge are interpolated to obtain a continuous relationship between OFT and aluminium thickness $t_{(AL)}$. Then the OFT value of the slice at the spot of interest is read and converted into a value of aluminium thickness (t_{AL}) that causes the same OFT. Then, the formula by Angmar *et al.* (1963) may be applied. When converted, the present day notation reads:

$$V = 100 \cdot \frac{[\mu_{A1} \cdot t_{A1} - \mu_0 \cdot t_0]}{[\mu_M \cdot t_S - \mu_0 \cdot t_S]}$$

in which t_S is the thickness of the slice and μ_{Al},μ_M, and μ_0 are the linear absorption coefficiants for aluminium, mineral, and organic material and /or water respectively. More directly, the x-ray absorbances of aluminium and slice can be equated :

$$\mu_{Al} t_{Al} = [(\mu/\varrho)_M \cdot c_M + (\mu/\varrho)_0 \cdot c_0] \cdot t_S$$

in which $(\mu/\varrho)_M$ and $(\mu/\varrho)_0$ are the mass absorption coefficients of mineral and organic material (+ water) in $m^2\ kg^{-2}$ respectively c_M and c_0 are the respective concentrations in $kg\ m^{-3}$.

When deriving the formula to enable calculation of mineral content from measured optical densities, Angmar *et al.* (1963) made certain assumptions:

1. Enamel consists of two major components:
   (i) an inorganic component of thickness "t_m", and
   (ii) an organic component of thickness "t_0".

Thus, the grey level for any point in the lesion has resulted from X-ray absorption by both components. Therefore, for any grey value of a point on the lesion, the absorption in enamel can be equated against the absorption in an aluminium step-wedge (Figure 2.25).

2. Mineral salts have a density of 3.15.

3. Normal enamel has an average composition of 37.1% Ca, 18.1% P, 43.3% O, 0.7% C and 0.3% H, which results in a Ca:P ratio of 2.05. In addition, it was assumed also that the Ca:P ratio for normal, demineralised and remineralised enamel were similar. Appendix D outlines in detail the derivation of the formula used to calculate the volume percent mineral from the optical density of the radiographic plate. It takes into account the absoption coefficients and thickness of the organic and inorganic elements of enamel, which is equated against the absorption coefficient of the aluminium.

For use in tables, these expressions can be broken into factors for the constituent elements:

$$(\mu/\varrho)_M \bullet c_M = (\mu/\varrho)_{Ca} \bullet c_{Ca} + (\mu/\varrho)_P \bullet c_P + \ldots.$$

and similarily for $(\mu/\varrho)_0$. Here (μ/ϱ) values are atomic attentuation coefficients. These can be found in the literature. Since, in this method, the mineral content is derived from a single quantity (OFT), assumptions are necessary to deal with all other parameters that influence the OFT. To calculate μ_M and μ_0, the elemental composition of both the mineral and the organic material plus water has to be assumed or be known. For use in the tables for atomic mass attentuation coefficients monochromaticity of the x-rays has to be assumed. The elemental concentrations needed for their calculations were taken from ash values by Gron (1978) to which was added 1.8 wt% firmly bounded water. It should be borne in mind that concentrations differ from subject to subject, from tooth to tooth, and even within the location on the same tooth. For major constituents, roughly ± 3%

of the average value is common; for trace elements, a factor of two may occur (Robinson *et al.*, 1983).

Geometric assumptions are also necessary. The slice must be homogenous over its thickness. The latter must be known. The film and x-ray beam should be homogenous over the area used by the step wedge and the sample. These assumptions have been addressed extensively (de Josslin de Jong *et al.*, 1987). Although most microradiographic work has been concerned with enamel and enamel caries, recent papers describe its application to dentine (Herr *et al.*, 1986) and dentine caries (Mellberg and Sanchez, 1986; Graves and Feagin, 1988; Arends *et al.*, 1989; Nyvad *et al.*, 1989). Care must be taken, however, to avoid shrinkage of dentine sections when dried in air.

2.3.2 Microradiographic Methods.

In the first stage of microradiography, the ground enamel sections are mounted between two layers of cling film (Figure 2.20). The area of one radiographic plate was used to accomodate a total of four sections. The number of the section was registered according to their location and sequence on the film, to help later identification of the samples. On the right side of the sections an aluminium step wedge (50-300 μm thick) is placed on the same film frame (Figure 2.21). The cling film is used to keep the specimens rigid during the x-ray exposure. During the cling film process the trapping of air bubbles has to be avoided between the cling film, the x-ray film and the specimens. If air bubbles get trapped in between those two

layers it could reduce the quality of the x-ray after development for the microdensitometry assessment.

The tooth sections were mounted onto a Kodak high resolution film (type SO 343) and secured in place on the plate holder. After closing the plate with the help of four screws, one at each corner of the plate, the film was protected from any light exposure (Figure 2.21). All these procedures were undertaken under strict darkroom conditions.

A Marconi TX 12 x-ray tube in an Enraf Nonius x-ray generator was used to expose the specimens on the plate to Cu Kα radiation (Figure 2.22). It operated at a voltage of 20 kV and a current of 30 mA for an exposure time of 20 min at a target specimen distance of 300 mm (Creanor, 1987). The microradiographs were developed under standard conditions, using Dacomatic Clearing Solution, Kodak D-19 Developer Solution and Kodak Unifix (Kodak, Rochester/N.Y.). The processed films were then thoroughly rinsed in running water and left to dry in a Kodak Film Drying Cabinet for about 30 min.. After the films were dried they were ready to be used for microdensitometry analysis.

Figure 2.25 represents a microradiographic film of enamel sections and an aluminium step-wedge as used in this project. There have been findings in previous studies that the quality of the x-ray beam was not homogeneous, and exhibited a variation from "north" to "south". However, horizontally, beam variation was found to be less than 1%. To ensure that the microdensitometric readings of the

step wedge were made at the same level as the measurements of the lesion whilst benefitting from the X-axis beam homogeneity, the step-wedge was placed along the Y-axis of the film (Creanor, 1987).

2.3.3 Microdensitometric Methods.

Microdensitometry was performed by using an `Advice ADV-2´ image analysis unit (Brian Reece Sientific Limited; Berkshire, England) (Figure 2.23) to quantify the mineral content within the lesion area of the specimen. The unit consists of a microscope (Leitz Dialux 22) fitted with a black and white video camera `COHU´ High Performance CCD Camera (COHU Elctronic Division, San Diego; CA.) and a computerised image analyser which digitised the video signals from the camera into 256 grey levels, with a resolution of 256 × 256 pixels. The procedure for evaluating the radiographs required a calibration of the operating system using an aluminium step wedge.

The grey level values (optical densities) of the aluminium step wegde is used for calibration. Initially, the radiographed film was positioned on the microscope stage so that the thickest aluminium wedge was in view. The intensity of the microscope light was then adjusted to give a high and a low light levels. Calibrations at the high and low levels were set using controls on the image analysis unit (Figure 2.23). The average grey level corresponding to that wedge in a chosen area on the screen, was calculated and transferred in turn to a PC. Next, the images of the lesions were scanned (PC-mouse), digitised and transferred to the PC (PC, IBM compatible,

4/86, DX2, 66Mhz, custom assembled). The grey levels for the other thicknesses were calculated and transferred in turn. A special software program (customized image analysis software package) was used, to redisplay the lesion in black and white image on the PC monitor. A PC-mouse was used to set the area of interest. This area was subsequently scanned by the software which averages the grey values of the pixels across the area scanned to give a mineral profile through the section. The pofile was then displayed at the site of the screen in an extra window. Using the PC-mouse the location of the area of interest could be enlarged or altered in position. The dimension of the area was presented by outlining it with a red band in rectangular formation that was flexible in width as well as length. The computer then displayed the calculated average microdensitometric profile at the side of the screen.

A fourth-order polynomial was then fitted to the aluminium step-wedge grey levels and the grey levels of the profile were converted to per cent volume mineral using the equation of Angmar *et al.* (1963). All the data were stored on on the hard disc of the PC and floppy disc for subsequent analysis.

To reduce the influence of second order effects on the calculated data (eg. accidental marks on the radiographic plate), all lesion tracings were firstly normalised to sound enamel mineral content of 80% (Groeneveld, 1974). Therefore the chosen value was considered well within the acceptable range (Creanor, 1987).

Figure 1.5 shows the various parameters used for the quantification of mineral content. The measurements include the:

1. Per cent volume mineral content of surface zone (SZ) taken at the maximum mineral content of the surface layer.

2. Per cent volume mineral content of lesion body (LB) taken at the lowest point of the mineral profile deep to the surface zone.

3. The lesion depth (LD) calculated as the distance from the 20% mineral content of the initial slope to an arbitary cut-off point at approximately 95% of the value of normal enamel. This arbitary cut-off point was selected because of the unreliability of determining depths.

4. Integrated mineral loss (Δz).

The limits of IML were taken from the 20% level on the initial slope of the profile to a point S in sound enamel, selected on the baseline profile of the lesion. The shaded area has units of percentage volume mineral $\times$ microns. However, IML is calculated in terms of fractional mineral content $\times$ depth. Hence, it has the units of microns (μm) (Creanor, 1987).

In this study, the mean values for, Δz, SZ, LB and LD were used for the statistical analyses. All data was available and stored on a floppy disc.

2.4 Visual Assessment Using The Caries Index Of von der Fehr.

Blind scoring of transparencies of the samples was done according to the criteria laid down by von der Fehr (1961), (Appendix B).

The 59 transparencies representing each tooth of the study were all given a preselected number. This number was allocated to a code. The transparencies were then made into slides and numbered accordingly. The total number was 59 slides which were placed in a `Kodak´ carousel slide projector and projected in darkness onto a large screen. The projections were scored by two examiners who were blind as to the nature of each specimens. A copy of the scoring sheet (Appendix C) and a copy of the von der Fehr Index was given to each of the two examiners. Prior the the actual scoring session, both examiners were calibrated using 7 samples representing the different grades of the von der Fehr Index as determined by the author. The slides of the demineralised samples were scored as 0, 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0. depending of the severity and extent of the white spot lesion. Within a week, the same group of slides was presented again to the two previous examiners. Before the second session, all 59 slides were randomised by the author. The new sequence for the second assessment was not know to the examiners as on the first occasion. After the scoring session, the code was broken and the 59 slides compared for each of the two examiners on the two different occasions.

2.5 In Vitro Study.

2.5.1 Material and Methods.

1. A total number of 60 sound bovine incisor teeth were extracted from bovine skulls. From the time of extraction onwards the teeth were stored in plastic containers with a 5% thymol solution.

2. The teeth were then cleaned by removing any parts of attached soft and hard tissue with a sharp scalpel blade. Then they were cleaned with a mixture of water and pumice to remove layers of plaque or debris if necessary and finally rinsed with water. After the cleaning process the teeth were assessed visually under the microscope (Figure 2.8) to ensure that the following criteria were fulfilled:

a) The teeth had no existing white spot lesions (Figure 2.1).

b) The teeth were sound and had no sign of caries.

c) The enamel on the labial surface was intact with no evidence of attrition, abrasion, erosion, pitting, hairline cracks or fractures.

d) The teeth were of normal morphology.

3. A hole was drilled through the apical third of the root to enable the tooth to be suspended via a piece of dental floss in a beaker containing demineralisation solution.

4. A band of sticky tape 1 mm wide and 6 mm long was cut to create the shape of a "band" for an upper and a lower window (Figure 2.12). They were used as a incisal (upper) lesion band and a cervical (lower) lesion band later on. The sticky tape band was stuck in the desired position (Figure 2.2) in the mid 1/3 portion of the labial surface of the crown.

5. The entire tooth (crown and root) was then painted with an acid resistant nail varnish (Max Factor; London; England). This measure was taken to protect the rest of the tooth from the effect of the demineralisation solution. The teeth were then dried for a period of 24 hours before the sticky tapes were removed (Figure 2.3).

Each tooth was then labelled with a number between 1 and 60 and stored in a container with the same number.

6. The labelled teeth were suspended from a length of a wooden stick in batches of 10. The sticks were placed lying on the top of a large beaker containing the demineralisation solution with the teeth completely immersed in the solution (Fig. 2.7). An acid buffer solution was containing 3.1 mM $CaCl_2$, 3.1 mM NaH_2PO_4, 50 mM glacial acetic acid pH 4.5, F < 0.02ppm (Theuns *et al.*, 1985) was used.

Appendix E gives the formulation of the demineralising solution and how it worked.

7. To control the pH of the demineralising solution and adjust it to the desired value of 4.55 (Figure 2.5) an "Orion" model EA 940 Ion Analyser was used. During the period of demineralisation the solution in which the teeth were suspended was stirred continously with a magnetic stirrer (Corning Hot Plate PC-101, Corning, N.Y.) (Figure 2.6).

8. After a period of 24 hours the teeth were examined visually under a microscope to assess the extent of demineralisation obtained. To categorize the teeth into groups of diferent degrees of demineralisation the caries Index of von der Fehr (1961) (Appendix B) was used. At this stage of time all 60 samples showed signs of decalcification at different stages (Figure 2.4). Some specimens showed a mild degree of demineralisation (Score 1), others represented a medium stage of demineralisation (Score 2) and the remaining teeth were characterized as heavy white spot lesions (Score 3). Only one single tooth showed signs of severe decalcification with extensive surface softening and cavitation that it was excluded from further investigation.

9. The demineralised teeth were then washed with deionised water and stored in individual boxes containing a 0.12% thymol solution.

10. Acetone was used as a solvent to remove the nail varnish from the teeth. Then they were again cleaned with deionised water and stored in boxes containing 0.12 % thymol solution.

11. Photographs of the buccal crown surface of all 59 samples were taken after the demineralisation procedure. The camera (Nikon F3, Japan) was attached to a microscope (type) (Figure 2.8). at a magnification rate of x-times.

12. The photographs were used to assess the white spot lesions according to the classification of the von der Fehr Index (1961), (Refer Appendix B), in the inter- and intra examiner comparison (Chapter 3.4).

13. The roots of the teeth were then sectioned apart from the crown. This was performed by using a mechanical operated (type) cutting blade which seperated the teeth 1mm below the amelocemental junction (Figure 2.14).

14. Longitudinal sections of the teeth were cut through the crowns using a microslice II Dual Purpose Saw (Malvern Instruments, Malvern, England) to prepare the samples for microsectioning. The machine (Figure 2.15) is equipped with a rotating diamond annular blade thickness 350 μm and was operated at a speed of 350 rpm. To stabilize and fixate the samples during the process of cutting, each tooth was mounted in tanwax mounting medium (Tanwax, Malvern, England) onto a chuck planoparallel to the longitudinal axis of the sections to be cut (Figure 2.16). The chuck was then mounted onto the microslice machine so that the blade

cut the tooth sample in a bucco-lingual direction through the window (Figure 2.17).

15. Before the section were taken for the examination, an initial facing cut was taken to guarantee that the following cuts would be deep enough within the two lesion bands. Due to the brittle nature of the enamel each section was supported by a rectangular piece of glass slide. It was fixed by using light cured ultraviolet light with an adhesive resin (Loctite Engineering Adhesive Resin, Loctite, U.K.) onto the free surface after each cut. The microslice was set to cut each section at a thickness of about 250 μm. The quality of each section was checked under the microscope (type). A minimum of 4 "good" sections through the window were obtained as a backup for the one which was selected for further investigation. Sections which showed any inconsistency, were broken or cracked were disregarded.

16. To remove the tanwax which surrounded the sample on the section, glass slide and adhesive resin were removed from the sections by immersion in acetone for about 20 min.. Subsequently, the sections were stored in a beaker of alcohol to neutralize the acetone fot 15 min.. Finally, they were rehydrated and stored in a thymol solution.

17. For the further microradiographic and microdensitometric evaluation only sections of the best quality were selected. If a section of a sample showed any signs of inconsistency or damage one of the four backup sections were selected

according to their quality. To grind the sections to their final thickness, a heavy brass plate covered in damp gauze and a slurry of carborundum powder (particle size 0.3 μm) and water, mixed on a ground glass plate (Figure 2.18) was used Each section was labelled to the order in which it was cut using a graphite pencil. Then it was placed on the moistened gauze and slowly rotated round the glass plate. At first one side of the sample was ground initially to the stage were all the roughness of the initial cut caused by the microslice blade was removed. Then this side was labelled and the opposite side was ground until the required thickness of 150 μm was achieved.

18. A digital micrometer (Mitutoyo) (Fig. 2.19) was used to assess the measurement of the section thickness (Appendix G). For this purpose the section was placed on a flat metal platform of the micrometer using a measuring probe which could be raised or lowered by the use of a camera extension cable. The sections were ground until the measurements were proved within an acceptable range of 140-150 μm.

19. The sections were then rinsed in water to clear them of any grinding material or other debris.

20. For microradiography and microdensitometry the sections were mounted on a Kodak high resolution photographic films (Kodak SO 343, Rochester, N.Y.). Their position was recorded on a special sheet (Appendix F) to reidentify the location of the specimen and its according number after development.

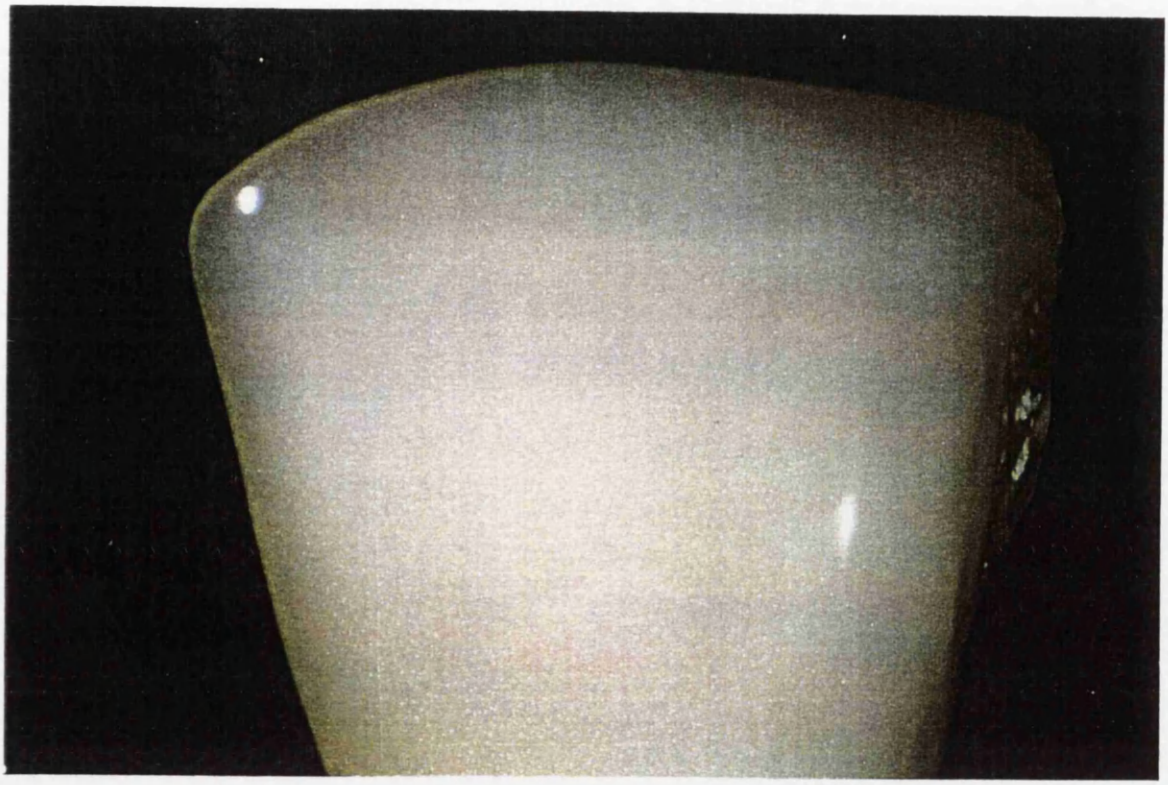


Figure 2.1 The buccal crown surface of a predemineralisation tooth.

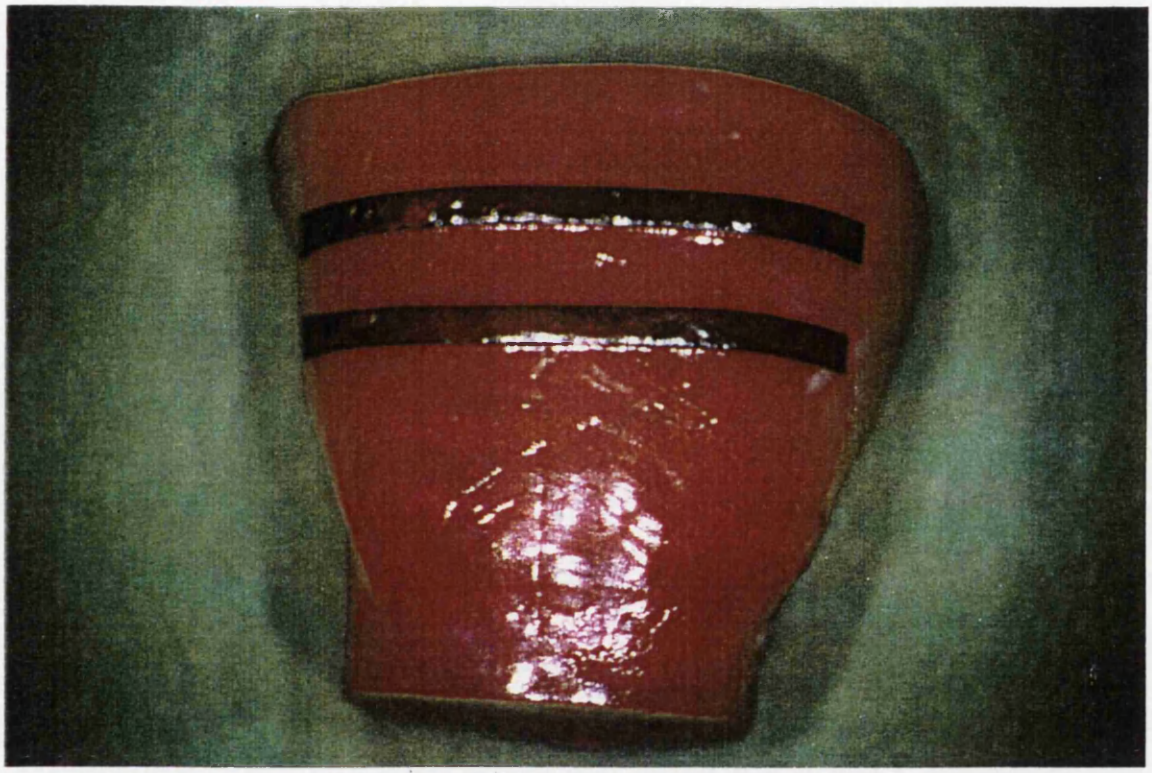


Figure 2.2 The nail varnished buccal surface of the crown of one of the samples prior to demineralisation. Two 1mm x6 mm bands of sticky tape have been attached for the creation of the two lesion windows according to the demineralisation protocol.

Figure 2.3 The nail varnished buccal surface of the tooth after removal of the sticky tapes exposing the two windows ready to be exposed to the demineralisation solution.

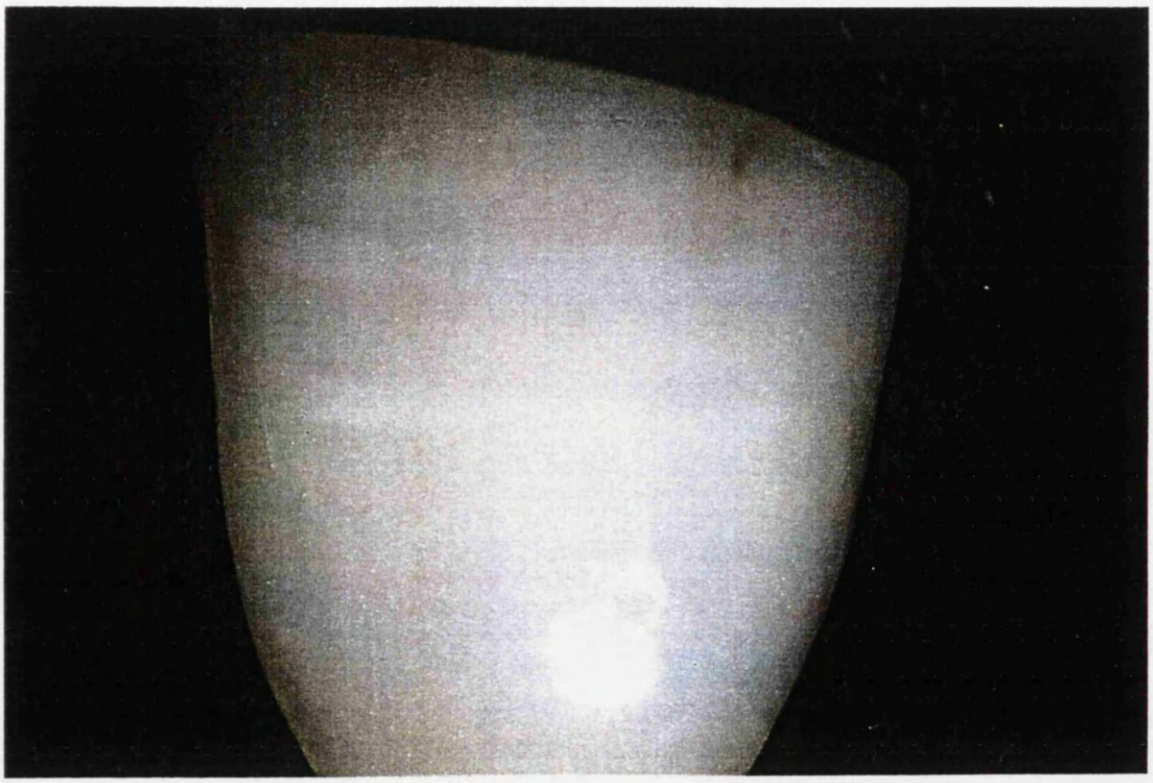


Figure 2.4 The buccal surface of the crown after demineralisation exposing the two created lesion bands as white spot lesions.

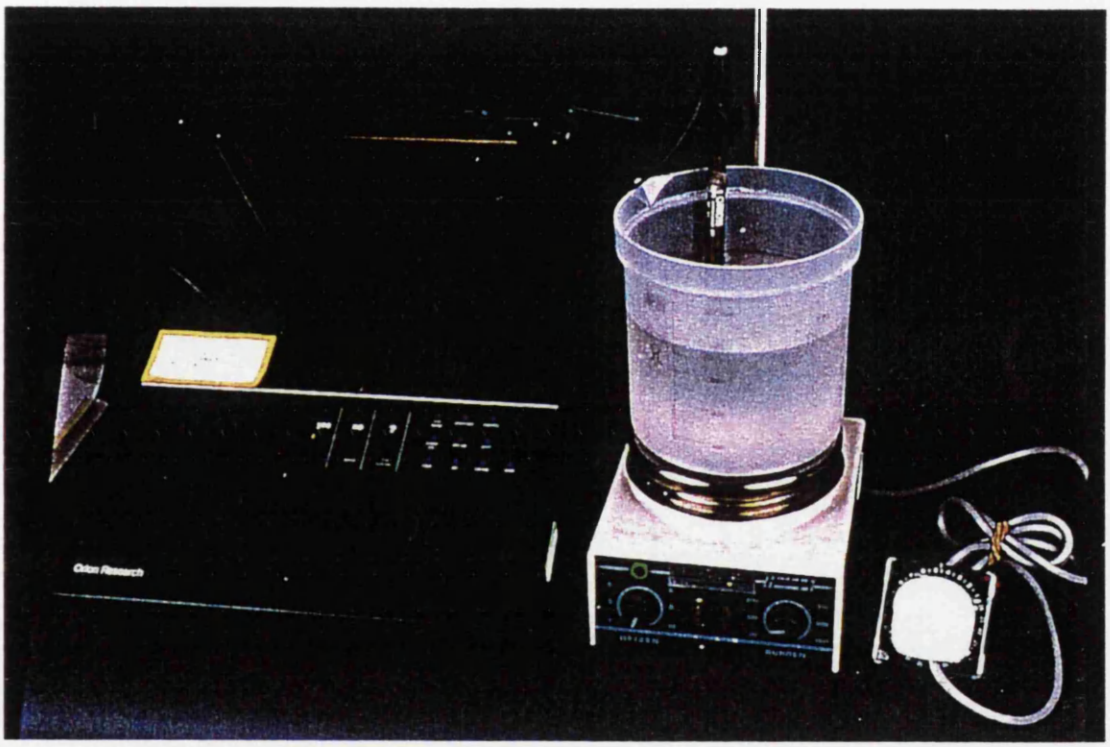


Figure 2.5 The Orion Model EA 940 Ion Analyser was used to check and adjust the pH of the demineralising solution during the exposure time of the samples.

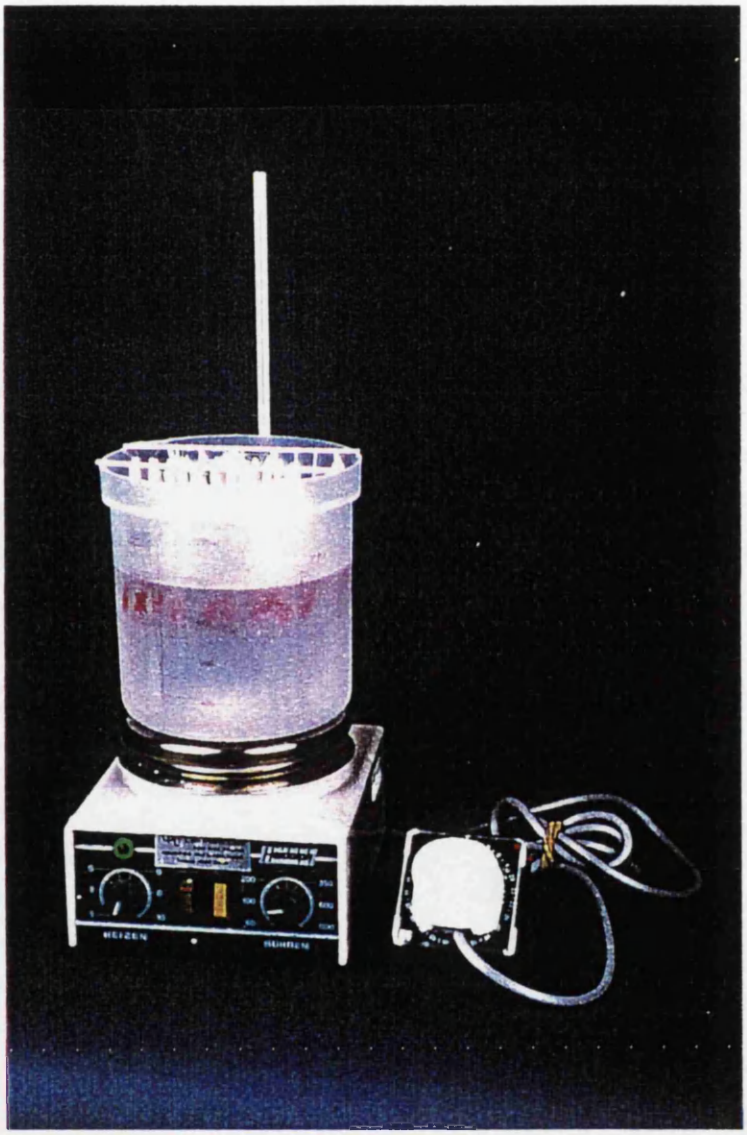


Figure 2.6 A magnetic stirrer kept the solution in motion during the whole demineralisation period.

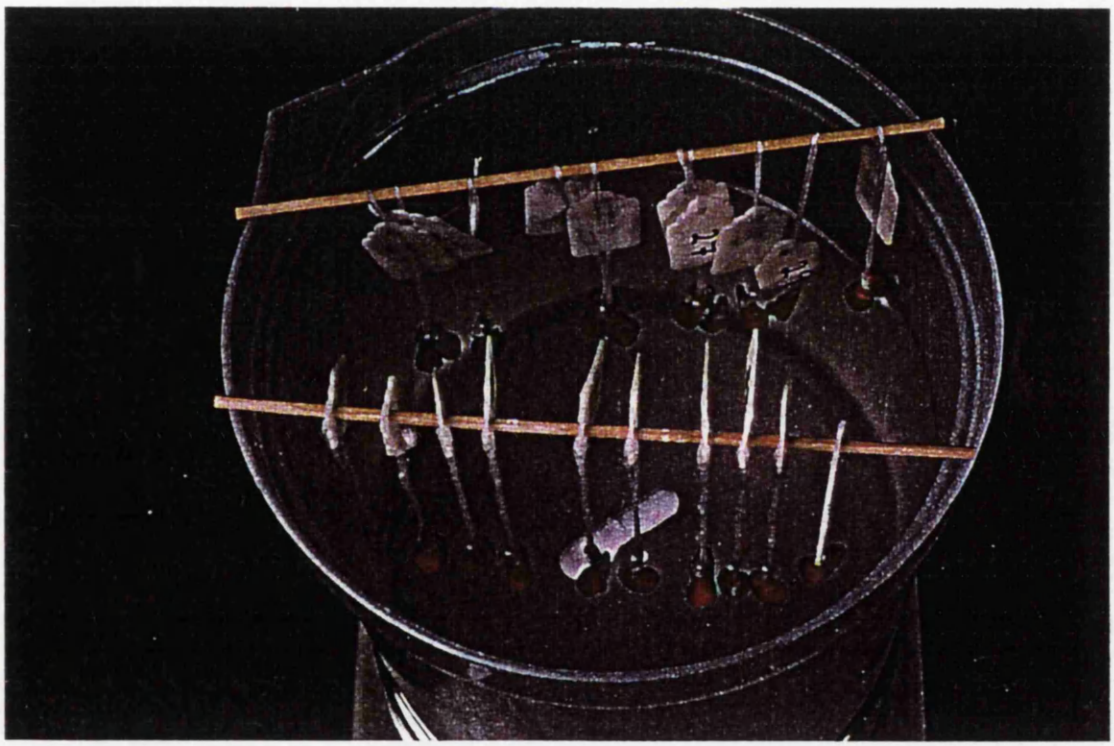


Figure 2.7 The teeth were suspended in groups of ten samples from a piece of wooden stick into a beaker containing the demineralisation solution.

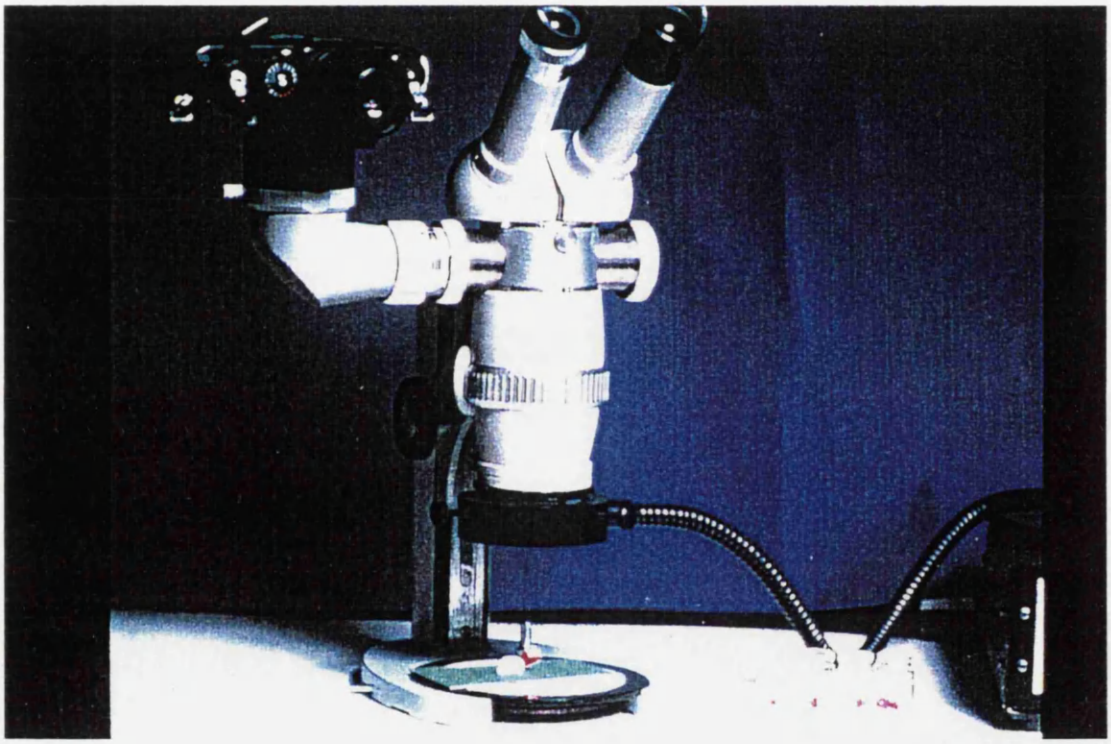


Figure 2.8 A Zeiss Stereomicroscope IV attached to a Nikon F3 Camera was used to take photographs of the samples.

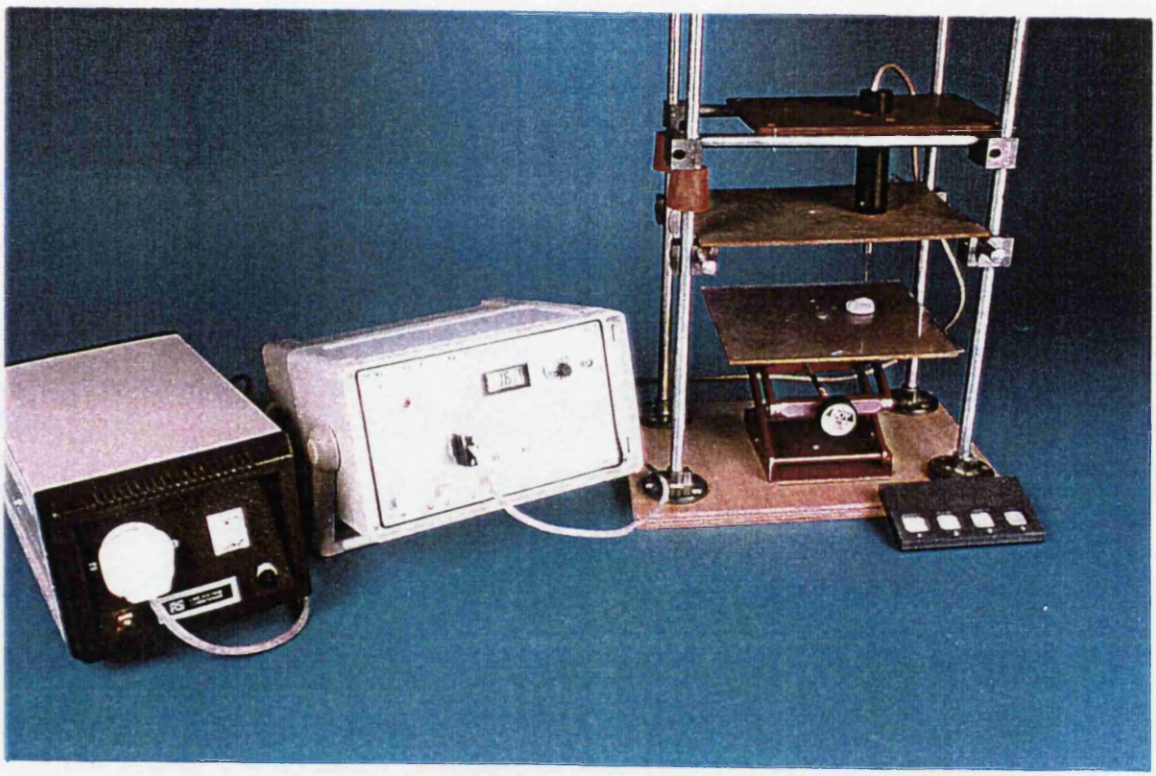


Figure 2.9 The Sensoptic optical caries monitor suspended for stabilisation purposes in a frame construction that holds the measuring head in a fixed position during measurement proceedings. The sample is fixed on a tray which can be altered in a vertical direction to get in contact with the measuring head. All readings will be displayed in the LED digital panel of the electronic unit which is connected to the probe. The electronic unit is connected to a voltage stabilizer which will balance fluctuations of current changes.

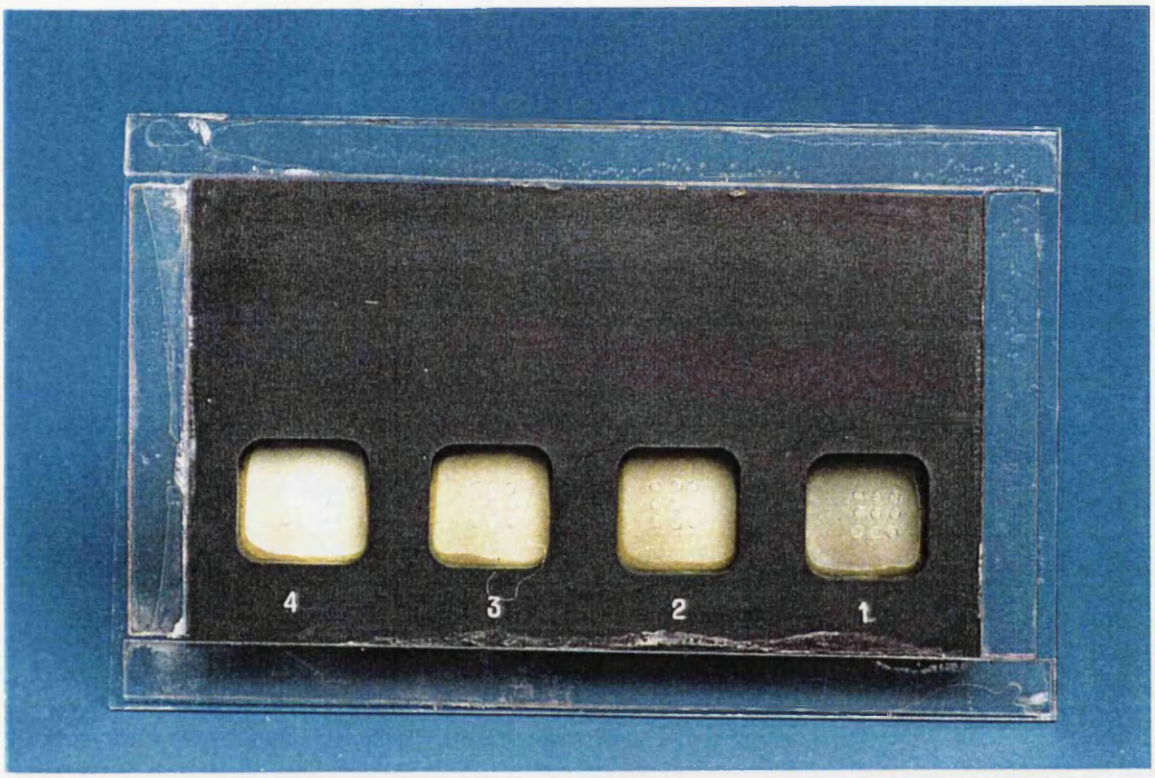


Figure 2.10 The four stable plastic calibration fields with the acrylic grid to superimpose during calibrations. The grid allows to repeat the calibration at the very same point each time when it is performed.

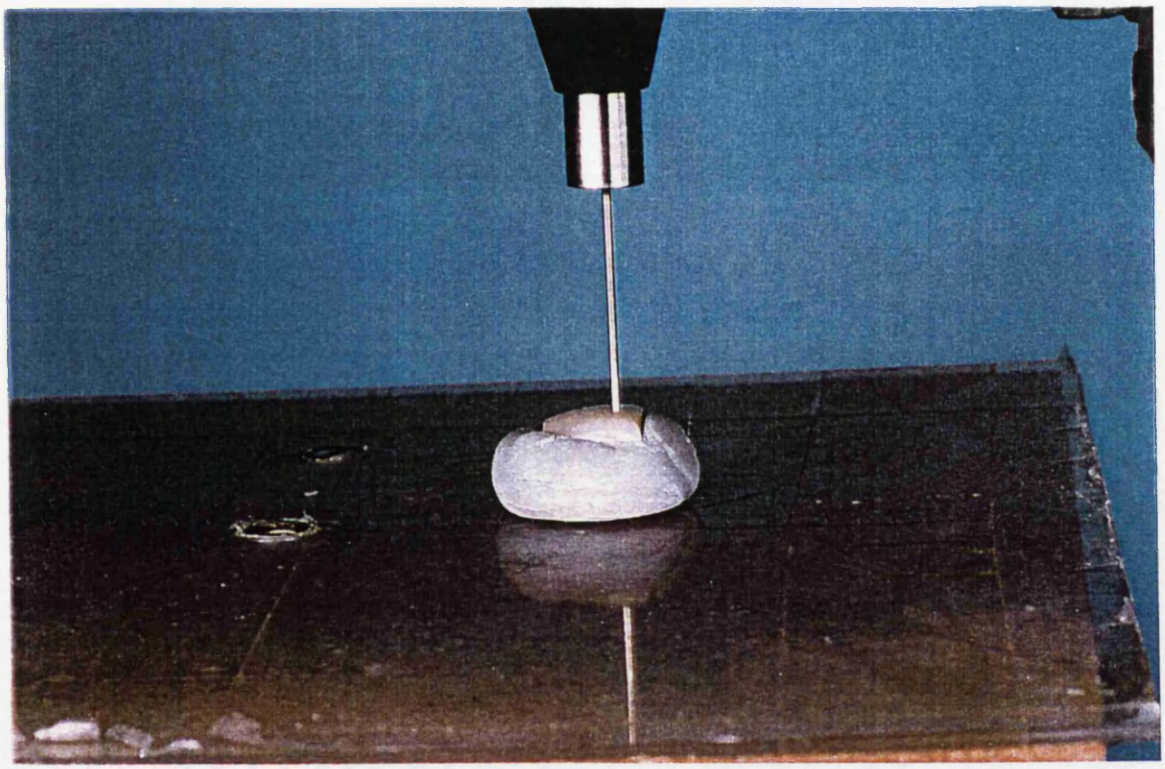


Figure 2.11 The head of the optical unit with the measuring pin in vertical direct contact with the area to be examined. The crown of the sample is fixed with blue tak to the underlying surface to prevent any kind of movement allowing to repeat the measurement at the very same point as often as required.

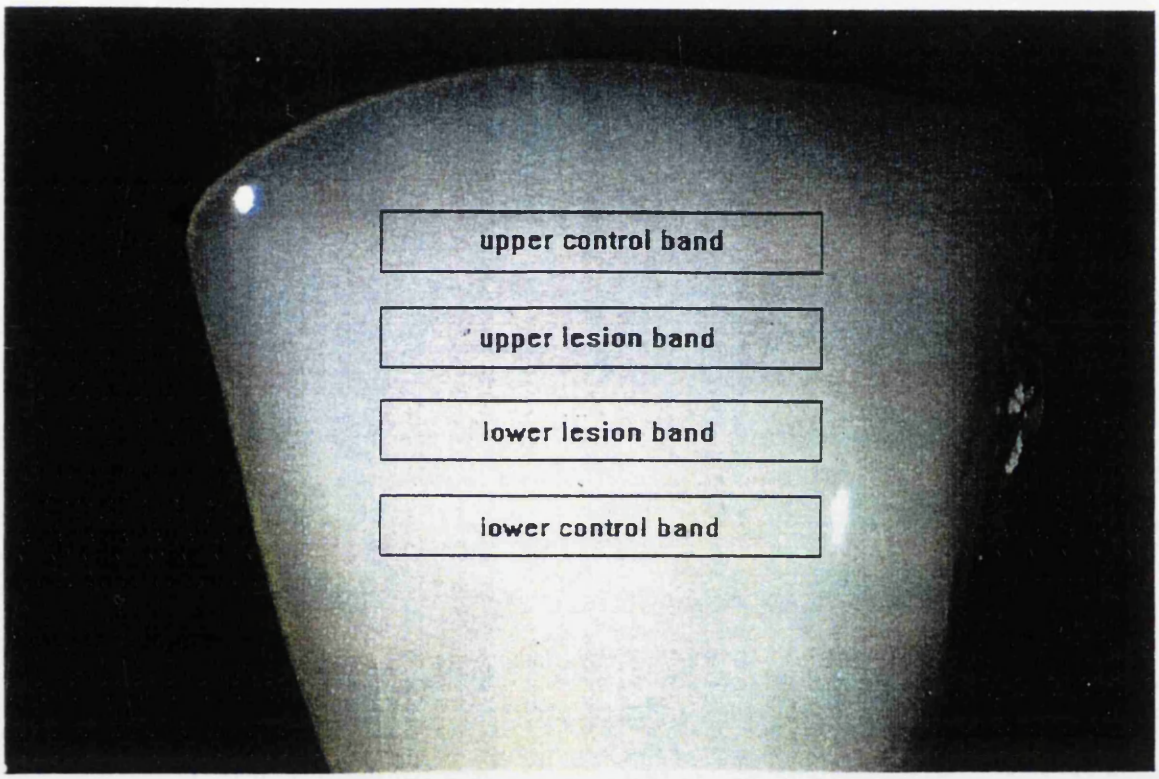


Figure 2.12 Areas of measurements to be taken for the caries lesion and the non demineralised control areas.

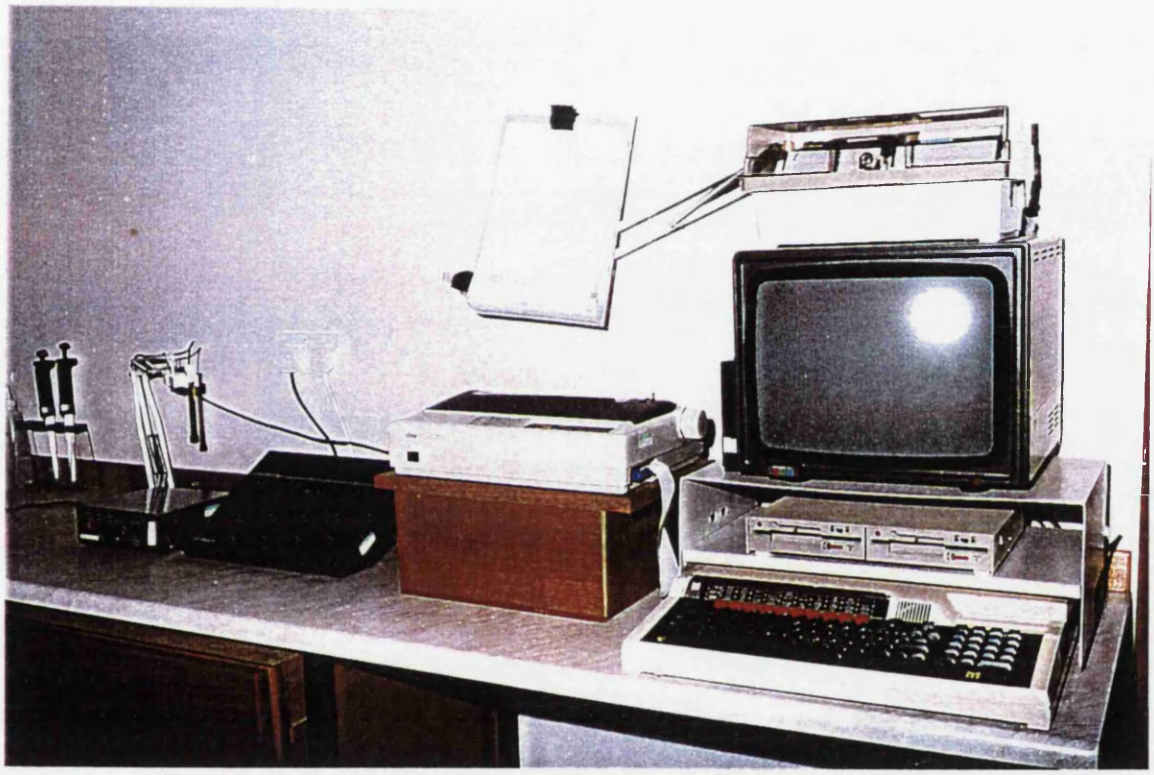


Figure 2.13 The BBC microcomputer which transferred the caries monitor readings according to their individual calibration values into sets of comparable data.

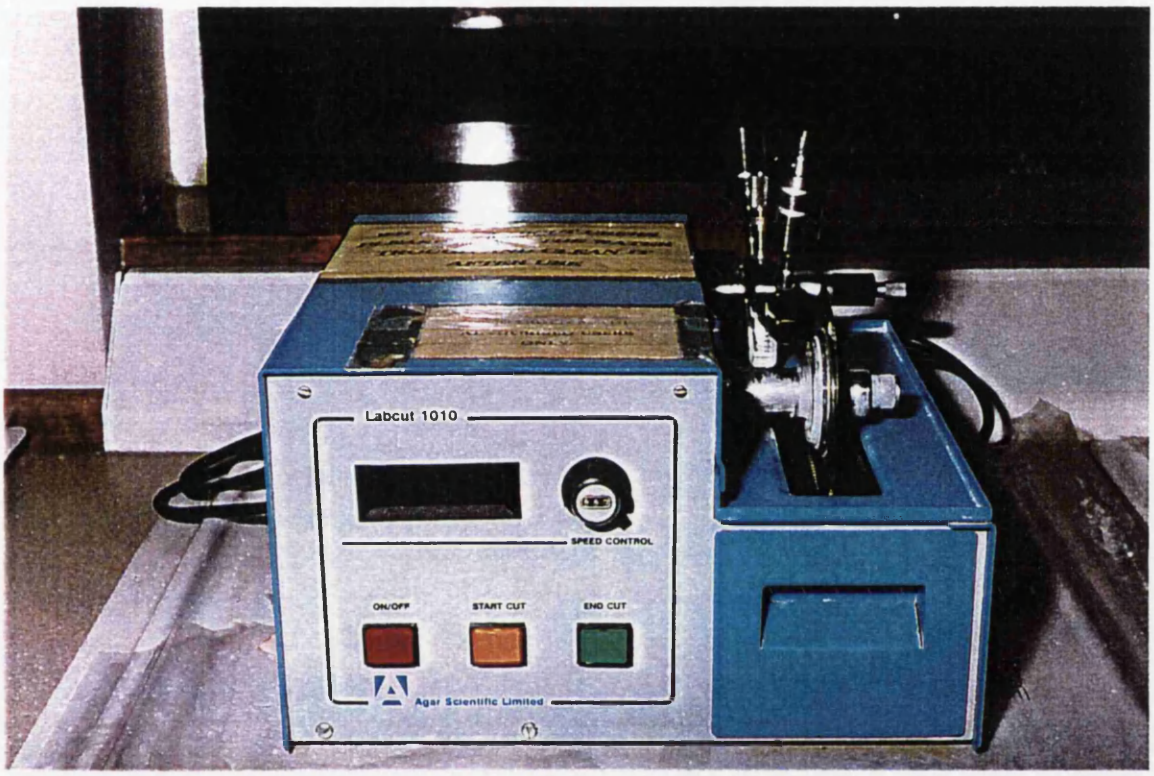


Figure 2.14 The Lab Cut 1010 mechanical saw that was used to cut the crown and the root of the samples apart from each other.

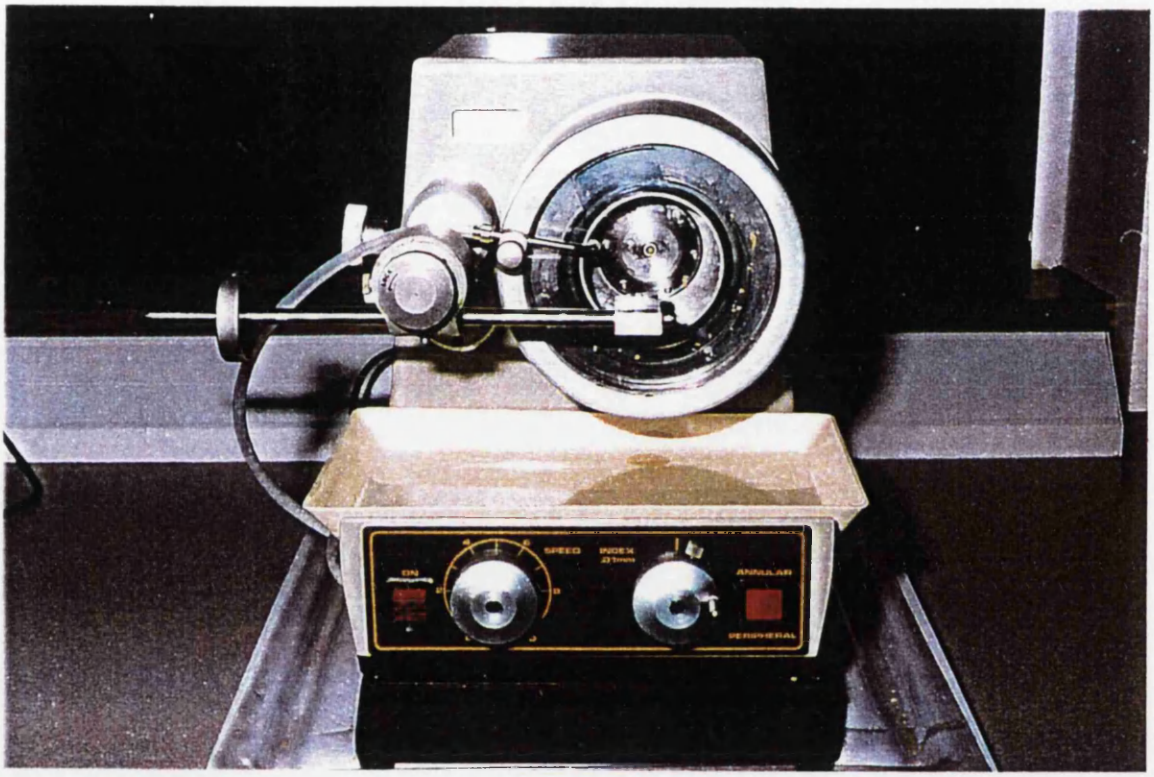


Figure 2.15 **The Microslice II precision slicing machine which was used to cut slices of 250 μm thickness. The chuck holds a crown embedded in tanwax for sectioning.**

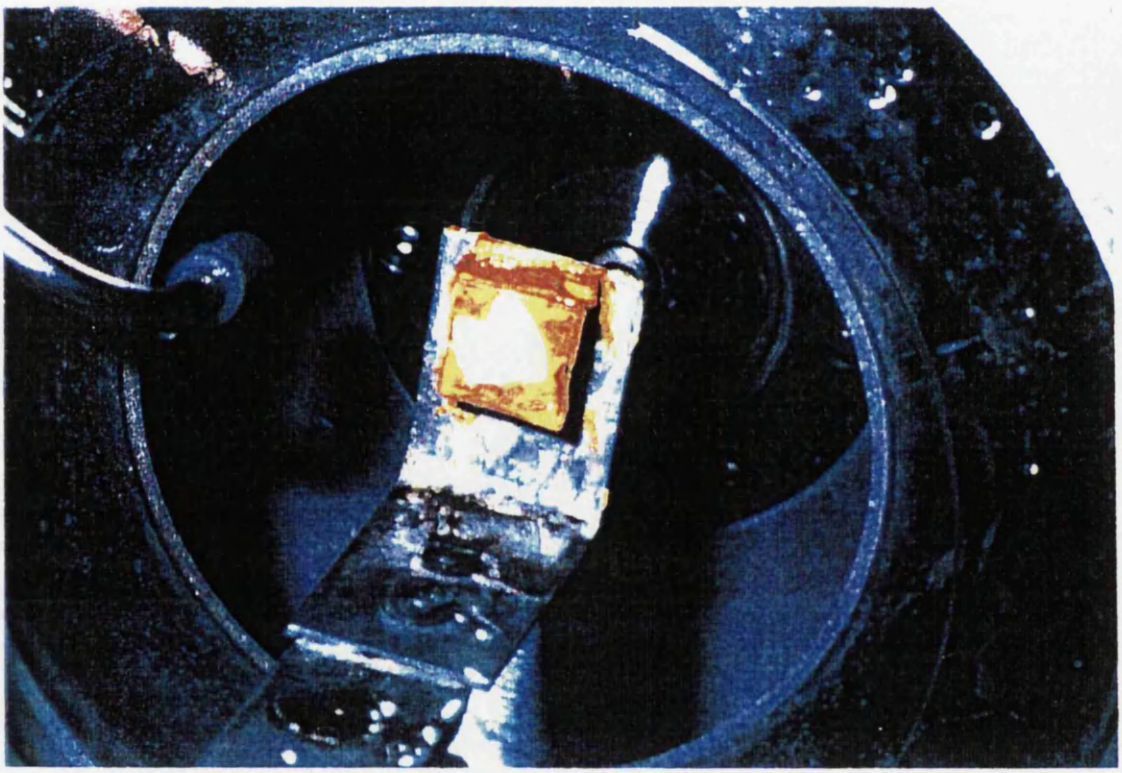


Figure 2.16 **A close up view of the Microslice II showing an already sectioned piece of crown embeded into a layer of tanwax. The glass plate on top prevents the fractioning of the section during the cutting process.**

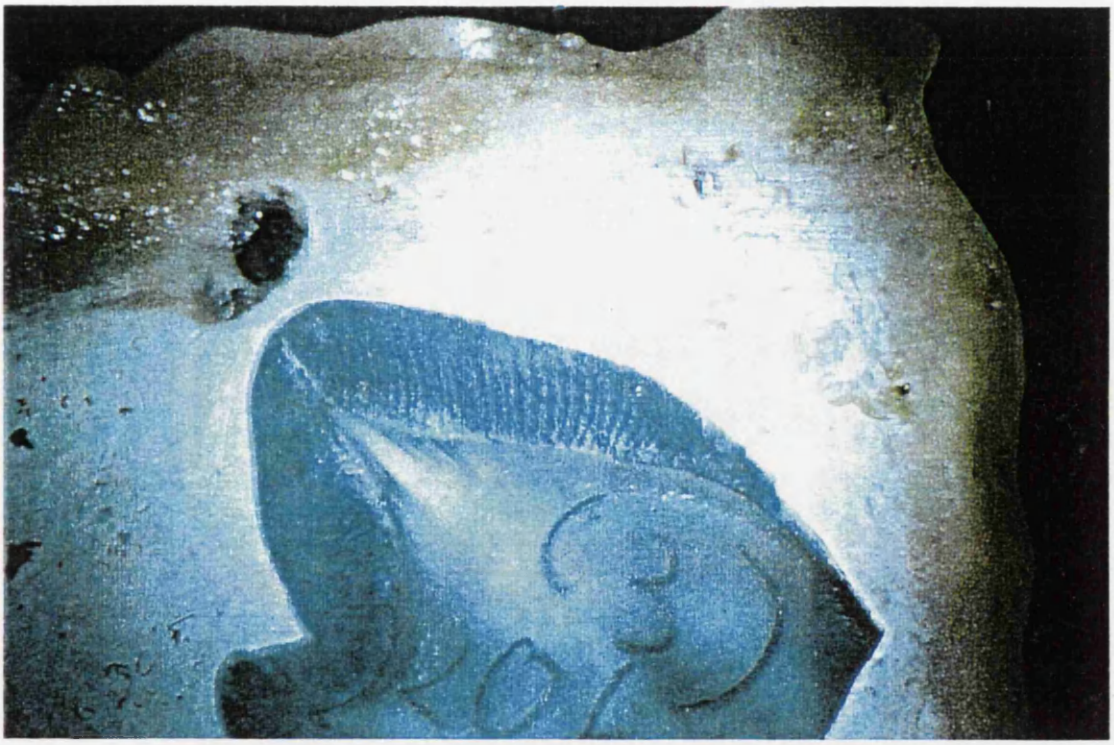


Figure 2.17 **An enamel lesion sample still embeded into tanwax as it appears under microscopic inspection after sectioning.**

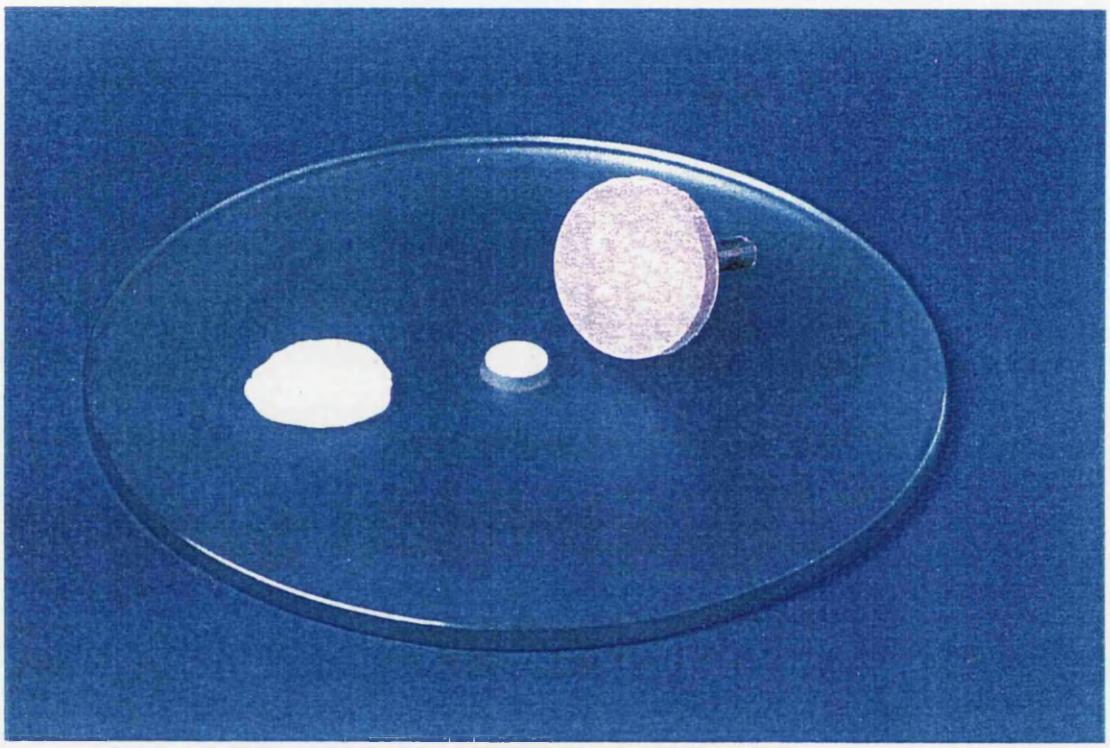


Figure 2.18 A set-up for the preparation of grinding the sections. The sections which were cut by the Microslice II were placed on the dampened gauze covering a heavy brass plate. The sections were ground by rotating the brass plate in a slurry of Bauxilite powder mixed with water on a ground glass plate.

Figure 2.19 A Mitutoyo digital micrometer used for determination of the thickness of a ground section.

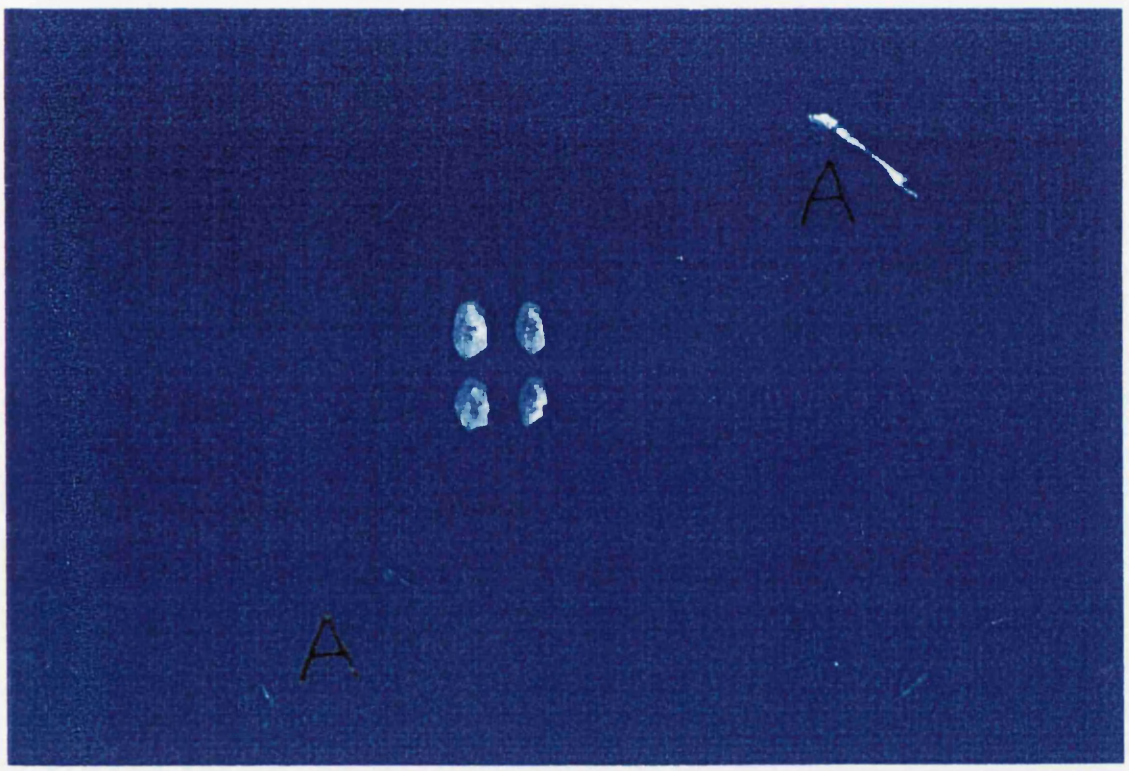


Figure 2.20 A group of four carious lesion sample sections is mounted into two layers of clingfilm for microradiographic examination purposes.

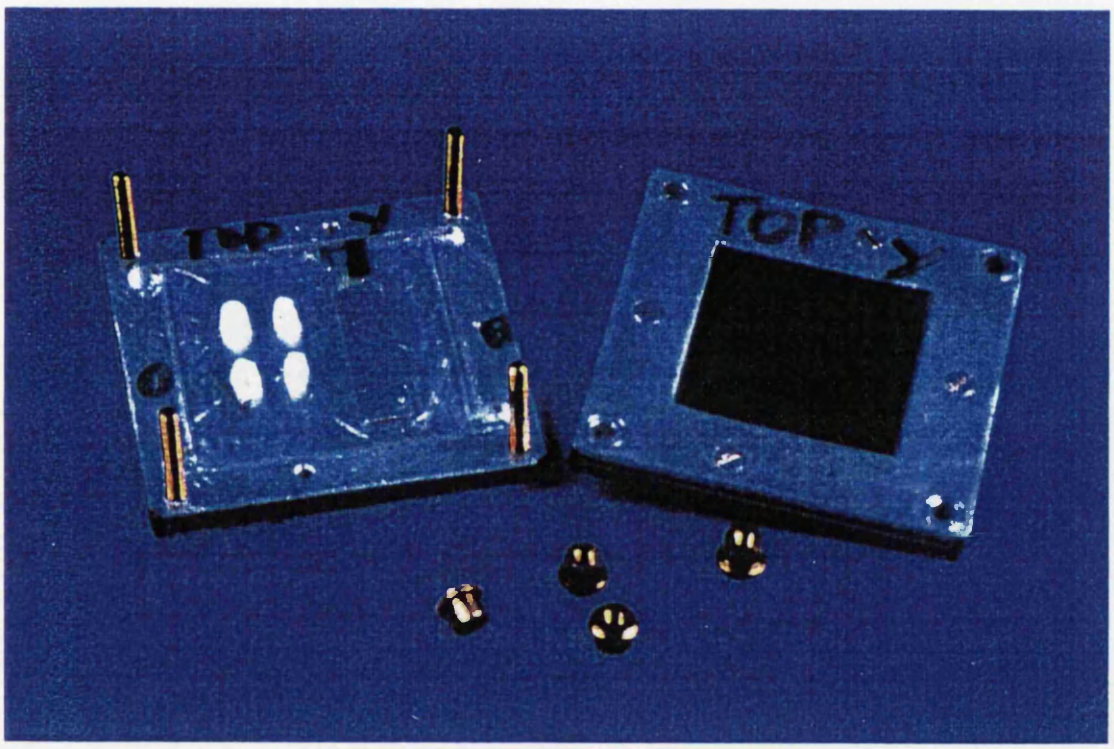


Figure 2.21 A photographic plate holder which holds the aluminium step-wedge, the radiographic film and the samples covered in cling film firm in its place prior to microradiography.

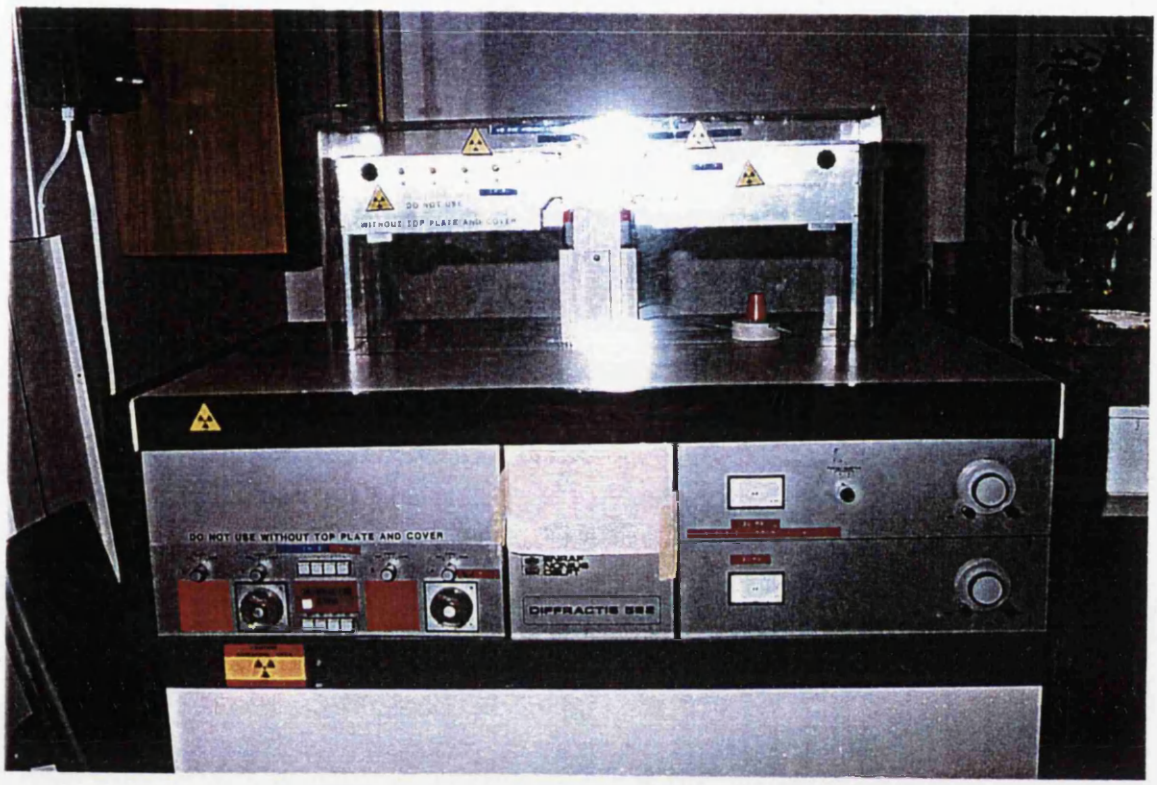


Figure 2.22 **Diffractis 582 Cu(K_a) X-ray source.**

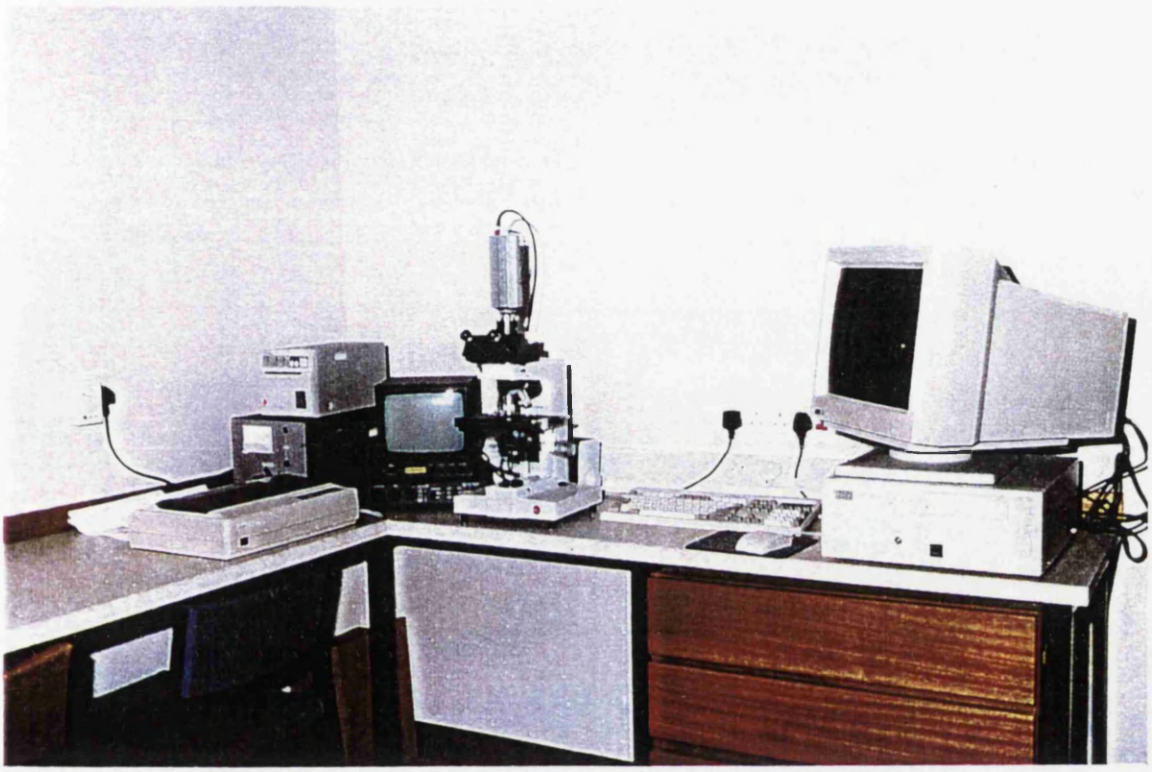


Figure 2.23 The system for the evaluation of the microradiographs by microdensitometry. The set-up includes a Leitz microscope connected to a video camera on top which is linked to an image analyzer unit and a personal computer to process and evaluate the data.

Figure 2.24 The typical image of a subsurface enamel lesion as it appears on a microradiographic film.

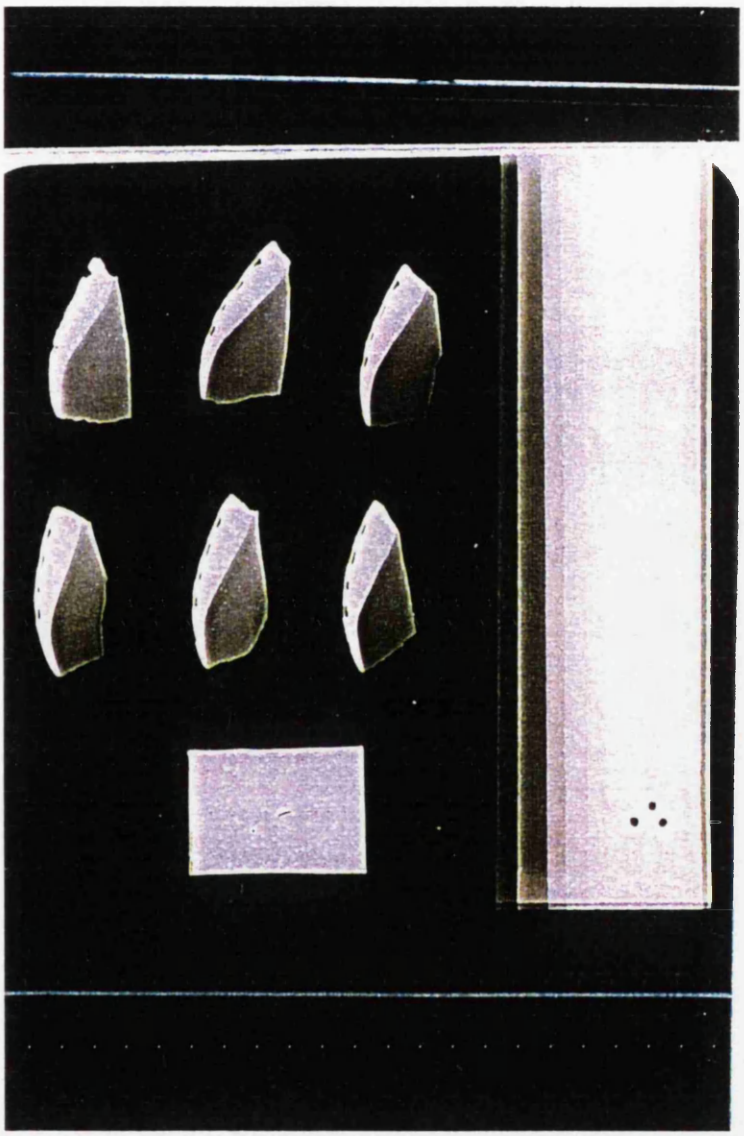


Figure 2.25 **A developed microradiographic film showing enamel sections with artificial carious lesions and the aluminium step-wedge on the side.**

Key Point	NSC121			
NSC121		DELTA Z	SZ	LB
	MIN CON	1177.156	59.76414	56.02743
	DEPTH	-	11.49911	24.63762
	POINT	-	14	30
		LESION I	NORM DEPTH	
	MIN CON	-	-	
	DEPTH	54.20509	87.05913	
	POINT	66	106	

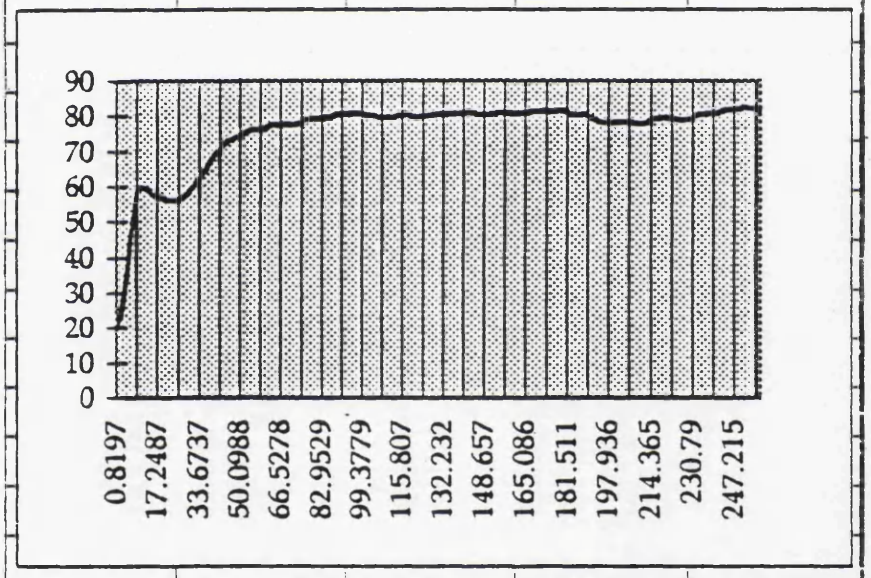


Figure 2.26 Computer analysed image of a microdensitometric reading.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Introduction.

This chapter describes the findings of the study. Results will be presented and discussed. In the first part of the investigation, the aim of the study was to determine the degrees of reproducibility and repeatability of the optical caries monitor readings. These were established by measuring the amount of tooth enamel surface mineralisation and demineralisation after being exposed to an artificial caries solution. In the second part of the investigation, the results from the evaluation of the data obtained from the optical caries monitor readings were compared to the microradiographic establishments. The results of the study also incorporates the visual scoring results between two different examiners.

In the following section the comparison of the data and discussion of the findings will be presented in the following sections:

i) results from the determination of the degrees of reproducibility and repeatability in measuring the amount of tooth enamel surface mineralisation and demineralisation

ii) results from individual methods of assessment- caries monitor, microradiograhy/microdensitometry and visual assessment

iii) correlations between the above listed methods of assessment

3.2 Aims Of Study.

The principal aim of the study was to investigate the reliability of the `Sensoptic' optical caries monitor as one of the available methods for a non-invasive caries detection technique. The appliance which is currently only designed for *in vitro* use could provide a useful tool in the field of early caries diagnosis and could provide vital information about the caries process and anassessment of the dynamics of a lesion at the time of the measurement. This is currently only available by exploitation and use of invasive techniques that lead inevitably to irreversible destruction of dental tissue and loss of the investigated sample material.

3.3 Material And Methods.

Enamel demineralisation *in vitro* was investigated in 60 bovine incisor teeth which were varnished in an acid resistant nail polish. Two windows had been prepared by covering the area selected for its location with sticky tape which was stretched over an area of 1 mm wide and 6 mm long. After the nail varnish dried the tape was peeled off with a thin pair of forceps revealing two white bands in the cental buccal surface of each tooth. They were placed horizontally with one of them being

more incisally and the other more cervically positioned. The first one was related to subsequently as the `upper' band and the later one as the `lower' band. They were placed parallel to the incisal edge within a distance of 4-5 mm regarding the upper one. The distance between these two parallel bands was in the range of 1.5 - 2 mm. All 60 samples were exposed to a demineralisation solution (Appendix E) for 24 hours. Photographs were taken of all 60 teeth after the exposure to the acid solution. One of the 60 teeth however had to be discarded because of surface damage. The varnish was subsequently removed with acetone and the teeth rinsed in water. Optical probe readings were taken in both window areas, in addition to an area above the incisal and below the cervical areas in the adjacent sound areas. In each of the windows four measurements were taken on four different points, totalling 32 readings with the optical caries monitor in both carious areas. In the sound control areas above and below the windows, the same number of measuring spots were chosen and the same amount of readings were performed at a distance of 1mm parallel to the neghbouring bands in a horizontal direction. The total number of measurements was therefore, 64 for each sample. With the exclusion of one out of the 60 samples, the number of caries monitor readings that were performed and available for statistical data evaluation was 3776.

All teeth were photographed after demineralisation for the purpose of visual scoring.

The demineralised samples were then prepared for microradiographic and microdensitometric investigations as described previously (Chapter 2.5).

The process and sequence of the experimental stages have been described previously (Chapter 2.5.1).

3.4 Results From Individual Methods Of Assessment.

3.4.1 Introduction.

The aim of this section of the study was to demonstrate the capability of the caries monitor, microdensitometry/microradiography and visual scoring to measure the changes between demineralised and non-demineralised tissue.

3.4.2 Caries Monitor Scores.

The optical caries monitor was calibrated on every occasion when it was switched on for measurement purposes. Due to the inconsistency of calibration measurements on each of the four stable plastic calibration surfaces, a grid with 10 predetermined mesurement points was superimposed (Figure 2.10) over the stable plastic area. The 10 points were marked by drilled holes in a perspex acrylic plate that allowed only the thin head of the probe to be lowered through those holes of ~1.0 mm diameter onto the calibration surface. The optical caries monitor was fixed in a solid metal frame construction (Figure 2.9) to avoid movements or shifting of the measuring probe during measurements. The objects that were to be measured were, therefore, placed onto a laboratory tray that was firmly fixed to a platform underneath to avoid any movement. The only direction the tray could be

moved was in vertical direction by turning the screw in an appropriate direction. This technique allowed the sample to approach the caries monitor from underneath until close contact was achieved. The teeth were embeded into `blue-tak' (Figure 2.11) during this process, to keep the specimens stable and allow the probe to be in a perpendicular position, allowing maximum contact between the surface of the probe and the tooth where the light is emitted into the investigated area of the sample. Table 3.1 shows typical readings from two samples, randomly taken from the study. In the table, the back scattered intensity (reflectivity) calculated by the caries monitor program using the calibration values as the standard. Every time the caries monitor was switched off between measurements, it was calibrated once again prior to commencing measuring. The calibration values were entered a the BBC-computer (Figure 2.13) which were subsequently transformed by the program on the basis of the calibration values to achieve values that could be compared to those from previously generated data. Reflectivity readings from various occasions can thus be compared. The results of the caries monitor readings in all four areas are shown in (Fig 3.1).

Table 3.1 A Set Of Typical Caries Monitor Readings And The Calculated Scatter Of A Sample For One Measuring Band.

mvolts	scat coef	LOG (scatt coeff)
1.50000	1.8930	0.07529
1.50000	1.89930	0.07529
1.42000	1.09045	0.03761
1.43000	1.10264	0.04243

mvolts=mmvolt reading of the caries monitor

scat coef=scatteringcoefficient of the BBC computer programm

LOG (scat coeff)=log used for transformation of caries monitor readings into calculated scatter of a sample

The mean optical caries monitor values for the upper and lower control areas (Figure 2.12) were 1.35 ± 0.34 and 1.41 ± 0.36 respectively. Comparative values for the upper and lower white spot lesions were 1.89 ± 0.64 and 1.82 ± 0.54 respectively. Comparing the two control areas showed no statistical difference (p=0.31) as was the comparison between the two lesions (p=0.53). The difference between the upper control (1.35) and the upper white spot lesion (1.89) was significant (p=0.00). The mean values and standard deviations for all four measurement areas are shown graphically in Fig: 3.2.

3.4.2.1. Discussion.

In conclusion, the optical caries monitor may prove to be a useful tool to discriminate areas of normal enamel and white spot lesions evident from its repeatability and reproducibility in measuring lesion and control areas.

Table 3.2 A Set Of Typical Caries Monitor Readings And Calculated Reflectivity Of Randomly Selected Samples At Different Measuring Bands.

	seub cm	seub ref	club cm	club ref	cllb cm	cllb ref	selb cm	selb ref
1.1	1.28	0.9695	1.56	1.3206	1.80	1.6514	1.47	1.2035
1.2	1.44	1.1654	1.59	1.3605	1.76	1.5945	1.68	1.4827
1.3	1.44	1.1654	1.51	1.2551	1.76	1.4552	1.64	1.427
1.4	1.49	1.2292	1.61	1.3873	1.70	1.5104	1.59	1.360
2.1	1.46	1.1908	1.48	1.2163	1.56	1.3206	1.57	1.3338
2.2	1.47	1.2035	1.45	1.1780	1.72	1.5382	1.55	1.3074
2.3	1.39	1.1028	1.50	1.2421	1.55	1.3074	1.59	1.3605
2.4	1.28	0.9695	1.67	1.4689	1.62	1.4008	1.54	1.2942

cm=Caries monitor readings

ref=calculated reflectivity scatter

seub=sound enamelupper band

club=caries lesion upper band

cllb=caries lesion lower band

selb=sound enamel lower band

1.1-1.4=First sample with four different measuring points

2.1-2.4=Second sample with four different measuring points

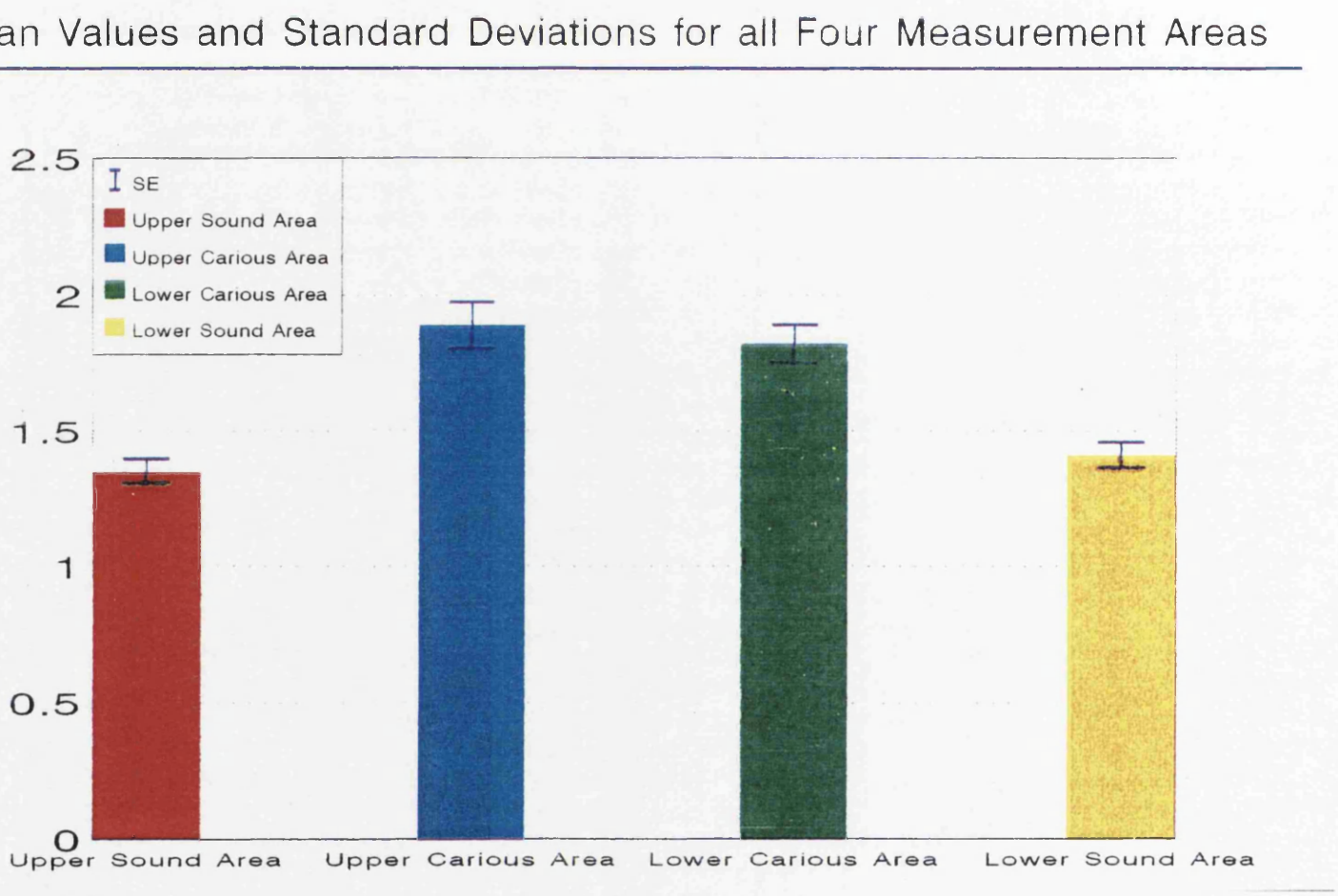


Figure 3.1 The mean values for caries monitor readings in all four measurement areas were established from four repeated readings on four selected measuring points.

Caries Monitor Readings In All Four Areas

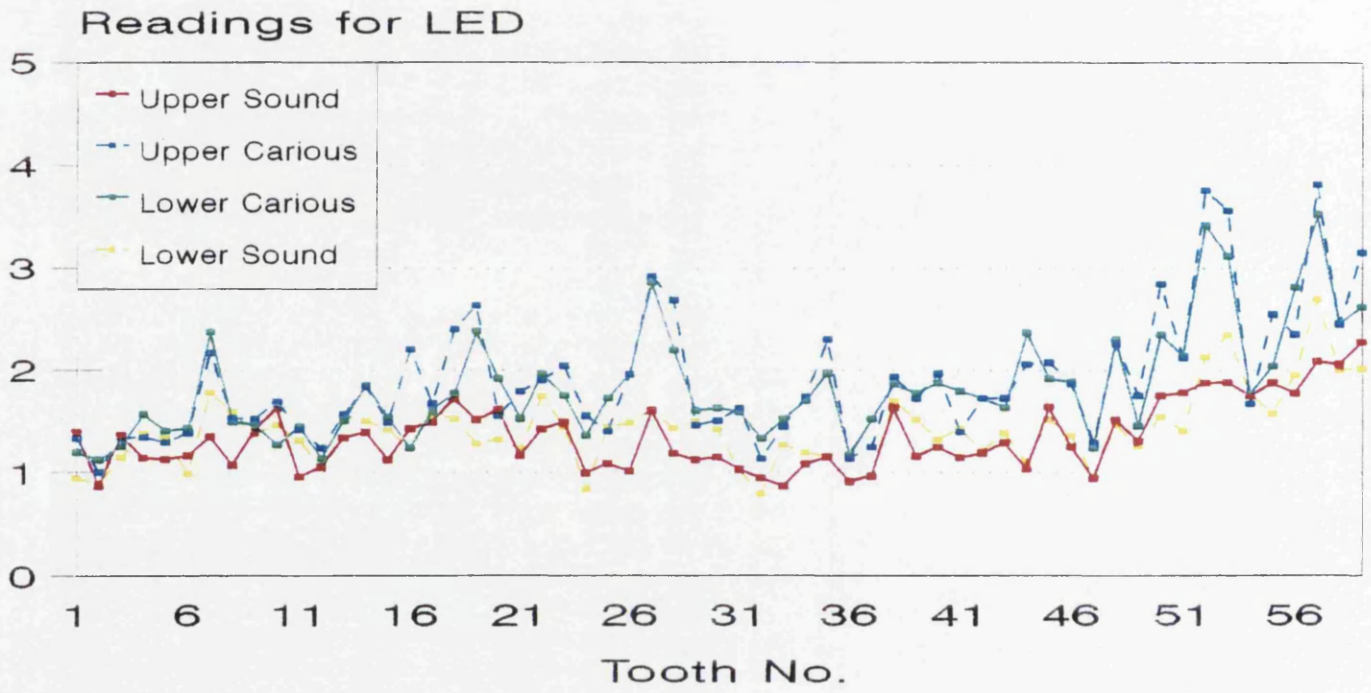


Figure 3.2 **The LED caries monitor readings represented by one mean value for each of the four areas from which they were collected. Each value represents the mean of 16 measurements that were taken on four different points at four times.**

3.4.3. Microradiography/Microdensitometry.

After the samples had undergone caries monitor scoring, they were subsequently photographed and prepared for the further investigation methods of microradiography and microdensitometry.

3.4.3.1 Reliability Of Selected Sections.

Although a minimum of four "good" sections of the area where the caries monitor readings were taken had been stored, only one of them was used for further microradiographic evaluation. Most of the ground sections were of good quality, although from a microscopic assessment preference was given to the section that was of the best quality, that is a specimen which had not been fractured, or had demonstrated no cracks.

3.4.3.2 Repeatability Of Microdensitometric Analysis Of The Microradiographed Sections.

For each of the 59 microradiographed samples, a total number of ten scans were made. In each of the two bands representing the upper and lower lesion band, five scans were made in a vertical direction. The first sequence of five scans was taken in the upper part of the incisal (upper) lesion band and the next five in the cervical (lower) lesion band. The ten scans gave ten Δz readings from each section and in Table 3.3 ten readings from two random samples are represented. In Fig.: 2.26 a

printout of a microdensitometric analysis is given with the values for Δz, surface zone, lesion body, lesion depth and normal depth attached.

Table 3.3 A Set Of Typical Readings Of Integral Mineral Loss (Δz) From Two Sections Of Randomly Selected Samples.

Tooth No.19	Section A	Section B	Section C	Section D	Section D
Δz1	2127.6	2035.7	2306.2	2440.6	2481.1
Δz2	1381.9	1348.7	1177.2	1519.2	1309.0
Tooth No. 22	Section A	Section B	Section C	Section D	Section E
Δz1	1584.8	1520.0	1294.4	1323.8	1190.8
Δz2	983.1	1259.2	1207.4	1073.4	1084.5

Tooth number 19 and 22 with the Δz-values for each of the five lesion scan readings (A-Z) taken in both carious lesion bands.

Δz1=mineral loss in the upper carious lesion band

Δz2=mineral loss in the lower carious lesion band

Table 3.4 A Set Of Typical Readings Of Integral Mineral Loss (Δz) From Randomly Selected Samples.

Tooth and section	Δz1	Δz2
Tooth No. 19, Section A	2127.6	1381.8
Tooth No. 22, Section A	1584.8	983.1
Tooth No. 38, Section A	842.6	1099.6
Tooth No. 39, Section A	1422.0	1267.1
Tooth No. 43, Section A	1239.5	2552.3
Tooth No. 53, Section A	1971.8	2735.9

Teeth number 19,22,38,39,43 and 53 with the averaged Δz-values out of five lesion scans readings (A-Z) taken in both carious lesion bands.

Δz1=Mean mineral lesion loss in the upper carious lesion band of the sample

Δz2=Mean mineral lesion loss in the lower carious lesion band of the sample

3.4.4 Visual Scoring.

The photographic images, which represent the buccal surface of the crown after exposure to the demineralisation solution, were examined for visual assessment using the von der Fehr Index. All procedures have been described previously in Chapter 2.

3.4.4.1 Intra-examiner Reliability.

The scores for the two examiners at the two different scoring sessions are represented for examiner A in Table 3.5 for the upper band and Table 3.7 for the lower band. For examiner B the results are represented in Table 3.6 for the upper and Table 3.8 for the lower band. These tables represent therefore the intra examiner reliability when using the von der Fehr Index. In those tables the diagonals represent the number of teeth which scored the same grade on both scoring sessions by the examiner concerned.

Table: 3.5 Intra-Examiner Agreement / Variability For Examiner In The Upper Lesion Band.

ROWS: RFUP 1 COLUMNS: RFUP 2

	10	15	20	25	All
10	3	2	0	0	5
15	1	8	5	0	14
20	0	1	1	3	5
25	1	10	4	6	21
30	0	3	7	4	14
All	5	24	17	13	59

RFPU 1 = Examiner 1 on the first occasion in the upper lesion band (rows)

RFUP 2 = Examiner 1 on the second occasion in the upper lesion band (columns)

No. of teeth scored the same grade on both sessions = 18

No. of teeth scored by a difference of 0.5 units = 20

No. of teeth scored by a difference of 1.0 units = 17

No. of teeth scored by a difference of > 1.0 units = 4

The total number of teeth scored on this occasion = 59

Table: 3.6 Intra-Examiner Agreement / Variability For Examiner B In The Upper Lesion Band.

ROWS: SCUP 1 COLUMNS: SCUP 2

	1.0	1.5	2.0	2.5	3.0	All
1.0	3	1	0	0	0	4
1.5	4	6	6	0	0	16
2.0	0	6	11	1	0	18
2.5	0	3	7	1	1	12
3.0	0	0	4	4	1	9
All	7	16	28	6	2	59

SCUP 1 = Examiner 2 on the first occasion in the upper lesion band (rows)

SCUP 2 = Examiner 2 on the second occasion in the upper lesion band (columns)

No. of teeth scored the same grade on both sessions = 22

No. of teeth scored by a difference of 0.5 units = 30

No. of teeth scored by a difference of 1.0 units = 7

No. of teeth scored by a difference of > 1.0 units = 0

The total number of teeth scored on this occasion = 59

Table: 3.7 Intra-Examiner Agreement / Variability For Examiner A In The Lower Lesion Band.

ROWS: RFLOW 1 COLUMNS: RFLOW 2

	0	1.0	1.5	2.0	2.5	All
0	2	0	0	0	0	2
1.0	0	5	2	1	0	8
1.5	0	4	2	6	0	12
2.0	0	1	7	3	3	14
2.5	0	3	4	2	2	11
3.0	0	0	3	6	3	12
All	2	13	18	18	8	59

RFLOW 1 = Examiner 1 on the first occasion in the lower lesion band (rows)

RFLOW 2 = Examiner 1 on the second occasion in the lower lesion band (columns)

No. of teeth scored the same grade on both sessions = 14

No. of teeth scored by a difference of 0.5 units = 27

No. of teeth scored by a difference of 1.0 units = 12

No. of teeth scored by a difference of > 1.0 units = 6

The total number of teeth scored on this occasion = 59

Table 3.8 Intra Examiner Agreement / Variability For Examiner B In The Lower Lesion Band.

ROWS: SCLOW 1 COLUMNS: SCLOW 2

	0	1.0	1.5	2.0	2.5	3.0	All
0	2	0	0	0	0	0	2
1.0	0	3	2	0	0	0	5
1.5	0	3	11	6	1	0	21
2.0	0	0	10	4	2	1	17
2.5	0	0	1	7	2	1	11
3.0	0	0	0	1	1	1	3
All	2	6	24	18	6	3	59

SCLOW 1 = Examiner 2 on the first occasion in the lower lesion band (rows)

SCLOW 2 = Examiner 2 on the second occasion in the lower lesion band (columns)

No. of teeth scored the same grade on both sessions = 23

No. of teeth scored by a difference of 0.5 units = 32

No. of teeth scored by a difference of 1.0 units = 4

No. of teeth scored by a difference of > 1.0 units = 0

The total number of teeth scored on this occasion = 59

3.4.4.2 Inter-examiner Reliability.

Table 3.9 and Table 3.10 represent the inter examiner agreement / variability for the scores of the upper and lower lesion bands. The diagonals show the number of teeth given the same grading by both examiners during the first and second occasion.

Table 3.9 Inter Examiner Agreement / Variability For Examiner A and B In The Upper Lesion Band.

ROWS: RFUP AV COLUMNS: SCUP AV

	0	1.00	1.25	1.50	1.75	2.00	2.25	2.50	2.75	3.00	All
0	0	0	0	0	0	0	0	0	0	0	0
1.00	0	1	2	0	0	0	0	0	0	0	3
1.25	0	2	0	0	1	0	0	0	0	0	3
1.50	0	0	1	3	2	1	1	0	0	0	8
1.75	0	0	0	0	3	3	1	0	0	0	7
2.00	0	0	2	3	3	2	1	0	0	0	11
2.25	0	0	0	0	0	5	1	1	3	0	10
2.50	0	0	0	0	2	3	4	3	1	0	13
2.75	0	0	0	0	1	0	0	1	1	1	4
All	0	3	5	6	12	14	8	5	5	1	59

Examiner (1) RFUP AV (rows) and examiner (2) SCUP AV (columns) with averaged values on the first and second occasion in the upper lesion band.

No. of teeth were examiners scored the same grade = 14

No. of teeth were the examiners scored by a difference of 0.5 units = 39

No. of teeth were the examiners scored by a difference of 1.0 units = 6

No. of teeth were the examiners scored by a difference of > 1.0 units = 0

The total number of teeth scored on this occasion = 59

Table 3.10 Inter Examiner Agreement / Variability For Examiner A and B In The Lower Lesion Band.

ROWS: RLOW AV COLUMNS: SRLOW AV

	0	100	125	150	175	200	225	250	275	300	All
0	2	0	0	0	0	0	0	0	0	0	2
100	0	1	3	0	0	1	0	0	0	0	5
125	0	2	1	2	1	0	0	0	0	0	6
150	0	0	0	1	3	0	0	0	0	0	4
175	0	0	1	5	6	2	2	0	0	0	16
200	0	0	0	2	2	1	1	1	0	0	7
225	0	0	0	0	3	1	3	0	1	0	8
250	0	0	0	1	1	1	2	3	0	0	8
275	0	0	0	0	0	0	1	0	1	1	3
All	2	3	5	11	16	6	9	4	2	1	59

Examiner (1) RFUP AV (rows) and examiner (2) SCUP AV (columns) with averaged values on the first and second occasion in the lower lesion band.

No. of teeth were examiners scored the same grade = 19

No. of teeth were the examiners scored by a difference of 0.5 units = 36

No. of teeth were the examiners scored by a difference of 1.0 units = 4

No. of teeth were the examiners scored by a difference of > 1.0 units = 0

The total number of teeth scored on this occasion = 59

Table 3.11 Statistic Values For The Reapetability Study.

Examiner	Band	Unweighted Kappa Values	Linear Weights	Quadratic Weights
A	upper	0.124	0.224	0.333
B	upper	0.161	0.382	0.592
A and B	upper	0.117	0.455	0.685
A	lower	0.058	0.272	0.470
B	lower	0.171	0.466	0.718
A and B	lower	0.208	0.583	0.811

3.4.4.3 Discussion.

The two examiners showed similar consistency in using the von der Fehr Index for the lesion assessment. Examiner B scored with slightly higher Kappa Values than examiner A (Tab. 3.11). Examiner B was a clinician and examiner A was a non-clinician although with extensive experience in the use of this index. Examiner B scored also more teeth at the same grade for the upper and the lower lesion band than examiner A. He also did not score teeth on either occasion with a greater difference than one unit either in the upper or the lower band, whereas examiner A did so on four occasions in the upper band and six occasions in the lower band.

In the lower band, both examiners achieved a more repeatable scoring result when results were averaged.

3.5 Regression Analysis Of Caries Monitor Readings - Correlations Between The Two Methods Of Assessments.

3.5.1 Introduction.

In this section the scores of the optical caries monitor will be compared with the more conventually used methods of microradiography and microdensitometry.

Correlations were be done between the Caries Monitor Readings (CrsMtr) and the Microdensitometry Readings (McrDns) as well as the regression of the

Microdensitometry Readings on each of Delta Z (DelZ), Surface Zone (SurZ), Lesion Body (LesB) and Lesion Depth (LesD).

3.5.2 Results.

Plots will be given in Figures 3.7-3.14 for upper and lower band seperately, and for a regression of the Caries Monitor reading on each of Delta Z, Surface Zone, Lesion Body are only poorly related with the Caries Monitor. With regard to the the R-Squared values (Fig. 3.12) the Surface Zone and Lesion Body are only poorly related to the Caries Monitor readings. Realistically, only Delta Z and Lesion Depth are sufficiently strongly related with the Caries Monitor reading to make a prediction possible.

Table 3.12 R-Squared Values Representing Relations Of Caries Monitor Readings With Other Data Obtained From Microdensitometry/Microradiography.

	upper lesion band	lower lesion band
Microdensitometry Score	0.554	0.590
Delta Z	0.554	0.594
Surface Zone	0.231	0.141
Lesion Body	0.154	0.042
Lesion Depth	0.423	0.558

For the evaluation of the microdensitometric data five different files were created. The files were defined as file number.1,2,3,4 and 5.

File No. 1 contained the data of six samples with all presented four caries monitor readings from the upper and five readings from the lower carious lesion band.

File No. 2 contained data from 30 teeth where at least one reading was taken from the upper or the lower carious lesion band.

File No. 3 contained the data of eight teeth where only one reading was available for upper and lower carious lesion band together.

File No. 4 contained data from 21 carious monitor readings in the upper carious lesions bands and nine readings in the lower, but they were divided into seperate data groups.

File No. 5 contained the data of six teeth with five caries monitor readings from the upper and five from the lower carious lesion bands but here only the mean values were taken into consideration for each of the five readings in each band to be compared to the mean values of the caries monitor readings in the same area.

For the separate statistical analysis of the upper and lower bands, the results of the files No. 5, No. 4 and No. 3 were combined. This meant that, for each band one average caries monitor reading and one average microdensitometer reading was

available and the upper and lower bands could be directly compared. In total, a number of 35 teeth with upper band readings and 23 teeth with lower band readings were available for analysis.

Caries monitor readings were regressed on the microdensitometry readings. The fitted lines for predicting the average caries monitor reading were as follows:

upper band: $0.7143 + (0.0009233 \times \text{microdensitometer reading})$;

lower band: $0.9830 + (0.0006697 \times \text{microdensitometer reading})$;

These lines are shown in the following plots as presented by Figure 3.3 for the upper and 3.4 for the lower band. The estimated slope fitted to the lower band is somewhat smaller than that for the upper band. The standard errors of these slopes (0.0001443 and 0.0001218 respectively) suggest that the slopes of the fitted lines are not significantly different from one another. Also shown on the plots are confidence bands (the inner curves) and prediction bands (the outer curves). These can be compared in two ways.

First, the prediction bands can be used to predict the likely caries monitor reading of a lesion with a given microdensitometric value. This is illustrated in the diagram of Figure 3.5. A lesion with a microdensitometer reading of 2000 in the upper band would be most likely to have a caries monitor reading of about 2.5. However, due to the experimental error in the method, the caries monitor reading for such a lesion could be between 1.5 and 3.5.

Second, the confidence bands can be used for calibration. In other words, to help to determine the microdensitometry reading of a lesion with a known caries monitor reading. This is illustrated in Figure 3.6, for a lesion with a caries monitor reading of 2.4 in the lower band. Reading in the horizontal direction , this is compatible with a microdensitmetry reading from about 1700 to 2600.

3.5.3 Correlations Between Caries Monitor Scores And Delta Z.

Figure 3.7 gives the plot of the caries monitor scores for the upper band against the depth of the lesion. The correlation is 0.554.

Figure 3.8 gives the plot of the caries monitor scores for the lower band against the depth of the lesion. The correlation is 0.594.

3.5.4 Correlation Between Caries Monitor Scores And The Surface Zone.

Figure 3.9 gives the plot of the caries monitor scores for the upper band against the surface zone. The correlation is 0.231.

Figure 3.10 gives the plot of the caries monitor scores for the lower band against the surface zone. The correlation is 0.141.

3.5.5 Correlation Between Caries Monitor Scores And The Lesion Body.

Figure 3.11 gives the plot of the caries monitor scores for the upper band against the lesion body. The correlation is 0.154.

Figure 3.12 gives the plot of the caries monitor scores for the lower band against the lesion body. The correlation is 0.042.

3.5.6 Correlation Between Caries Monitor Scores And The Lesion Depth.

Figure 3.13 gives the plot of the caries monitor scores for the upper band against the lesion depth. The correlation is 0.423.

Figure 3.14 gives the plot of the caries monitor scores for the lower band against the lesion depth. This correlation is 0.558.

3.6 Disscusion.

The correlation of optical caries monitor readings and microdensitometric / microradiographic readings could be established. With regard to the results and data given by microdensitometry readings only two out of four aspects of the lesion represented in the given data could proove a statistical correlation when compared to the caries monitor readings. These were Delta Z and Lesion Depth

which could be associated with caries monitor scores whereas Lesion Body and Surface Zone failed to provide significant links to them.

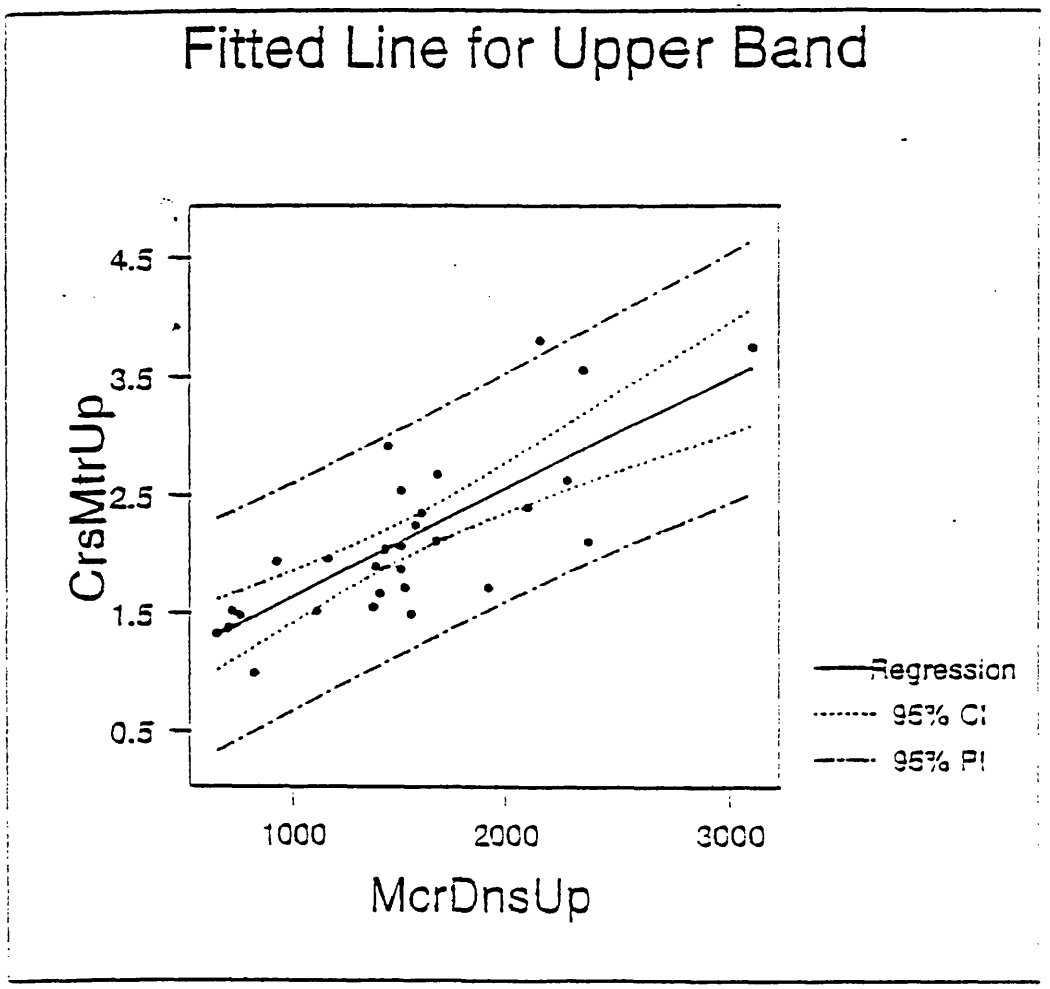


Figure 3.3 **Plot of Caries Monitor upper readings vs Microdensitometry upper readings.**

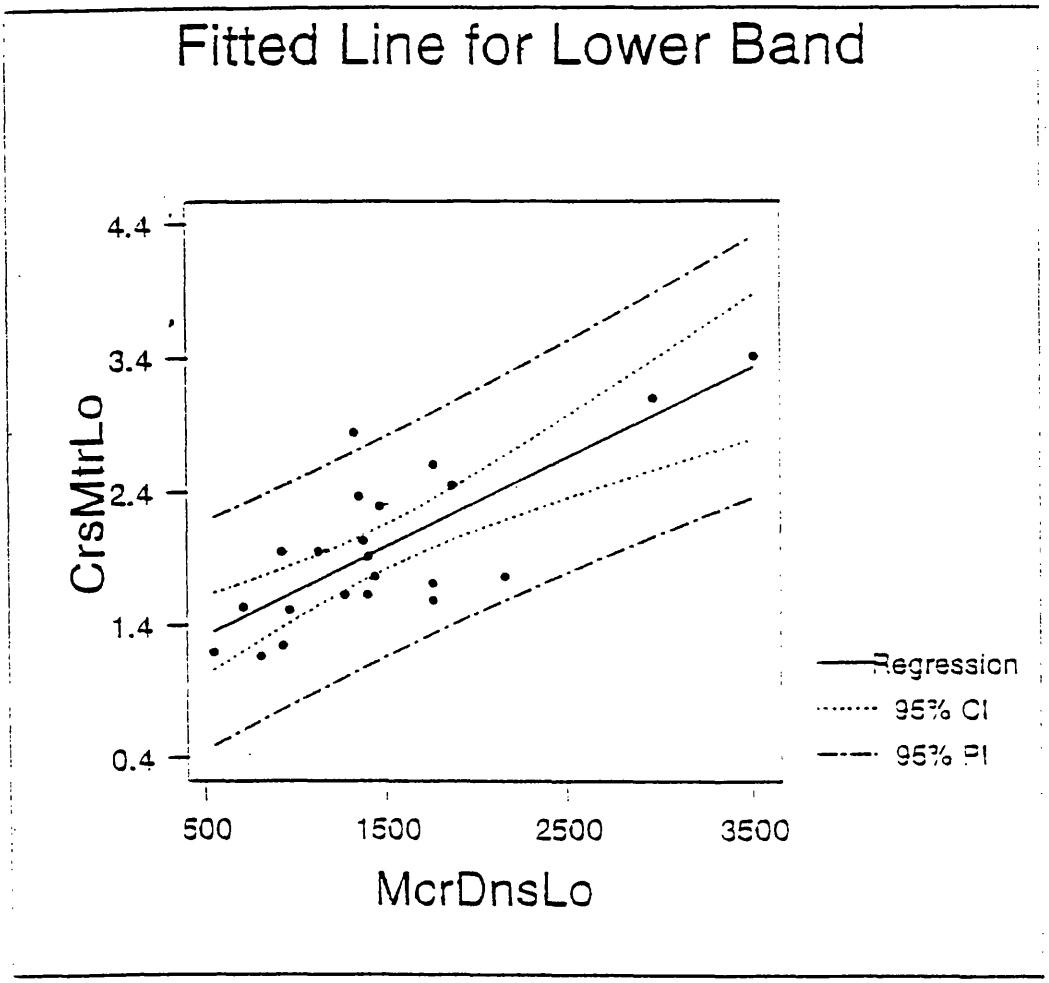


Figure 3.4 **Plot of Caries Monitor lower readings vs Microdensitometry lower readings.**

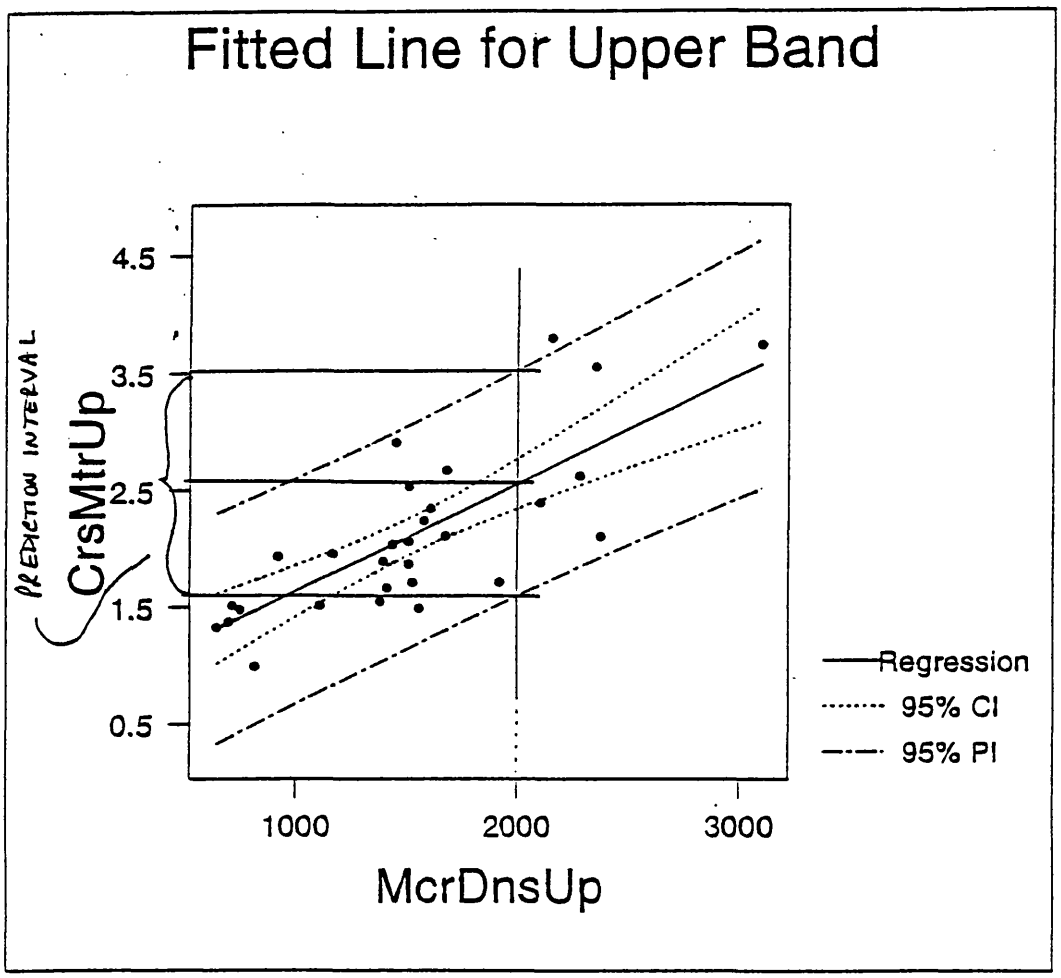


Figure 3.5 **Plot of Caries Monitor upper readings vs Microdensitometry upper readings (Prediction Interval).**

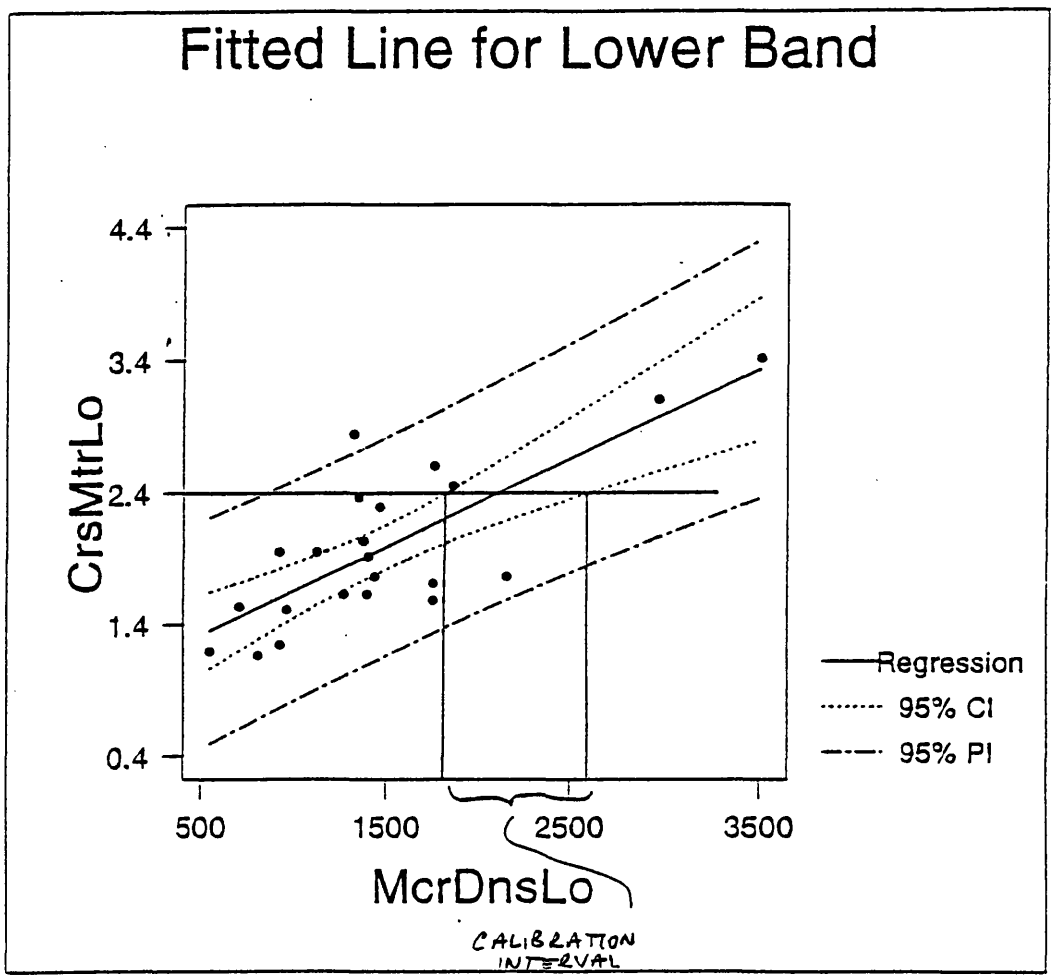


Figure 3.6 **Plot of Caries Monitor lower readings vs Microdensitometry lower readings (Calibration Interval).**

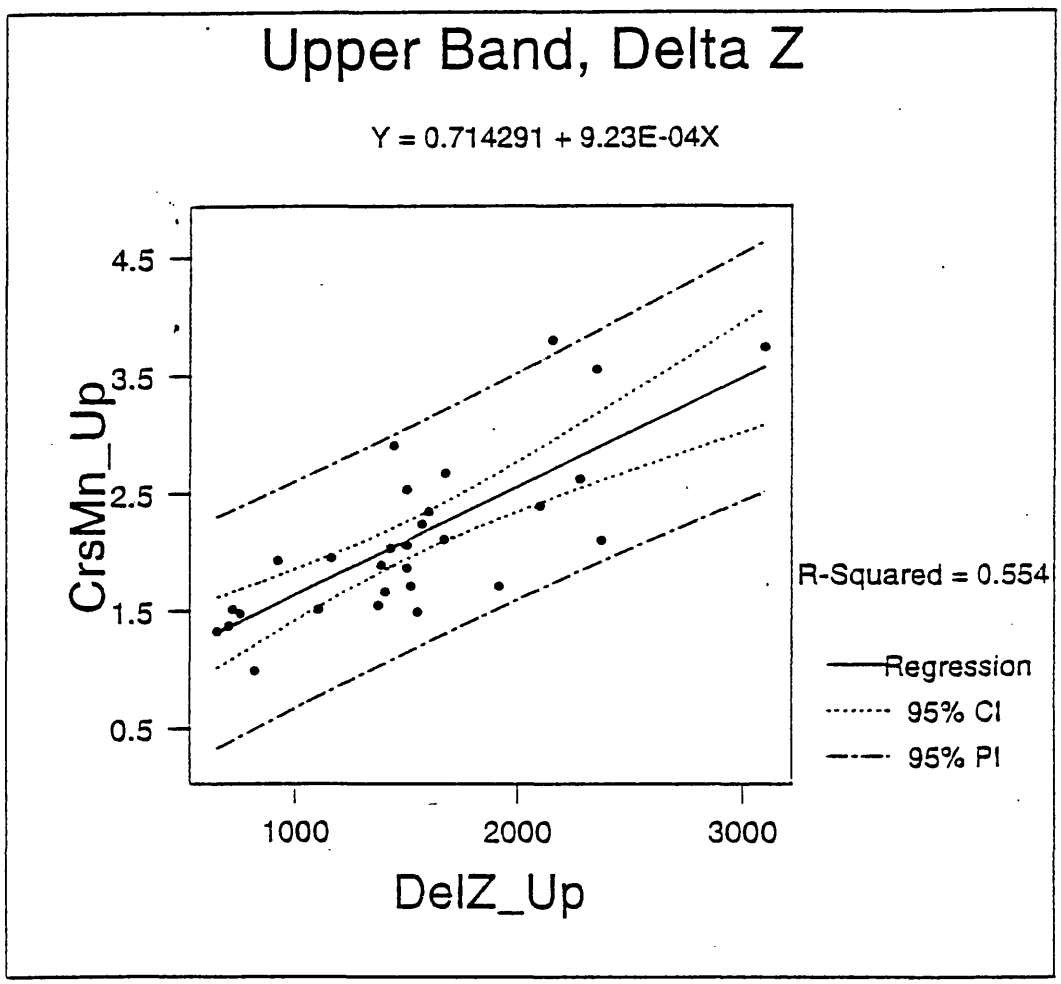


Figure 3.7 **Plot of Caries Monitor upper readings vs Delta Z upper readings.**

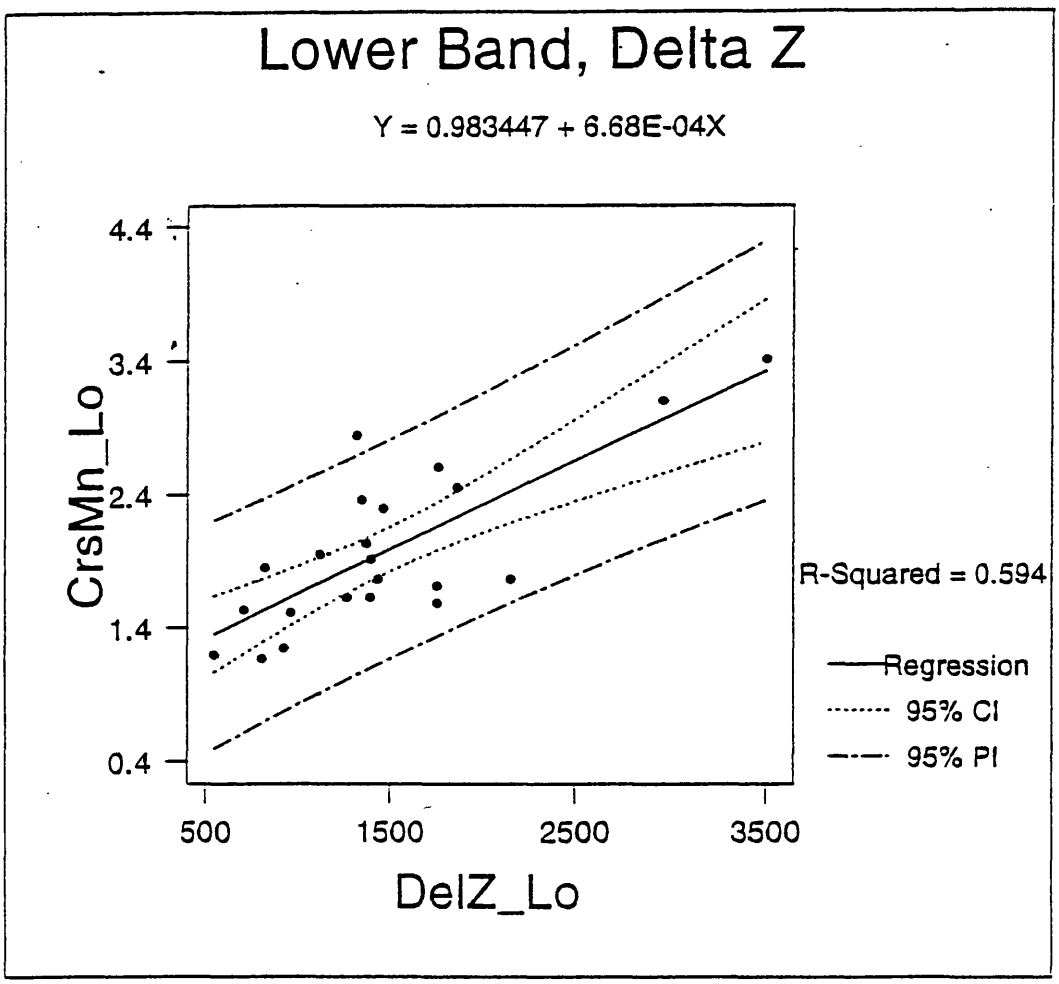


Figure 3.8 **Plot of Caries Monitor lower readings vs Delta Z lower readings.**

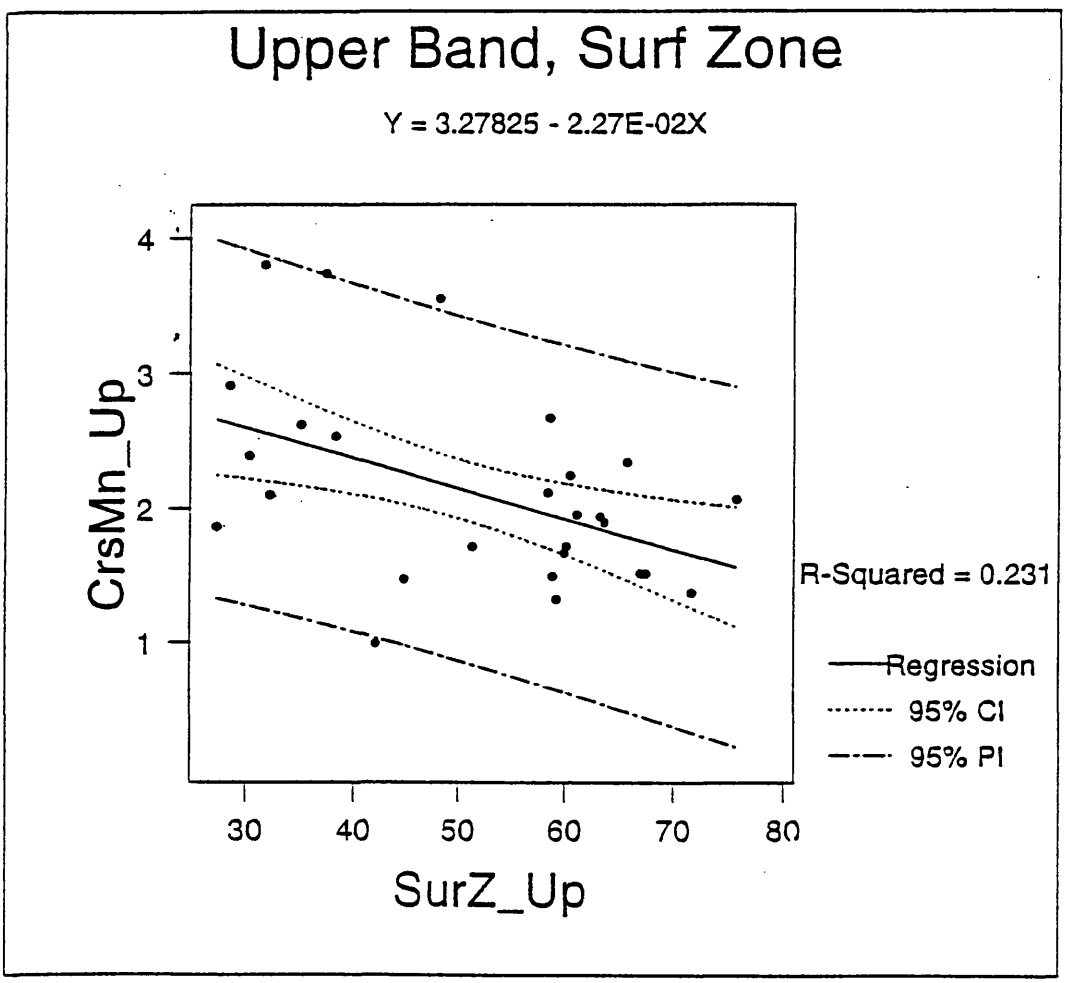


Figure 3.9 **Plot of Caries Monitor upper readings vs Surface Zone upper readings.**

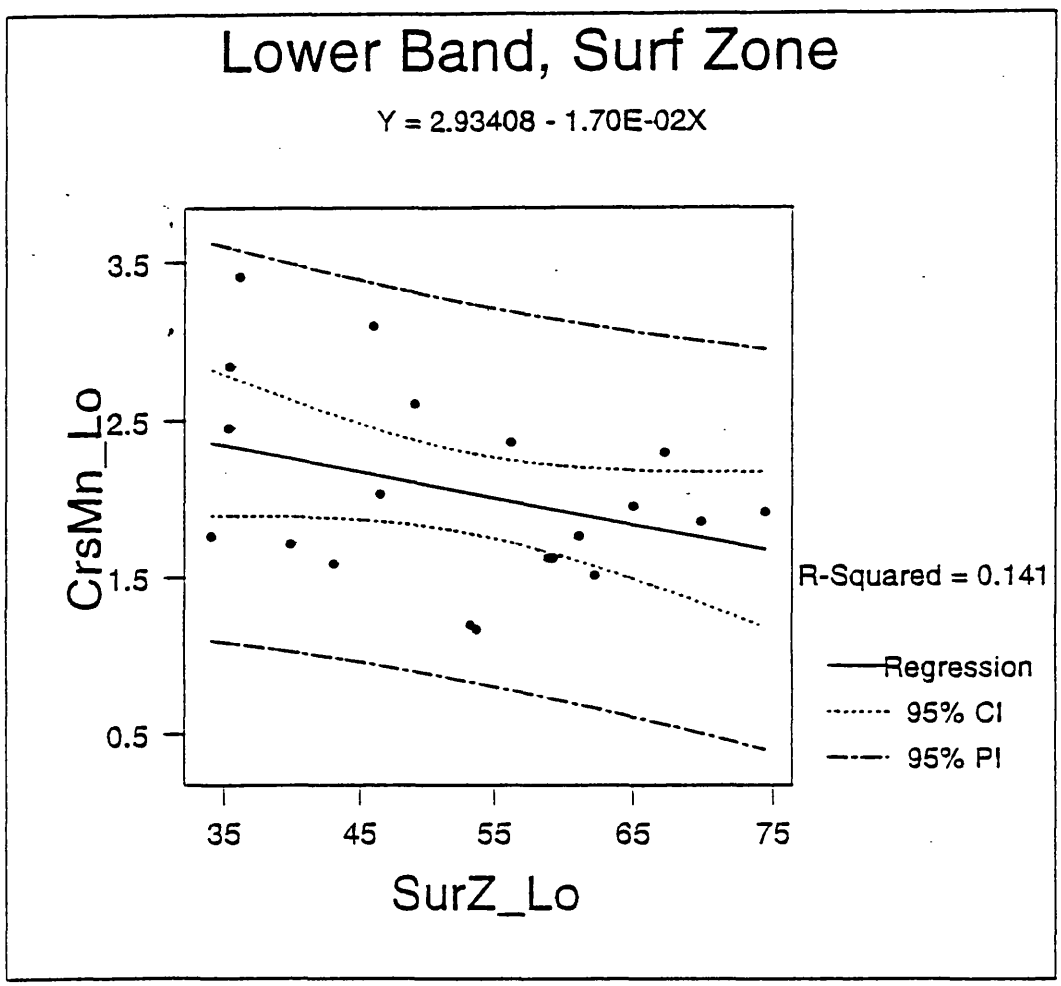


Figure 3.10 **Plot of Caries Monitor lower readings vs Surface Zone lower readings.**

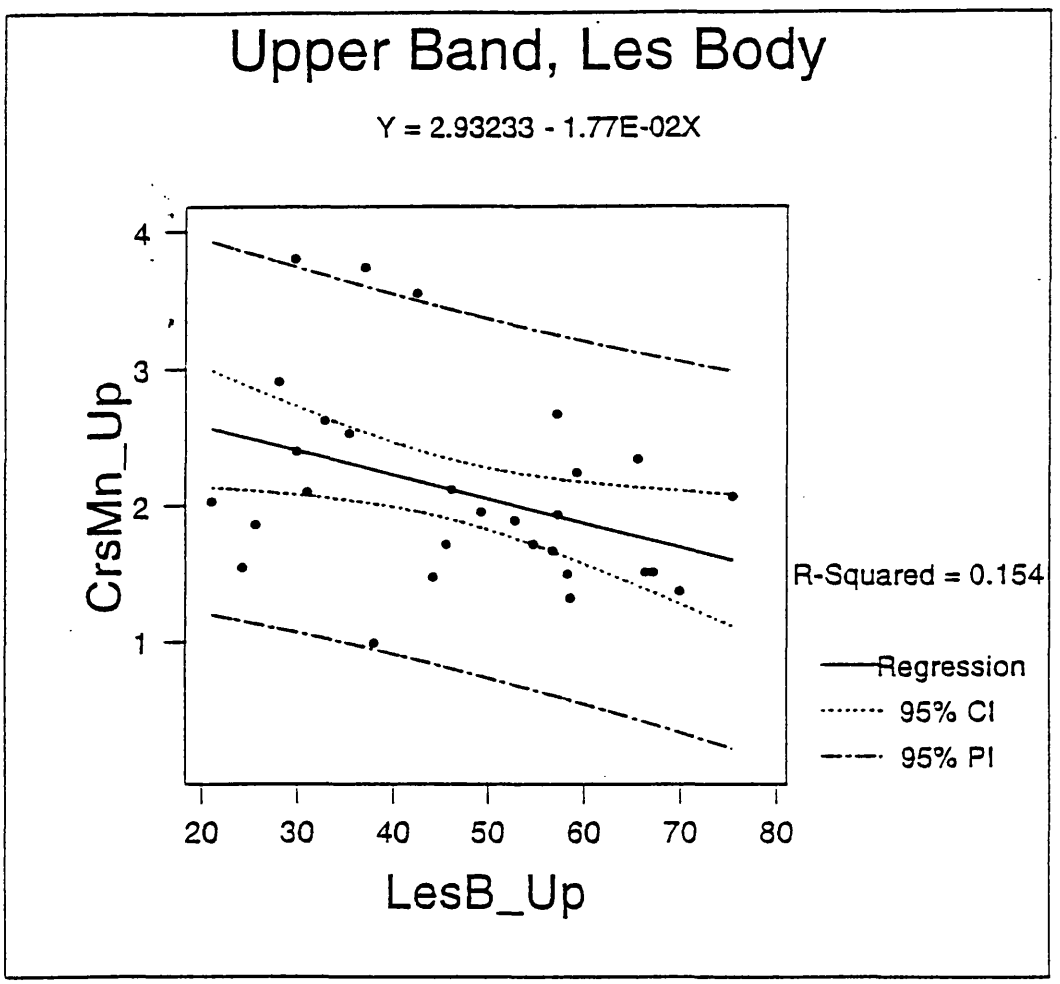


Figure 3.11 **Plot of Caries Monitor upper readings vs Lesion Body upper readings.**

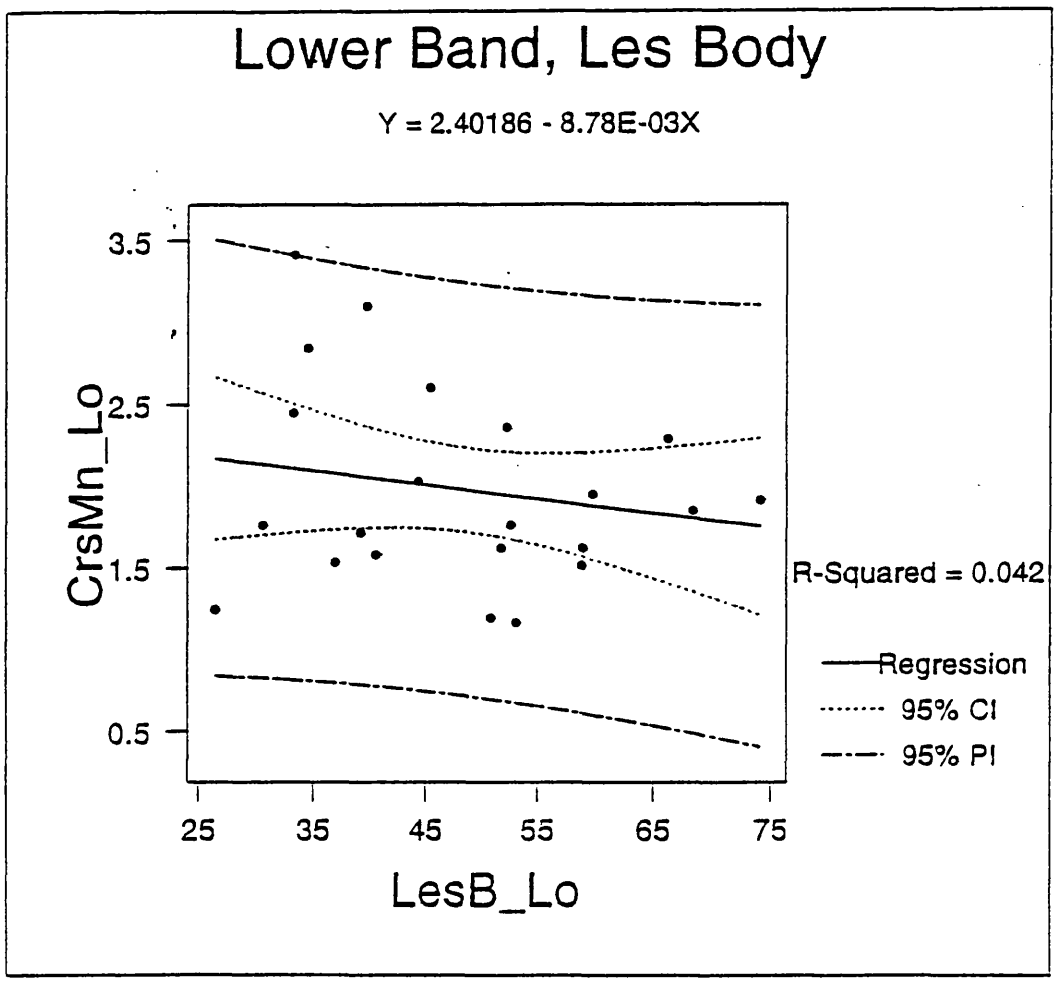


Figure 3.12 **Plot of Caries Monitor lower readings vs Lesion Body lower readings.**

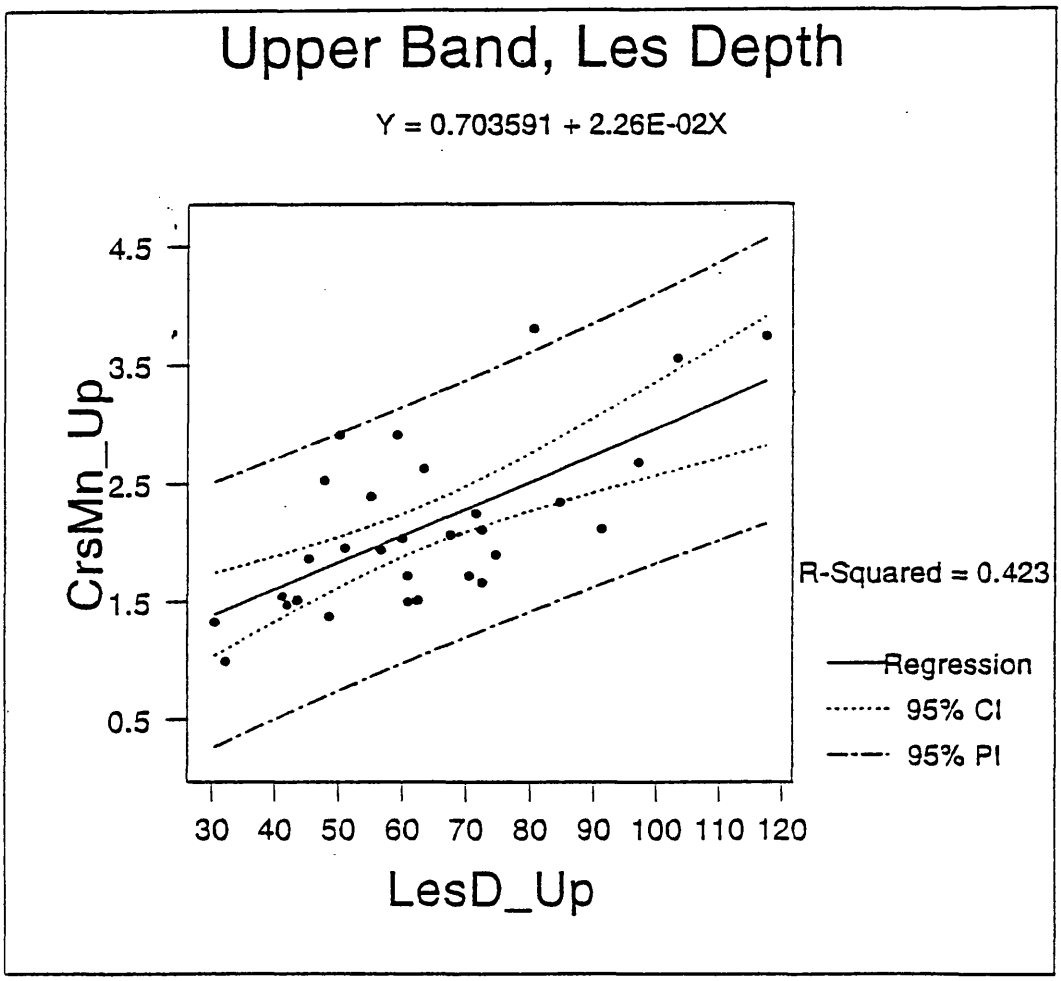


Figure 3.13 **Plot of Caries Monitor upper readings vs Lesion Depth upper readings.**

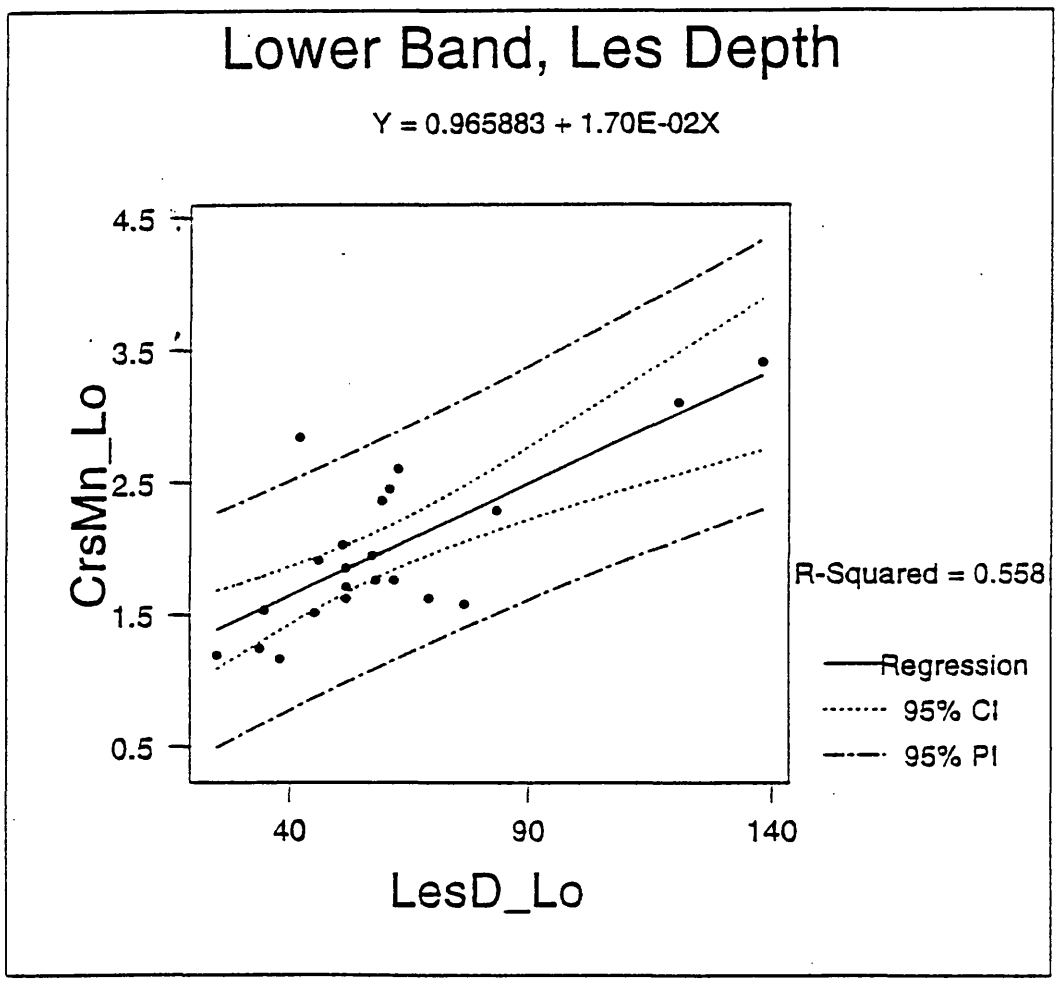


Figure 3.14 **Plot of Caries Monitor lower readings vs Lesion Depth lower readings.**

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

4.1 Introduction.

The findings and conclusions of the investigations and the experimental studies will be discussed in this Chapter.

A brief discussion has already been presented in the previous Chapter which described the results of the experimental procedures and the subsequent findings. Furthermore, recommendations which resulted from the experience of having worked with the optical caries monitor during the investigation and might contribute to further improvements regarding its handling and use and are given at the end of this section.

4.2 Conclusions.

1. The optical caries monitor is a reliable instrument for measuring changes in the structure of enamel mineralisation / demineralisation. It showed good results in differentiating between samples of decalcified and non-decalcified tissue samples in terms of good reproducibility and repeatability.

2. Microradiography / microdensitometry also proved to be a method for providing a good quality of reproducible readings which allowed the measurement of the changes in mineral tissue due to decalcification.

3. The von der Fehr Index seems to be a method of lesion assessment which can give a fairly consistent assessment of visual changes caused by enamel surface demineralisation. Nevertheless, its use was examiner-dependent, with better results coming from an examiner with a wide range of clinical experience in this subject. Only when the scores of both examiners were averaged did the comparability as well as the variability decrease.

4. From previous studies with the optical caries monitor, it was suggested that at least five measurements should be scored with the caries monitor and at least three readings with the microdensitometric method. In this study, caries monitor readings were collected at four different locations and repeated four times on each of the two bands to obtain a mean value that was compared to a microdensitometric mean value which resulted from five different readings per band. Furthermore the total number of samples should not be lower than 50. A possible explanation for this is the likliehood of sections being unacceptable for further evaluation due either to cavitation at an early stage of the investigation, or a loss of surface enamel during the section grinding process.

5. The correlations between the two methods in their ability to monitor the changes in enamel surface mineralisation provided suitable results in two out of the

four parameters. A correlation between the caries monitor readings and the microdensitometry readings could be established, as well as the Delta Z and Lesion Depth proved to be parameters comparable to caries monitor readings. Despite these results, Lesion Body and Surface Zone remain the two parameters which failed to be statistically correlated to the optical caries monitor readings.

The resuts from the visual scoring assessment were not strong enough to encourage a direct comparison to the two other methods of assessment and were very examiner dependent. It has to be concluded therefore from the results of the study that the visual scoring method does not provide a reliable means of estimating the amount of mineral change that had occured.

6. From the practical experience during the handling of the optical caries monitor, it has to be concluded that it is a valuable tool for research purposes with regard to the fact that it has to be handled with great care. Change in measurement results if the needle is only moved about 50% of the width of its diameter (~1mm) on the same measurement location. This can cause large differences with regard to an individual score. For clinical purposes, this demands a great deal of precision to ensure that the probe is placed on exactly the same spot. This will undoubtably prove to be an unrealistic condition for clinical every day use. Another point would be the size and proportion of the probe and measuring head which are, at the moment, too large and bulky to be used for an assessment within the oral cavity. Also, with the needle not being flexible this remains another obstacle with regard to the various areas of potential use to which it would be exposed in clinical

performance. The issue of calibrating the monitor is another problem regarding its clinical application because of the amount of time required for this process.

4.3 Recommendations.

The caries process and its early formation as a white spot lesion remains one of the greatest challenges for dentistry. This is especially so for orthodontics. The success of improving the dental irregularities of a dentition by fixed appliance treatment should not be endangered by the occurence of carious lesions which may be a threat to longevity, aesthetics and stability of a dentition. The concern for the orthodontist is the difficulty to predict patients', ability to maintain a high degree of oral hygiene which can be achieved during the period of treatment and the stability and continuity of its quality throughout a lengthy period, sometimes more than two years. Even the most motivated patient can show signs of decline in terms of oral hygiene after the treatment with fixed appliances has been started. The decision to stop fixed appliances treatment, which would automatically involve a debond of the fixed appliance is only taken in cases where there has been a dramatic decline in patient cooperation. Fortunately, this happens only rarely. Usually the use of oral hygiene instruction and motivation combined with the application of fluoride rinses and fluoridated toothpastes helps to minimize the risks considerably.

To monitor the development or presence of white spot lesions a reliable and efficient diagnostic tool is desirable for the early detection of surface changes in

enamel mineralisation. This could be further extended to the use of controlling and surveying the effectiveness of preventive and therapeutic measures.

The great advantage of the optical caries monitor compared to other methods is its non-destructive, non-invasive nature. Furthermore, the patient is not exposed to the use of x-ray radiation, allowing repeatable use, independent to any limitations that may exist due to medical conditions and without concern for long term consequences. The apparatus is harmless to tooth, patient and clinician. These aspects, therefore, give a sound basis for the use of frequent long term monitoring of a patients dental state. However, for its clinical use it has been suggested that the following recommendations would improve its ease of use and encourage it to be used routinely.

1. The design and shape of the optical caries monitor has to be improved to allow an easier handling during its clinical use. A much more versatile design is desirable for intra-oral usage and storage purpose at the chair side.

2. The capability to produce repeatable results is entirely dependent on the correct placement of the probe-head. This must always be in a perpendicular direction to the centre of the buccal tooth surface, and in close contact. The length and inflexibility of the needle attached to the measuring head does not allow any other approach than from the anterior labial segment if it is to be brought into contact with a sample. This makes it almost impossible to use it to monitor any lingual, palatal, mesial or distal surfaces, especially of those teeth which are located

in the buccal segments with regard to the molar fields in the mandible and maxilla. The presence of orthodontic attachments would make mesurements even more difficult in this respect. From experience with the fluctuation of readings when the probe was hand held onto an *in vitro* sample without any interference from soft tissues in earlier trials, this study tried to eliminate any interference from this source by fixing not only the probe but also the sample as much as possible. To gain a maximum reliability in an attempt to guide the probe onto the same spot when a repeated measurement was taken probe and sample were fixed in the apparatus shown in figure 2.9. These conditions though are not applicable for clinical use.

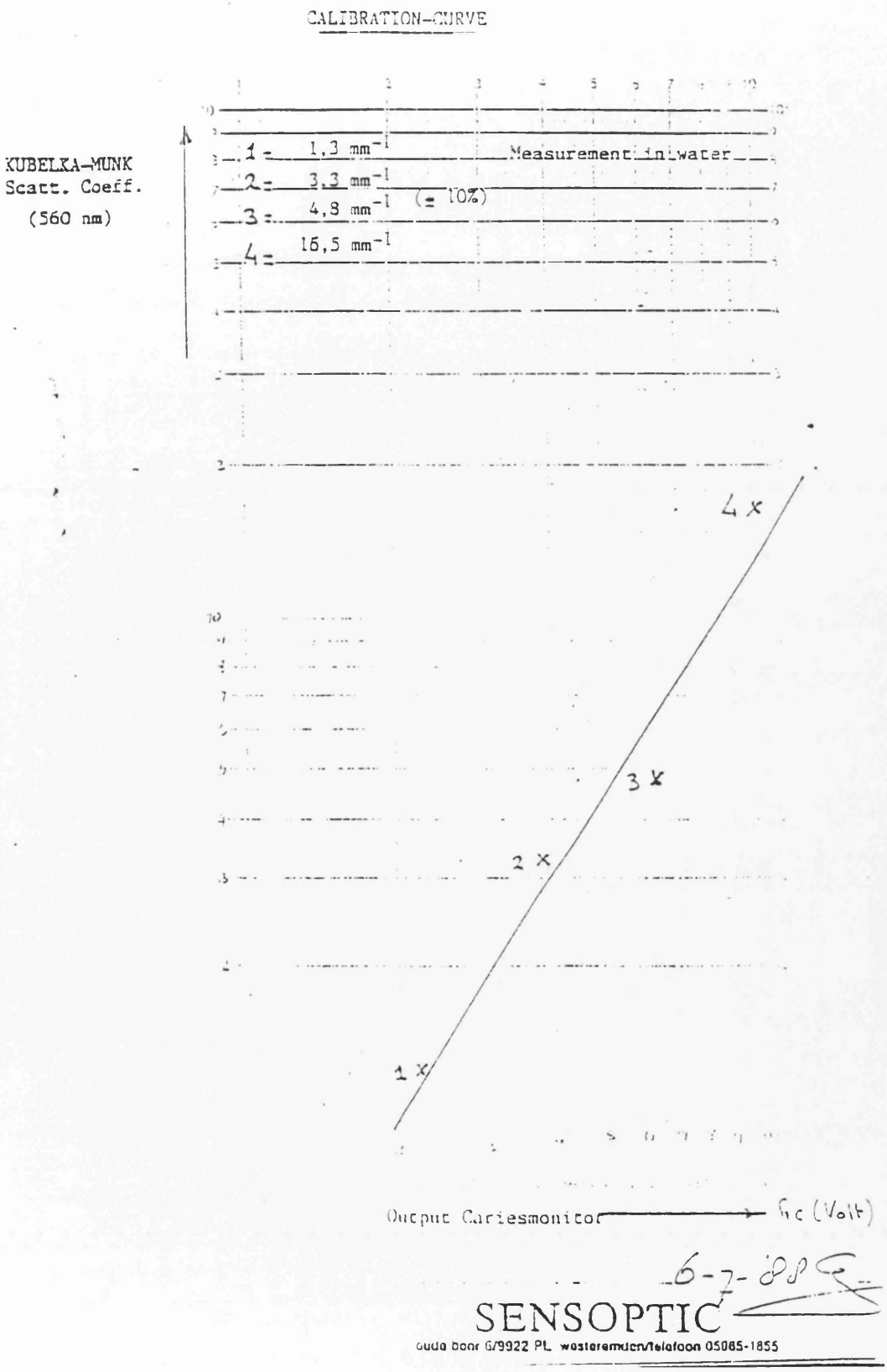
3. The calibration procedure remains one of the greatest practical obstacles for its clinical use as a diagnostic aid. The time period of 30 min. which has to be spent to achieve a level of calibration that gives a reliable measurement is not a realistic condition for routine clinical use. Especially as the readings then have to be entered into a computer software program that converts the back-scattered intensity for comparison between different sets of readings. A set up which would eliminate this process is highly desirable and would save time and manpower. In this study, it was observed that the calibration fields exposed different results on different areas within the same calibration field. To get more reproducible results to control the performance of the appliance a grid was developed that was superimposed on the calibration field to have comparable data between different calibrations. A higher grade of homegeneity of the quality of the four stable plastics would be desirable.

4. For future investigations it might be interesting to compare directly preselected well-defined areas of the lesion with a maximum size of 1.5mm diameter for caries monitor readings and afterwards select the same areas for microradiographic investigation. This would relate the measurements directly to each other instead of averaging the readings achieved in the same lesion band or microradiographic scan. The problem is that the caries monitor readings represent a score for an area of 1000 μm whereas the microdensitometry reading is restricted to only 130-160 μm, which is less than a fifth of the caries monitor area. It might be possible to get a better correlation between microradiographic readings and microdensitometric readings if the areas were similar.

5. An interesting field of research in this area might be the evaluation of effects achieved by remineralising lesions by the use of fluoride application and compare the readings of the caries monitor before and after. Now that it was proven that it can differentiate between demineralised and non-demineralised tissue it might be possible to investigate this further.

APPENDICES

APPENDIX A


CALIBRATION-CURVE
KUBELKA-MUNK
Scatt. Coeff.
(560 nm)
1 = 1,3 mm-1
2 = 3,3 mm-1
3 = 4,8 mm-1
4 = 16,5 mm-1
Measurement in water
(± 10%)
4 x
3 x
2 x
1 x
Output Cariesmonitor
Uc (Volt)
6-7-88
SENSOPTIC
Oude Baan 6/9922 PL Westeremden/Telefoon 05965-1855

APPENDIX B

CARIES INDEX SYSTEM (VON DER FEHR, 1961)

The criteria for the use of the Caries Index System proposed by von der Fehr for assessment of the opacity of white spot lesions are as follows:

Score 0: Surface appears intact.

Score 1: Limited greyish tinge, with or without accentuated perikymata.

Score 2: Perikymata well accentuated, in some areas confluencing into greyish white spots.

Score 3: Pronounced white decalcification.

Lesions that did not fit definetively into one of the above stages were given intermediate scores (0.5, 1.5, or 2.5).

The Caries Index for the subject was calculated as the average for the areas examined.

Appendix C

VISUAL SCORING SHEET

1	16	31	46
2	17	32	47
3	18	33	48
4	19	34	49
5	20	35	50
6	21	36	51
7	22	37	52
8	23	38	53
9	24	39	54
10	25	40	55
11	26	41	56
12	27	42	575
13	28	43	8
14	29	44	59
15	30	45	-

APPENDIX D

DERIVATION OF THE EQUATION BY ANGMAR *ET AL.* (1963)

The grey level for any point in the lesion, which has resulted from x-ray absorption by both organic and inorganic components (thickness t_m and t_o respectively), can be equated against an equivalent aluminium (Al) grey level. Hence, for a particular level of absorption of x-ray (i.e. the grey value of a part of the lesion), the absorption can be equated against the absorption in an aluminium stepwedge.

Thus

$$\mu_a \cdot t_a = \mu_m \cdot t_m + \mu_o \cdot t_o$$

where

μ_a = linear absorption coefficient of the aluminium.

μ_m = linear absorption coefficient of the mineral component.

μ_o = linear absorption coefficiant of the organic component.

t_m = thickness of the minerral element

t_o = thickness of the organic element

t_a = equivalent thickness of aluminium to give that grey value

but

$$t_o = t_o + t_o$$

where

t_s = section thickness

and where

V_m = volume of mineral component

$$\frac{V_m}{V_s} = \frac{t_m}{t_s}$$

V_s = section volume

thus

$$\frac{V_m}{V_s} \times 100 = 100 \; \frac{(\mu_a \cdot t_a - \mu_o \cdot t_o)}{(\mu_m - \mu_o)\, t_s}$$

The absorption coefficients depend on the radiation source (eg. kV, target, filter, etc.). Angmar *et al.* (1963) employed Cu Kα radiation; μ_a, μ_m and μ_o were found from known data and thus the equation reduces to:

$$\% \text{ vol. min.} = \frac{52.77}{t_s} \times t_a - 4.54$$

Thus the only unknown is t_a, since t_s can be measured. Therefore, for every point in the enamel, the equivalent aluminium thickness (t_a) is derived and percent volume mineral caculated.

APPENDIX E

DEMINERALISING SOLUTION FOR THE CREATION OF ARTIFICIAL LESIONS IN ENAMEL

	1 L	5 L
2mM Calcium chloride (anhydrous) $CaCl_2$	0.222 g	1.11 g
2mM Sodium dihydrogen orthophosphate (anhydrous) NaH_2PO_4	0.24 g	1.2 g
Deionised (or destilled) water	800 ml	4000 ml
50 mM Glacial Acetic Acid	2.87 ml	14.35 ml

Weigh out the Ca and P and place it in a beaker. Add about 800 ml (or 4000 ml) of de-ionised water and dissolve with stirring. Add the acetic acid and adjust the pH to 4.55 with 0.1 m sodium hydroxide solution. After cooling, pour into a volumetric flask. Rinse the beaker with a little de-ionised water and add the contents of the flask. Add 1ml of 10 ppm F per litre of solution to give a final concentration of 0.01 ppm F. Make up to the mark and decant into a plastic container of suitable size. Store at 4°C until required for use.

1M Sodium hydroxide solution

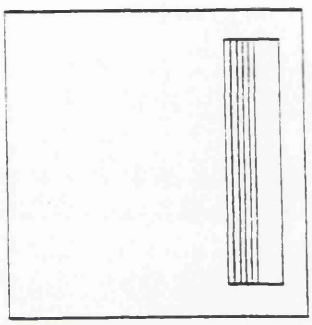
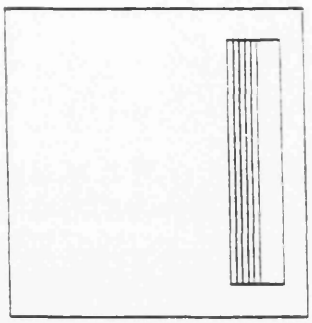
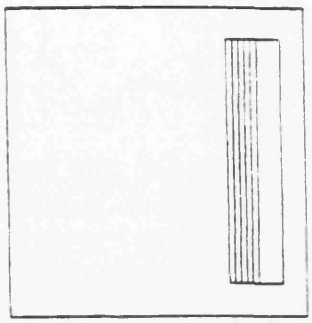
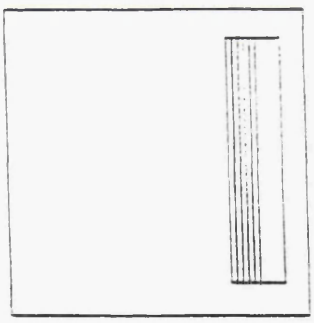
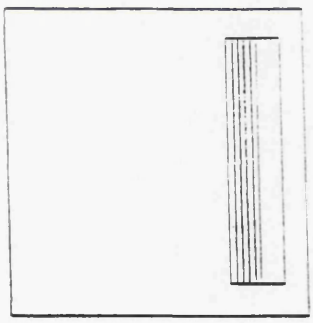
Sodium hydroxide pellets	4 g
Deionised (or distilled) water	100 ml

Dissolve the pellets slowly in the water, stirring continously. After cooling, decant the solution into a plastic bottle, Sodium hydroxide absorbs carbon dioxide from the atmosphere and this solution should be made up as soon as possible before use.

All solutions should be labelled with the relevant information, initialled and dated, before storage.

APPENDIX F

RADIOGRAPHIC PLATE DETAILS

_____  Date _____ _____ kV _____ mA Time _____	_____  Date _____ _____ kV _____ mA Time _____	_____  Date _____ _____ kV _____ mA Time _____
_____  Date _____ _____ kV _____ mA Time _____	_____  Date _____ _____ kV _____ mA Time _____	_____  Date _____ _____ kV _____ mA Time _____

APPENDIX G

SECTION DETAIL SHEETS

Tooth code:

Date specimen received: / / . Source: Tooth:

Section	Thickness	Date of preparation

Tooth code:

Date specimen received: / / . Source: Tooth:

Section	Thickness	Date of preparation

APPENDIX H

Calibration Scoring Sheet Of The Four Stable Plastic Fields

On Nine Different Measuring Points

Specimen Number	Plate	Section Number	Thickness	Wedge Filename	Image/Results Filename

APPENDIX I

Scoring Sheet For Recording The Section Number, The X-ray Number, The Section Thickness And The Wedge File Number

Date		Callibration	No		for tooth No
Run	Field 1	2	3	4	
1					
2					
3					
4					
5					
6					
7					
8					
9					
Mean					

Date		Callibration	No		for tooth No
Run	Field 1	2	3	4	
1					
2					
3					
4					
5					
6					
7					
8					
9					
Mean					

Date		Callibration	No		for tooth No
Run	Field 1	2	3	4	
1					
2					
3					
4					
5					
6					
7					
8					
9					
Mean					

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