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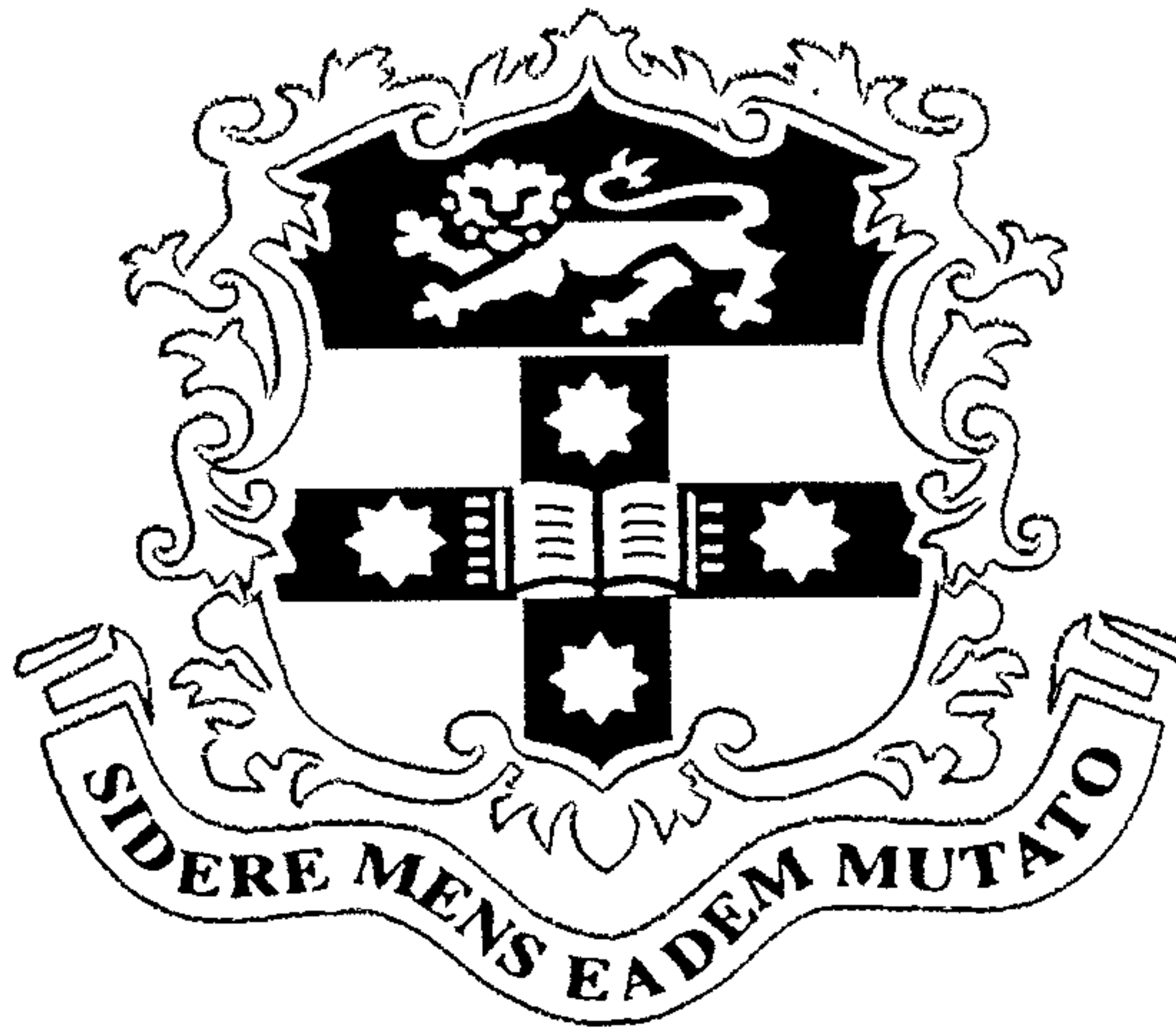
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# Erosion of Primary Teeth

Erin Kathleen Mahoney

BDS (Otago) FRACDS

A thesis submitted in partial fulfilment  
of the requirements for the degree of  
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---

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### Erosion of Primary Teeth

**Introduction:** Tooth wear is a normal physiological process and its cause is most often multifactorial. It can result from erosion, attrition, abfraction and/or abrasion. In children generally erosion is thought to be the dominant process. One of the principle causes of erosion is the consumption of acidic beverages. *In vitro* testing has dominated the recent literature on determining the erosive potential of these acidic beverages although each *in vitro* method is subject to significant experimental error.

**Aim:** The aim of the first part of this study was to determine the hardness and elastic modulus of primary enamel and dentine using a novel Ultra Micro Indentation System (UMIS) and determine its appropriateness for dental research. The aim of the second part of this study was to observe the change in hardness and modulus of elasticity of primary enamel and dentine upon exposure to potentially erosive beverages and finally to attempt to relate the degree of change in mechanical properties to the chemical composition of the test beverages.

**Material and Methods:** For the first part of the experiment eight mandibular first primary molar teeth were sectioned, set in resin and polished. In each tooth, thirty indentations were made in both the enamel and the dentine using a Berkovitch indenter. Fifteen of these indents were subject to a load of 50 mN and fifteen indents to as load of 150 mN. An automated computerised system

converted the force/penetration graph for each indentation in to a hardness vs depth graph from which values for the mean hardness and elastic modulus were calculated. For the second part of the study 20 primary mandibular molar teeth were prepared as for part one of this study. Each tooth then had fifteen indents in each of enamel and dentine at a load of 50 mN. Each tooth was immersed for 10 minutes in one of the following beverages- distilled water (control), Ribena Toothfriendly tropical flavour, Orange Juice, Orange Cordial and Fanta. After immersion the teeth were re indented as before. The statistical analysis of the change in hardness was determined from the hardness vs depth graphs produced by the automated computer system.

**Results:** From experiment one, the hardness of enamel ranged from (4.66 +/- 0.23) GPa to (5.15 +/- 0.53) GPa under a load of 50 mN and (4.67 +/- 0.18) GPa to (5.12 +/- 0.34) GPa under a load of 150 mN, whilst the hardness of dentine ranged from (0.78 +/- 0.07) GPa to (1.07 +/- 0.07) GPa under 50 mN load and (0.76 +/- 0.03) GPa to (1.03 +/- 0.08) GPa under 150 mN. Young's modulus for enamel varied from (74.47 +/- 2.85) GPa to (99.94 +/- 3.95) GPa at 50 mN and (73.31 +/- 3.53) GPa to (95.15 +/- 8.36) GPa under the 150 mN load. For dentine the modulus varied from (18.23 +/- 0.81) GPa to (23.97 +/- 0.72) GPa at 50 mN and (16.39 +/- 0.31) GPa to (22.53 +/- 1.03) GPa at a 150 mN load. The mean hardness of enamel and dentine was calculated to be (4.88 +/- 0.35) GPa and (0.92 +/- 0.11) GPa respectively. The average modulus of elasticity for enamel and dentine was (80.35 +/- 7.71) GPa and (19.89 +/- 1.92) GPa respectively. Using linear regression analysis a significant relationship could be shown between the hardness and the elastic modulus for both enamel

and dentine when loaded to 150 mN but only for dentine at 50 mN ( $p < 0.01$ ). The second part of the study revealed that the teeth immersed in distilled water showed close to zero change in hardness and modulus of elasticity. Of the other beverages, the hardness reduction in enamel varied from 2.7% for Orange Juice to 47.7% for Fanta. In dentine the reduction in hardness varied from 4.5% for Ribena to 28.2% in Fanta. In enamel the reduction in modulus varied between 0.3% for Ribena to 23.7% for Fanta. In dentine the reduction in modulus varied between 1.0% for Ribena to 16.0% for Orange Juice. Fanta and Orange Cordial were the only test beverages to show a statistically significant reduction in the enamel hardness in comparison to the control and only Fanta showed a statistically significant reduction in dentine hardness. None of the test beverages statistically reduced the enamel or dentine modulus of elasticity. Using stepwise multiple regression analysis the pH of the beverage followed by the phosphate concentration were the most statistically significant factors in predicting erosive potential in enamel. For dentine, the calcium content and titratable acidity were the most statistically significant factors in predicting erosive potential. The statistical analysis showed that fluoride concentration could be used in predicting the erosive potential of a beverage on enamel only.

**Conclusion:** The UMIS offers a simple and reproducible method of measuring basic mechanical properties of small samples of enamel and dentine and has application in determining the change in hardness and modulus of elasticity with exposure to potentially erosive beverages. The pH, titratable acidity, fluoride, phosphate and calcium content of beverages are important factors in determining the potential erosiveness of an acidic beverage.

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# Chapter 1. LITERATURE REVIEW

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## Introduction

With the reduction in the caries experience of children in the developed world there is an increasing awareness of tooth tissue loss. Of interest in the paediatric dental population, due to its reported high prevalence is erosion. The focus of this literature review will be dental erosion.

The first section of this literature review will briefly define the terms of tooth wear before focusing on the prevalence and aetiology of dental erosion. The prevalence of dental erosion will be discussed for both children and adults. Dental erosion can have an intrinsic or extrinsic cause and the aetiologies of these are discussed in turn.

The second section of this literature review will review the assessment of erosion. This will be done by detailing both *in vivo* and *in vitro* research and approaches the different clinical indices that have been reported for epidemiological assessment of dental erosion prevalence.

The third section will discuss the mechanical properties of the dental hard tissues. Knowledge of the mechanical properties of the dental hard tissues is advantageous when determining the changes to these tissues after exposure to a potentially erosive substance. The available research is summarised on the enamel and dentine hardness and modulus of elasticity.

The final section of this literature review discusses the prediction of the erosive potential of a beverage. The dental literature has identified the pH, titratable acidity, calcium, phosphate and fluoride content of a beverage to be potential predictors of the erosive potential of a beverage. The relevance to dental erosion of each of these beverage components are lastly discussed.

## Part 1. Tooth wear

Tooth wear or non carious loss of tooth tissue is a normal physiological process and occurs throughout life (Flint S & Scully C 1988). Tooth wear is usually a multifactorial process that is the result of erosion (non bacterial acid dissolution), attrition (direct tooth-tooth rubbing), abfraction (eccentric occlusal loads) and abrasion (wear of tooth surface by substances other than another tooth) (Milosevic A 1988). These terms are used to describe specific etiological factors that are associated with the loss of hard dental tissues. Problems arise when the loss of dental hard tissue is substantial enough to cause tooth sensitivity, aesthetic problems and functional problems for a person.

Wear is defined in material science as the progressive loss of material from its surface due to relative motion, and wear of materials during sliding or rolling contacts (Reid CN, Fisher J & Jacobsen PH 1990). The wear mechanism of dental materials and tissues in the mouth are complex due to the variable movements and loadings which can occur and the hostile biological and chemical environment. Due to the surface roughness the contacts between real surfaces take the form of interactions between the surface asperities which can deform both elastically and plastically. When two surfaces slide against one another, frictional forces result from both adhesion between and deformation of the asperities which come into contact (Reid CN, Fisher J & Jacobsen PH 1990). Four types of wear mechanisms have been described; adhesive wear, abrasive wear, fatigue wear and corrosive wear (Dowson 1981). Corrosive wear is the removal of the protective oxide layer or chemical modification of

the surface, which produces increased wear rates (Reid CN, Fisher J & Jacobsen PH 1990). This term helps explain the multifactorial nature of dental hard tissue wear.

## Definitions of Tooth Wear

### **Abrasion**

Dental abrasion is used to describe the pathological wearing away of dental hard tissue through abnormal mechanical processes, involving foreign objects or substances repeatedly introduced into the mouth and contacting teeth (Imfeld 1996). Abrasion is usually the result of toothbrushing using either a particularly abrasive toothpaste, or a normal toothpaste with heavy pressure, and often with a horizontal brushing action. This is why abrasion tends to be most obvious at the necks of teeth where that dentine and cementum are more easily abraded (Eccles JD 1982).

### **Attrition**

The term dental attrition describes the physiological loss of tooth tissue as a result of tooth-to-tooth contact with no foreign substances intervening (Imfeld 1996). Attrition therefore primarily affects the occlusal and incisal surfaces of the teeth (Kelleher M & Bishop K 1999). One of the more commonly recognised cause of attrition is parafunctional activity or bruxism (Eccles JD 1982).

### **Erosion**

Erosion is defined as the removal of hard tissue from the tooth surface by chemical means, in the absence of dental plaque. Usually the agent is an acid but some tooth loss is possible through chelating action near normal pH (Eccles

JD 1982). The clinical erosive lesion is smooth, polished and rounded with loss of tooth surface characteristics (Eccles JD 1982, Imfeld 1996) and any restorations present become proud (Deery *et al.* 2000). The acids responsible for erosion are not products of the intraoral flora but they stem from exogenous, endogenous or unknown sources (Imfeld 1996). More recently the term corrosion is the physical deterioration of a material by chemical or electrochemical attack. This term is postulated to more precisely describes the process dentists describe as erosion. For this thesis in concurrence with the bulk of the literature the term erosion will be used for this process (Grippio JO & Simring M 1995).

### **Abfraction**

Abfraction is the loss of hard tissue from eccentric occlusal loads that lead to compressive and tensile stresses at the cervical fulcrum area of the tooth. The tensile stresses weaken the cervical hydroxyapatite which creates a special form of wedge-shaped defect with sharp rims at the cemento-enamel junction (Imfeld 1996, Grippo JO 1991). This process predispose the cervical enamel to erosion. Stress induced lesions in the mouth are a hypothesis to explain a clinical finding with no obvious aetiology (Milosevic A 1988).

Most cases of tooth wear are thought to be the result of a combination of all or some of the above processes. However often one mechanism will dominate. In children generally erosion is thought to be the dominant process. As such the following literature review will concentrate on erosion. Awareness of the deleterious effects of erosion has increased in the past 5 years as indicated by the increased frequency of literature produced on this subject. Erosion can be a

particular problem for young children as the enamel and dentine layers of the primary dentition are much thinner than in adults. Once dentine becomes exposed loss of dental tissue accelerates (Shaw L & Smith A 1994). Although there is no doubt that tooth wear in general can have a multifactorial origin, in children there is anecdotal to support the suggestion that erosion may be increasing (Shaw L & Smith A 1994, Harley K 1986).

## Erosion

### Prevalence

It is difficult to assess the prevalence of tooth wear in general and erosion in particular. It is often difficult to distinguish erosion from other forms of tooth wear and, as stated before, tooth wear is usually a combination of abrasion, attrition and erosion. There have been many scales and indices suggested to measure tooth wear and their very diversity suggest that not one of them is ideal (Shaw L & Smith A 1998).

Table 1.1 summarises the data available to date on the prevalence of tooth wear in adults. It would appear that between 5 and 43% of adults show evidence of some form of tooth wear however it is uncommon for these studies to identify erosion specifically. All adult studies have shown that tooth wear increases with increasing age. This is not surprising as tooth wear is a known normal physiological process and occurs throughout life (Flint S & Scully C 1988).

However in the paediatric population studies it would appear that erosion is often assessed more specifically with up to 80% of all primary incisors

showing signs of erosion (Millward A, Shaw L, *et al.* 1994). Table 1.2 summarises the data available on the prevalence of tooth wear and more specifically (when mentioned in the studies) erosion in children. In one study it was found that nearly half of the 178 children between the age of four and five showed signs of erosion. These authors also found that children from lower socio-economic backgrounds had lower level of erosion than their more affluent counterparts (Millward A, Shaw L, *et al.* 1994). After clinical assessment 101 children Millward *et al* (1994) found that 35% of the children examined showed signs of severe erosion and 65% of children that had mild to moderate erosion. As the authors admit, their results may be higher than expected as of the 101 children that they used in their study, 20 were specifically referred to the Dental School because of their obvious erosion (Millward A, Shaw L, *et al.* 1994). A large, less biased study utilising 14-year-old children found that 30% of the sample had at least one tooth with exposed dentine. They did not attempt to ascribe aetiology to the wear reported (Milosevic A *et al.* 1994).

Interestingly and very alarming is the finding in the UK National Diet and Nutrition Survey by Hinds and Gregory (1995) that up to 10% of children as young as one and a half years old had erosion associated with their buccal surfaces of their primary incisors.

| Authors (date)                | Population                                                 | Number                       | Criteria                                                             | Prevalence                                                                                                                                                                                                                 | Notes                                                                                                        |
|-------------------------------|------------------------------------------------------------|------------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| Smith and Robb (1996)         | 15 to 65+ yrs                                              | 1007                         | Smith and Knight tooth wear Index (TWI)                              | 57.3% in the 15-26 yrs<br>8.19% in the 56-65 year and 8.84% in the 65+ age groups had tooth surfaces worn to an unacceptable degree. All other intermediate age groups had lower overall values                            | No differentiation of aetiology of tooth wear                                                                |
| Robb, Grady, and Smith (1991) | 16 yrs+ and Skulls                                         | 884<br>151                   | Smith and Knight tooth wear Index (TWI)                              | 19.9% of skulls had extensive tooth wear and reported that 7.1% of this tooth wear due to erosion<br>Greater cervical wear of modern populations than skulls although similar buccal and lingual wear rates between groups | Difficult study to interpret as comparing quite different populations<br>No absolute values of erosion given |
| Jarvinen et al. (1991)        | Subjects identified as having erosion and matched controls | 106 subjects<br>100 controls | Eccles and Jenkins method (1974)                                     | 5%                                                                                                                                                                                                                         |                                                                                                              |
| Lussi et al. (1991)           | 26-30 yrs<br>46-50 yrs                                     | 197<br>194                   | Modified Linkosalo and Markkanen                                     | 16% overall had erosion on facial surfaces<br>30% of 26-30 yrs-old and 43% of 46-50 yrs-old had severe erosion on occlusal surfaces                                                                                        |                                                                                                              |
| Xhonga and Valdmans (1988)    | 14-80 yrs                                                  | 527                          | Using a perio probe and these divided into minor, moderate or severe | 25% of all teeth but different between cities                                                                                                                                                                              | Illustrations show that a large amount of wear could be due to attrition, not erosion                        |
| Sognnaes et al. (1972)        | Extracted teeth- all ages                                  | 10,000                       | ?                                                                    | 18%                                                                                                                                                                                                                        | Incisors most commonly affected                                                                              |

Table 1.1: Summary of tooth wear prevalence in adults (and erosion prevalence when specifically mentioned).

| Authors (date)                         | Population         | Number                  | Criteria                                         | Prevalence                                                                                             | Notes                                                                                                                                            |
|----------------------------------------|--------------------|-------------------------|--------------------------------------------------|--------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| Shaw, A. D. B. and Smith 2000 and 2001 | 14 yrs             | 118                     | Modified Smith and Knight tooth wear index (TWI) | 48% of children had low erosion<br>51% moderate erosion and<br>1% severe erosion                       | Asthmatic children significantly more erosion than children not affected with asthma<br>More erosion observed in lower socio-economic categories |
| Deery <i>et al.</i> 2000               | 11-13 year old     | 129 in USA<br>125 in UK | Modified Smith and Knight tooth wear index (TWI) | 41% of children in USA and 37% in the UK had erosion                                                   | No causal relationship found                                                                                                                     |
| Kunzel <i>et al.</i> 2000              | 12 yrs from Havana | 1010                    | Severity grading of incisors                     | 17.4% of children examined                                                                             | Authors found that most likely due to the high consumption of halved oranges.<br>Higher erosion in communities close to fruit plantations.       |
| Barlett <i>et al.</i> 1998             | 11-14 yrs          | 210                     | Smith and Knight tooth wear index (TWI)          | 57% of children with enamel erosion on 10+ teeth<br>2% incisal and palatal surfaces through to dentine | No aetiology of tooth wear elucidated in study                                                                                                   |
| Hinds and Stagon 1995                  | 1.5 - 4.5 yrs      | 10,000                  | Smith and Knight tooth wear index (TWI)          | 10% of children had erosion on buccal of incisors<br>19% had erosion affecting palatal surfaces        |                                                                                                                                                  |

**Table 1.2:** Summary of tooth wear prevalence in children and adolescents (and erosion prevalence when specifically mentioned).

| Authors (date)                 | Population          | Number | Criteria                                | Prevalence                                                                                                        | Notes                                              |
|--------------------------------|---------------------|--------|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|
| Millward, Shaw, and Smith 1994 | 4 - 5 yrs           | 178    | Smith and Knight tooth wear index (TWI) | >50% showed signs of erosion                                                                                      | Biased sample - some pts referred in with erosion. |
| Millward et al 1994            | Children 5 - 15 yrs | 101    | Smith and Knight tooth wear index (TWI) | >80% of incisor teeth showed erosion and 30% of molars had dentine exposed                                        | Low level of inter-examiner agreement              |
| O'Brien 1994                   | 5 - 15 yrs          | 10,000 | Modified Smith and Knight (TWI)         | >50% 1 incisors<br>23% 2 incisors                                                                                 | No aetiology of tooth wear elucidated in study     |
| Milosavljevic et al 1994       | 14 yrs              | 1,035  | Smith and Knight tooth wear index (TWI) | 30% dentine exposure (score 2 or 3)<br>8% had exposed dentine also had exposed dentine on occlusal and/or lingual |                                                    |

Table 1.2: Summary of tooth wear prevalence in children and adolescents (and erosion prevalence when specifically mentioned).

## Aetiology of Dental Erosion

Erosion may be caused by presence of acid in the mouth. The source of this acid may be intrinsic or extrinsic.

### **Extrinsic Erosion**

Extrinsic erosion is the result of exogenous acids i.e. from acids entering the mouth from an external source. Traditionally, factors that may cause extrinsic erosion have been identified as originating from the environment, diet, medications and lifestyle choices of a patient.

### *Environmental Erosion*

Environmental erosion occurs when patients are exposed to acids in their workplace or during leisure activities (Centerwall BS, Armstrong CW, *et al.* 1986). Any occupation or activity that involves daily exposure to acids, presents some degree of increased risk of dental erosion (Zero DT 1996).

The most commonly quoted examples of environmental erosion is erosion found in factory workers and long term swimmers in gas-chlorinated swimming pools (Centerwall BS, Armstrong CW, *et al.* 1986, Tuominen ML, Tuominen RJ, *et al.* 1991, Filler SJ & Lazarchik DA 1994). Ten Bruggen Cate (1968) investigated the amount of erosion in factory workers who were exposed to acid fumes and aerosols in comparison to a control population who were also factory workers but worked in acid free environments. Battery factory workers were found to have the highest incidence of dental erosion (Ten Bruggen Cate 1968). Petersen and Gormsen (1991) also investigated

battery factory workers who were exposed to sulphuric acids. These authors report that the workers in the factory reported having sharp, short, or fragile teeth. The prevalence of erosion amongst this group was 31%, although a large number of teeth (average, 5.3 teeth per person) had been crowned previously. These authors concluded that severe erosion due to sulphuric acid mists should be recognised as an occupational disease.

More recently there have been reports in the literature of wine tasting and its link to environmental erosion. Wine has a pH of between 3.3 and 3.8 (Gray A, Ferguson MM, *et al.* 1998), and has been reported to cause significant erosion in multiple publications when wine tasters are exposed up to 30 times per day to wine over long periods of time (Gray A, Ferguson MM, *et al.* 1998, Chaundhy SI, Harris JL, *et al.* 1997).

### *Medications*

There have been multiple reports associating medications and over-the-counter dental products with erosion (Bhatti SA, Walsh TF, Douglas CW. 1994). Bhatti *et al.*, (1994) reported on the ethanol and pH level of a number of mouthwashes and found that the majority of mouthwashes were acidic, some with a pH as low as 3.4 (Bhatti SA, Walsh TF, Douglas CW. 1994). These authors did not report on whether the mouth rinses caused dental erosion but it is an area of concern in susceptible patients. Rytomaa and colleagues (1989) reported on their findings of the erosive potentials of mouthwashes. They found that one of the mouthwashes that they investigated (Calcusan) produced four times the surface erosion *in vitro* than a known erosive sports drink. The authors stated

that the ethylenediaminetetraacetic acid (EDTA) contained in this mouthwash is a known dental tissue demineraliser and suggested that its presence was responsible for the high erosive potential. Two of the mouthwashes (Salisan and Salivin) produced no erosion and while the fourth mouthwash (Veadent) investigated had a slight erosive potential. These authors concluded that it is important to recognize that the organic acids and chelating agents in various oral hygiene products may have deleterious effects on teeth (Rytomaa I, Meurman JH, *et al.* 1989). Conversely Lussi and Hellwig (2001) tested seven oral care products' (including products containing ethanol) erosive potential, of which the pH values were all below the critical pH for enamel dissolution of 5.5 (except one toothpaste with a pH of 7.25). Using change in surface microhardness these researchers reported that all the fluoride containing mouthwashes and toothpastes caused an increase in surface hardness. The only oral care product that led to a decrease in surface microhardness did not contain fluoride therefore indicating an erosive potential (Lussi A & Hellwig E 2001).

Medications in liquid form have been known to have varying pH ranges (James and Parfitt 1953). They investigated iron tonic products, which had a pH range from 1.5 to 8.6, leading them to conclude that they had the potential to damage the teeth. More recently medications in tablet form such as aspirin and vitamin C have been reported to have an erosive potential (Sullivan RE & Kramer WS 1983, Meurman JH & Murtomaa H 1986, Giunta JL 1983).

Common asthma medications have a pH value of between 4.31 (Bricanyl, powder form, Astarzenece Proprietary Limited) and 9.30 (Ventolin, aerosol form, Allen and Hanburys Ltd.) (O'Sullivan EA & Curzon MEJ 1998). The powder forms of all common asthma medications have a significantly lower pH than the aerosol form of the medication. In the powdered form almost all commonly used drugs have a pH less than 5.5. This could be a significant variable in the erosive potential of asthma medications, if used multiple times per day (O'Sullivan EA & Curzon MEJ 1998). Because of the erosive potential of asthma medications, authors have begun to investigate whether these commonly used drugs really pose a clinical problem. McDerra and colleagues (1998) sampled 100 asthmatic children and examined them for tooth surface loss along with other dental conditions. In comparison to the controls, who did not suffer from asthma, the children with asthma had a significantly larger number of teeth affected by tooth surface loss. Furthermore there was more severe tissue loss on the labial surfaces of the anterior teeth and occlusal surfaces of the posterior teeth. Shaw and colleagues (2000) have recently reported in their random sample of 14 year-old children that children with asthma have significantly more erosion and of greater significance than children who do not suffer from asthma. The possibility that children with asthma have an increased incidence of gastro-oesophageal reflux (Wilson NM, Charette L, *et al.* 1985) has also been implicated as a co-factor in the aetiology of dental erosion (Jarvinen VK, Rytomaa II, *et al.* 1991, Rytomaa I, Jarvinen VK, *et al.* 1990).

An interesting report in the literature by Sullivan and Kramer (1983) suggested, but did not back up with any statistical analysis, that juvenile rheumatoid arthritis appeared, in some children, to be associated with varying degrees of clinical erosion. The authors found that teeth were affected by erosion only in those children who found it necessary to chew their aspirin tablets. Teeth were unaffected in children who swallowed the aspirin tablets.

A controlled comparison of 40 children with phenylketonuria and their siblings was conducted by Kilpatrick and colleagues (1999). These authors concluded that children with phenylketonuria did have a significantly higher incidence of tooth wear, of which erosive wear appeared to be the predominant process. The authors identified the amino acid supplements used in the management of the condition to be potential cause of the erosion due to the low pH and high titratable acidity.

### *Lifestyle*

Lifestyle habits, choices and conditions may have a direct effect on the amount of erosion that is experienced by individuals. This is highlighted in multiple case reports and studies. High (1977) reported on a man who held Coca Cola in his mouth until all the dissolved gas had come out of solution. This patient had a large amount of erosion that was associated by the high frequency of Coca Cola consumption but may also have been influenced by the unusual way in it was consumed (High AS 1977).

Millward and colleagues (1994) found very little erosion in children from low socio-economic background in comparison to four-year-old children from higher socio-economic group (Millward A, Shaw L, *et al.* 1994). The authors concluded that the differences might be due to the different dietary habits or oral hygiene practices. Davis and Winter (1988) reported that abrasion was accelerated by toothbrushing following demineralisation from dietary acid exposure (Davis WB & Winter PB 1980).

Over the past few years there has been an increasing amount of literature produced on other, less common lifestyle choices that may be linked to dental erosion and tooth wear. Milsovic and colleagues reported that 60% of 3,4-Methylenedioxyamphetamine (ecstasy) users had tooth wear through the enamel into dentine in comparison to only 11% of non users. The main risk factors identified in this study were dry mouth, tooth grinding and increased consumption of carbonated beverages. Although the authors made no conclusion as to the aetiological factor that caused the tooth wear, the common ingestion of acid beverages to alleviate the dry mouth which are potentially erosive to teeth (Milosevic A *et al.* 1999).

There appears to be a lifestyle conditions and choices that can have a bearing on the severity of erosion in individuals.

### *Dietary*

Food and beverages, which can contain a variety of acids, have the ability to potentially cause dental erosion.

### **Acidic Drinks:**

There is an abundance of studies conducted *in vivo* and *in vitro* in the literature linking acidic drinks and erosion (Zero DT 1996, O'Sullivan EA & Curzon ME 2000, Al-Dlaigan YH, Shaw L, *et al.* 2001). Table 1.3 summarises a number of studies in the literature. The acids most frequently found in drinks are citric, malic, phosphoric and carbonic (Milosevic A 1988), but the most frequently consumed erosive acids are the fruit acids and phosphoric acids contained in fresh fruit, fruit juices and soft drinks (Allan DN 1967, Asher C & Read MJF 1987, Levine RS 1973, Lussi A, Jaggi T, *et al.* 1993, Miller CD 1950, Al-Dlaigan YH, Shaw L, *et al.* 2001). The pH of most fruit juices, sports drinks and soft drinks vary between 2.1 and 4.46 (Eccles JD 1982 & Jenkins WG 1974, Smith AJ & Shaw L 1987, Milosevic A 1997), which is well below the critical pH value for enamel demineralisation (Milosevic A 1997, Smith AJ & Shaw L 1987). O'Sullivan and Curzon (2000) found in their case controlled study that the young patients with erosion consumed a statistically significant larger amount of carbonated beverages and cordials. Interestingly they also found that swishing or sucking habits while drinking were found in 43% of patients presenting with erosion. This was in stark contrast to the 3% of controls with similar habits (O'Sullivan EA & Curzon ME 2000). Therefore not only is the amount of a beverage important in the erosive process but also the way the beverage is consumed.

There have been at least two reports in the literature of alcoholic soft drinks having a high erosive potential (Rees JS, Burford K, *et al.* 1998, O'Sullivan EA

& Curzon ME 1998). Rees and co workers (1998) reported in an *in vitro* study that the pH of alcoholic lemonade, depending on the flavour, ranged from 2.57-3.54. The authors concluded that all products had a greater erosive potential than Orange Juice. After one hour immersed in the product, the enamel surface loss was between 1.8-3.28  $\mu\text{m}$ , which was (all except the blackcurrant flavour) greater than Orange Juice.



| Author and date        | Beverage/s Studied                                    | Dental tissue tested | Method of investigation                             | Results                                                                                                                                                                | Factor of beverage as identified as the possible erosive potential                                                   |
|------------------------|-------------------------------------------------------|----------------------|-----------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| Hussain et al. 1995    | Various carbonated and uncarbonated juices            | E                    | <i>In vitro</i> surface microhardness               | Apple juice > schweppes > orange soft drink > grapefruit juice. All produced significant decreases of surface microhardness.                                           | PH, titratable acidity, fluoride content, phosphate concentration used to predict the erosive potential of beverages |
| Mistry and Grenby 1993 | Apple juice, still Orange Juice and carbonated orange | E                    | <i>In vivo</i> digital image analysis on rats teeth | Intact surface remaining after 6 week exposure was 47% for apple juice, 27% on still Orange juice and only 6% on carbonated orange                                     |                                                                                                                      |
| Lamminen et al. 1991   | Soft drinks and sports drinks                         | E                    | <i>In vivo</i> epidemiological study                | Considerable risk of dental erosion if soft drinks were consumed daily or if sports drinks were consumed weekly                                                        |                                                                                                                      |
| Grobler et al. 1990    | Orange and apple juice, Pepsi and Diet Pepsi cola     | E                    | <i>In vitro</i> , calcium dissolution               | Highly significant increase in calcium concentration of all beverages at 4 to 40 minutes emersion of enamel in drinks. Orange juice > apple juice > Pepsi > diet Pepsi | Calcium content of drinks                                                                                            |
| Grobler et al. 1989    | Apricot, grape, guava, apple and Orange Juice         | E                    | <i>In vitro</i> , etching depth                     | Erosion Apple > grape > guava > apple > orange over 6 minute exposure period                                                                                           |                                                                                                                      |

Table 1.3: Showing a summary of the available literature on erosive potential of acidic foods. E= Enamel and D= Dentine, NI= not identified.

| Author and date      | Beverage/s Studied                                                                 | Dental tissue tested | Method of investigation                             | Results                                                                                                                                                                                                                            | Factor of beverage as identified as the possible erosive potential |
|----------------------|------------------------------------------------------------------------------------|----------------------|-----------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|
| Sorvari 1989         | Sports drink                                                                       | E and D of rats      | <i>In vitro</i> , animal, stereomicroscopy          | Marked erosion on lingual surfaces of mandibular molars (statistically significant)                                                                                                                                                | pH                                                                 |
| Avionneau et al 1988 | Various beverages including cola and sport drinks, beer, buttermilk, coffee etc... | E bovine             | <i>In vitro</i> , profilometry and SEM              | Beer, buttermilk, coffee and mineral water had no detectable erosive effects. Cola beverage and Orange Juice statistically significant erosion. Sports drinks also significant erosion but less than cola and Orange Juice drinks. | pH<4<br>type of acid                                               |
| Asher and Read 1987  | Citric acid containing drinks                                                      | E and D              | Case report                                         | Only predisposing factor to erosion was consumption of acidic drink                                                                                                                                                                | -                                                                  |
| Smith and Shaw 1987  | Baby fruit juices                                                                  | E                    | <i>In vitro</i> , light microscopy                  | Appreciable destruction of the enamel                                                                                                                                                                                              | pH                                                                 |
| Stanton et al 1983   | Orange Juice                                                                       | E and D              | <i>In vivo/in vitro</i> on exfoliated primary teeth | Slight demineralisation of those participants teeth that had had OJ for 12-18 months                                                                                                                                               |                                                                    |
| Thomas 1957          | Fruit Juices                                                                       | E                    | <i>In vivo</i> , light microscope                   | 4-6 <sup>th</sup> week of study microscopic changes only 2 of 60 participants showed marked macroscopic changes orange juice erosion > grapefruit or carbonated beverages                                                          |                                                                    |

Table 1.3: Showing a summary of the available literature on erosive potential of acidic foods. E= Enamel and D= Dentine, NI= not identified.

### **Acidic foods.**

Much less research exists on the potential erosive effects of acidic foods. Table 1.4 summarises the available literature on erosive potential of acidic foods. In a case controlled cohort study, Jarvinen and colleagues (1991) showed that people who consumed citrus fruits more than twice a day had a 37 times greater risk of erosion than individuals who consumed citrus fruit less often (Jarvinen VK, Rytomaa II, *et al.* 1991). This finding is reinforced by multiple other reports (Eccles JD & Jenkins WG 1974, Smith AJ & Shaw L 1987, Grobler SR, Senekal PJC, *et al.* 1989, O'Sullivan EA & Curzon ME 2000). O'Sullivan and Curzon (2000) report in their recent case controlled study that the most important factor in relation to erosion and food consumption was when fruit was consumed twice or more per day the patients with erosion had significantly more erosion than age matched controls. These authors also noted that a larger number of the study group appeared to enjoy vinegar based foods than the control population.

Acids are present in foods such as tomato sauces, in baked beans and also in vinegar (acetic acid). Vinegar is used as an accompaniment with food or as an ingredient in cooking (Milosevic A 1988) these foods have the potential to cause erosion. The erosive potential from these foods is probably dependant on individual eating habits (Milosevic A 1988). A group of individuals who consumed a lactovegetarian diet were reported to have a clearly higher frequency of erosion. This is not surprising as lactovegetarian diets contain plenty of coarse, fresh food, citrus fruits, acidic drinks and honey which may increase the frequency of erosion. The lactovegetarians had a much higher

frequency of ingestion of acidic berries, vinegars, vinegar conserves and acidic drinks (Linkosalo L & Markkanen H 1985).

| Author and date                  | Food studied                                               | Dental tested | tissue | Method of investigation                                        | Results                                                                                                        | Factor in food identified as the possible erosive potential                    |
|----------------------------------|------------------------------------------------------------|---------------|--------|----------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Al-Dalgaan, Shaw and Smith, 2001 | Fresh fruit, Vitamin-C tablets and other acidic foodstuffs | E and D       |        | <i>In vivo</i> epidemiological study                           | Statistically significant association between these foods and erosion in random sample of 14 year old children | Acidity                                                                        |
| Kunzeller et al, 2000            | Oranges                                                    | E             |        | Case report                                                    | 17.4% of children examined                                                                                     | Higher erosion in communities close to fruit plantations                       |
| O'Sullivan and Curzon, 2000      | Fresh fruit                                                | E and D       |        | <i>In vivo</i> case controlled study                           | Consumption of fresh fruit twice per day or greater statistically significant more erosion than controls       |                                                                                |
| Dodds et al, 1997                | Mexican citric acid snacks                                 | E             |        | <i>In vitro</i> microhardness                                  | Statistically significant reduction in Vickers hardness number for snacks                                      | NI                                                                             |
| Grobler et al, 1994              | Honey                                                      | E             |        | Scanning electron microscopy and <i>in vitro</i> microhardness | No erosion noted with either method                                                                            | PH, buffering capacity, fluoride content, calcium and phosphorus concentration |
| Jarvinen et al, 1991             | Citrus fruits                                              | E             |        | <i>In vivo</i> epidemiological study                           | Considerable risk of dental erosion if citrus fruits consumed more than twice a day                            |                                                                                |
| Grobler et al, 1989              | Minced fruit                                               | E             |        | Etching depth and spectrophotometer                            | Apricots > grape > guava > apple > orange<br>juices much greater than actual minced fruit itself               | PH, amount of solution, types of organic acids important                       |

Table 1.4: Showing summary of the available literature on erosive potential of acidic foods. E= Enamel and D= Dentine, NI= not identified.

| Author and date              | Food studied         | Dental tissue tested | Method of investigation                | Results                                                                                                         | Factor in food identified as the possible erosive potential                 |
|------------------------------|----------------------|----------------------|----------------------------------------|-----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Ryomaa et al. 1988           | Strawberry yogurt    | E (bovine)           | <i>In vitro</i> , profilometry and SEM | No appreciable erosion                                                                                          | type of acid, lactic acid shown previously to have little effect on erosion |
| Linkosalo and Markkanen 1985 | Lactovegetarian diet | E and D              | <i>In vivo</i>                         | Increased consumption of vinegar and vinegar conserves, citrus fruits and acidic berries predisposes to erosion |                                                                             |

**Table 1.4:** Showing summary of the available literature on erosive potential of acidic foods. E= Enamel and D= Dentine.

## **Intrinsic Erosion**

The term intrinsic erosion implies the presence of gastric acid in the oral cavity (Milosevic A 1988). Vomiting, regurgitation, gastroesophageal reflux or rumination can result in the presence of gastric acids in the oral cavity.

## ***Vomiting and Eating Disorders***

Vomiting has many causes including disorders of the alimentary tract, neurological disorders, metabolic and endocrine disorders, side effects of many drugs and finally a large group of psychosomatic disorders including eating disorders and stress induced psychogenic disorders (Scheutzel P 1996) (see Table 1.5). The effects of vomiting are well documented in patients suffering from bulimia nervosa as this condition can mean that individuals are vomiting multiple times per day over a long period of time (Burke FJT, Bell TJ, *et al.* 1996, Robb ND, Smith BG, *et al.* 1995, Stege P, Visco-Dangler L, *et al.* 1982). Rytömaa and colleagues found that the incidence of tooth erosion, abrasion and attrition was 1.5 to 6 times more common amongst bulimics than controls. The most common sign of this condition is the presence of perimolysis-erosive lesions localised on the palatal aspect of the maxillary teeth. The severity of oral sequelae of bulimia has been related to the following: 1. frequency of vomiting, 2. duration of habit, 3. oral hygiene habits, 4. degree of acid dilution by water rinsing or drinking following vomiting and 5. timing and technique of toothbrushing (Burke FJT, Bell TJ, *et al.* 1996).

Patients suffering from bulimia often binge eat food with high energy and sugar foods which may also have a high erosive potential (Rytomaa I, Jarvinen VK, *et al.* 1998). Bidwell and colleagues (1999) report a male adult patient with extensive erosion that they associated with the patient,s self reported bulimia disorder. However these authors made no mention of possible confounding factors such as the 12 pack of beer and wine consumed daily by this patient, which undoubtedly increased the severity of the erosion. Other authors have noted the relationship between chronic alcoholism and erosion (Smith BG & Robb ND 1989, Simmons MS & Thompson DC 1987). A study of 37 chronic alcoholics reported significantly more pathological tooth wear than aged matched controls although no definitive aetiology of the tooth wear was found .

| Causes of vomiting          |                                                                                                      |
|-----------------------------|------------------------------------------------------------------------------------------------------|
| Psychosomatic               | Stress induced psychogenic vomiting<br>Eating disorders<br>Bulimia nervosa<br>Anorexia nervosa       |
| Metabolic and Endocrine     | Uraemia<br>Diabetes<br>Pregnancy                                                                     |
| Gastro-Intestinal disorders | Peptic ulcer, gastritis<br>Obstruction<br>Nervous system disorders<br>Encephalitis<br>Cerebral palsy |
| Drug induced                | Primary, eg. Cytotoxics<br>Secondary to gastric irritation eg. alcohol, aspirin, NSAID               |

**Table 1.5:** Showing the causes of vomiting. From Shaw and O’Sullivan (2000).

### *Regurgitation and Gastroesophageal Reflux*

Gastroesophageal reflux is the involuntary passing of gastric contents into the oesophagus. This condition is due to laxity of the lower oesophageal sphincter, allowing the contents of the stomach into the oesophagus. Gastroesophageal reflux is a frequent finding in patients with cerebral palsy, bronchitis, other respiratory disorders, hiatus hernia, pregnancy and has been reported to occur two to three times more commonly in children with asthma (O'Sullivan EA, Curzon MEJ, *et al.* 1998, Mansfield LE 1992, Milla PJ 1988, Kumar PJ & Clark ML 1990). Gastric acidity ranges from pH 1 to 3 and therefore if the contents of the stomach reach the oral cavity there is no doubt that these acids have the potential to cause dental erosion. Some dietary components (chocolate, fat or coffee), smoking, chronic alcoholism and certain types of exercise can also cause reflux (Bishop K, Kelleher M, *et al.* 1997, Kumar PJ & Clark ML 1990).

Although not all patients with reflux disease develop erosion (Meurman JH, Toskala J, *et al.* 1994), regurgitation of gastric acid into the oral cavity has been associated with tooth erosion in multiple studies (Jarvinen VK, Rytomaa II, *et al.* 1991, Rytomaa I, Jarvinen VK, *et al.* 1990, Howden GF 1971, Bartlett DW, Evans DF, *et al.* 1996). Bartlett *et al.*, (1996) demonstrated a strong association between gastro-oesophageal reflux and erosion of the palatal surface of maxillary teeth. Patients with erosion but no previous history or gastroesophageal reflux were all tested for gastroesophageal reflux. This well designed study recorded the pH change over a 16-23-hour period and continuously measured the pH at three positions, the distal oesophagus,

proximal oesophagus and the oral cavity (palate). Of the patients with palatal erosion, 64% (n=36) had pathological gastro-oesophageal reflux as measured by distal oesophagus pH measurements over a 16- 23-hour period. Although there was a relationship noted between the oral acid exposure of pH <6 and the tooth wear index, this relationship was not very strong ( $r=0.37$ ) in statistical terms. The authors concluded that palatal erosion was strongly associated with gastroesophageal reflux from the distal oesophageal results but not the intraoral pH monitoring.

Most studies have evaluated reflux and erosion in adults however a similar relationship in children has been suggested (Taylor G, Taylor S, *et al.* 1992), although this relationship may well be less clear (Dodds AP & King D 1997, Gudmundsson K, Kristleifsson G, *et al.* 1995). Gudmundsson and colleagues (1995) used a 24-hour pH monitoring system to evaluate gastroesophageal reflux in 14 children with clinical erosion. None of these children demonstrated significant pH changes in the oral cavity despite recording 339 reflux episodes. These authors stated that considering the high prevalence of gastroesophageal reflux, it is tempting to assume that the mere presence of acid is enough to cause erosion. This conclusion is partially reinforced in a study by O'Sullivan and colleagues (1998) who investigating the amount of erosion in children with previously confirmed moderate to severe gastroesophageal reflux (24-hour pH monitoring). This study did not have a control group of patients, but used the reported rate of erosion in of the 1993 UK Child Dental Health Survey (O'Brien M 1994 41) to compare the level of erosion to a control child population. Although the authors admitted that a number of the children

investigated were being treated before the study with anti-reflux medicine, there was no attempt to compare the erosion experienced by patients who had been on preventive medicine with those that had not. They did conclude that dental erosion might not be as great a problem in children as it is in adults suffering from gastroesophageal reflux due to the low frequency of erosion seen in their potentially at risk population (O'Sullivan EA, Curzon MEJ, *et al.* 1998).

Further confirmation of the link between gastroesophageal reflux and erosion can be found in a study by Shaw *et al.*, (1998). In this study 51 medically compromised children were investigated. The only factor that was found to be statistically associated with erosion was the gastroesophageal reflux, especially in children with cerebral palsy. Diet, feeding habits, and toothbrushing habits were not associated with erosion in their study.

### *Rumination*

Rumination is the voluntary regurgitation of gastric contents into the mouth. In comparison to the general population, rumination is more commonly seen in institutionalised and non-institutionalised mentally disabled children and adults suffering from stress or significant psychological disturbances (Reis S 1994, Amarnath RP, Abell TL, *et al.* 1986). The contents of the stomach are often held in the mouth leading to erosion on the palatal surface of the teeth as well as on the buccal aspect of teeth in the posterior segment (Gilmour AG & Beckett HA 1993). This makes recognition of a patient suffering from rumination difficult to diagnose from the oral condition, as no clear clinical

picture is evident (Scheutzel P 1996). In general there is little known about the rumination syndrome and the prevalence of erosion associated with it (Milosevic A 1988).

### **Idiopathic Erosion**

Idiopathic erosion is the result of acids of unknown origin. This is where erosion results from an unknown source and no tests or anamnesis are capable of providing an aetiology (Imfeld 1996).

### **Multifactorial Aetiology of Tooth Wear**

It is not always possible to differentiate between erosion, attrition, and abrasion as these conditions frequently occur in combination (Smith BG, Bartlett DW, *et al.* 1997, Grippo JO & Simiring M 1995), but with differing proportional effects (Nunn J, Shaw L, *et al.* 1996). Erosion is a destructive process that renders the eroded tooth surface hypomineralised, making this surface more susceptible to the effects of abrasion, abrafraction and attrition (Imfeld 1996, Jaeggi T & Lussi A 1999). Erosion can also lead to substantial loss of enamel, which acts to protect the underlying dentine which, being softer (Mahoney EK, Holt A, *et al.* 2000) is more susceptible to abrasion and attrition.

## Part 2. Assessing Erosion

Erosion as previously defined is the removal of hard tissue from the tooth surface by chemical means, in the absence of dental plaque. Clinical and *in vivo* data are obviously important although difficult to statistically correlate and analyse. *In vitro* assessment of erosion on extracted teeth is essentially measurement of the erosive potential of a substance. *In vitro* methods of assessing erosion and erosive potential provides a means of a) understanding the erosive process, b) establishing what poses an erosive risk and c) determining methods of prevention.

### *In vivo* Assessment of Erosion

Dental erosion is a continual process (Hunter ML, West NX, *et al.* 2000a, Lussi A & Schaffner 2000) and is often clinically assessed in combination with abrasion and attrition under the heading of tooth wear. Some authors have tried to single out erosion in their research and attempted to eliminate the other forms of tooth wear. There are several different methods of evaluating erosion clinically. These include case histories and clinical examination with a variety of clinical indices. At present there is no single clinical index that is perfect for the task of assessing and monitoring erosion.

### **Case histories and clinical examination**

The rate of erosion progression was investigated by Xhonga and colleagues (1972) using clinical examination. These investigators took impressions of erosive lesions at the beginning and then, 5 months later, at the end of the study. They then used profile tracings on these impressions to find that the

daily average erosion rate was 1  $\mu\text{m}$  in patients that had erosive lesions already present. Erosion measured *in vivo* offers the advantage of direct measurement of the clinical condition. These authors also measured the effect of fluoride mouth rinses on erosion and found that no significant difference between the teeth treated with 33.33% NaF and the control teeth. Errors may have been introduced into this study because the authors were measuring the erosion from models (silicone), poured from Coe-flex (GC, America) impressions (light weight rubber base impression paste) and the amount of erosion was very small overall (1  $\mu\text{m}$ ). A split mouth design was used for the testing of the fluorides effect on erosion. Each patient had only half of the mouth isolated for fluoride application, however because fluoride could have become mixed into the saliva and been incorporated into the control teeth. This study is somewhat weak.

### **Clinical indices**

To overcome the difficulties of the assessing and measuring the clinical loss of dental hard tissue a number of investigators have designed clinical indices. Smith and Knight (1984) proposed a tooth wear index (TWI). Their aim was to design a tool for use in research into the aetiology, prevention and management of tooth wear problems. It would seem that this is what has happened as a large number of the studies into tooth wear have utilised this or a modification of this index (Milosevic A *et al.* 1994, Bartlett DW, Coward PY, *et al.* 1998, Hinds K & Gregory JR 1995, O'Brien M 1994, Robb ND, Cruwys E, *et al.* 1991) . Their index (Table 1.6) visually records each surface of the tooth that is susceptible to wear, so that 128 surfaces scores in total can be recorded (4 surfaces per tooth for 32 teeth).

| Score* | Surface         | Criterion                                                                                                                                                                                              |
|--------|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 0      | B/L/O/I<br>C    | No loss of enamel surface characteristics<br>No change of colour                                                                                                                                       |
| 1      | B/L/O/I<br>C    | Loss of enamel surface characteristics<br>Minimal loss of contour                                                                                                                                      |
| 2      | B/L/O<br>I<br>C | Loss of enamel exposing dentine for less than 1/3 <sup>rd</sup> of the surface<br>Loss of enamel just exposing dentine<br>Defect less than 1mm deep                                                    |
| 3      | B/L/O<br>I<br>C | Loss of enamel exposing dentine for more than 1/3 <sup>rd</sup> of the surface<br>Loss of enamel and substantial loss of dentine, but not exposing pulp or secondary dentine<br>Defect 1-2 mm deep     |
| 4      | B/L/O<br>I<br>C | Complete loss of enamel, or pulp exposure, or exposure of secondary dentine<br>Pulp exposure or exposure of secondary dentine<br>Defect > 2mm deep, or pulp exposure, or exposure of secondary dentine |

**Table 1.6:** Showing the Smith and Knight Index for tooth wear.

Legend: B= buccal or labial; L= lingual or palatal; O=occlusal; I= incisal; C=cervical; \* in case of doubt the lower score is given.

This is a relatively simple tooth wear index, and has been widely used in prevalence studies. This index is not however specific for the measurement of erosion as it encompasses all tooth wear irrespective of aetiology.

Since the TWI was first introduced researchers have modified the index so as to help identify specific components of tooth wear such as erosion (Deery *et al.* 2000, O'Brien M 1994). Millward *et al* (1994) used the TWI to study erosion in 4-year-old children but the children were classified into one of three groups depending on their TWI based erosion score. Low erosion was scores of 0 and 1 for all teeth, moderate erosion was any surface with scores of 2 and severe erosion as any surface with scores of 3 or 4. The 1993 Survey of Children's Dental Health in the UK (O'Brien M 1994) also utilised a modified TWI index.

The researchers in this large study examined the buccal and lingual surfaces of the primary and permanent maxillary incisors and recorded the loss of surface enamel characteristics and exposure of dentine or pulp. The rationale being that erosion is likely to predominate on the buccal and lingual as opposed to the occlusal surfaces which are more susceptible to abrasion and attrition.

Prior to the introduction of the TWI, Eccles and Jenkins (1974) created their own criteria specifically for erosion. They used dental casts and photographs taken from patients with dental erosion and defined a set of criteria for diagnosing erosion and also for grading the severity.

Criteria for diagnosis of erosion:

- Absence of developmental ridges on enamel
- Concavities primarily in the cervical region of the enamel
- Edges of amalgam or silicate restorations raised above the level of tooth surface
- Depression on the cusps of posterior teeth (cupping) or grooves of incisal edge of anterior teeth

Grading of severity

- Grade 0: no involvement
- Grade 1: loss of surface features on labial, lingual or occlusal enamel surfaces, no dentine involvement
- Grade 2: Involvement of the dentine for  $< 1/3^{\text{rd}}$  of the area of the tooth surface

- Grade 3: involvement of the dentine for  $> 1/3^{\text{rd}}$  the area of the tooth surface

This method of assessment of the presence and severity of erosion has been used in number of studies (Lussi A, Schaffner M, *et al.* 1991, Linkosalo L & Markkanen H 1985, Jarvinen VK, Rytomaa II, *et al.* 1991) when specifically investigating erosion.

Øilo and colleagues devised (1987) an index for clinical wear, based on the Ryge's system in which the decision to treat or not is the basic criterion (Ryge G 1973). This index had three acceptable criteria (R (Romeo), S (Sierra) and M (Mike)), all of which indicated that no treatment was required at that time, whilst T (Tango) and V (Victor) indicated that treatment was required (Øilo G, Dahl BL, *et al.* 1987). This index has been evaluated and the authors found the index to be well suited for the evaluation of dental wear in large populations after investigator training (Øilo G, Dahl BL, *et al.* 1987). This index does not however differentiate between the different forms of tooth wear.

Larsen and co-workers have proposed a clinical index based on a clinical examination, photographic and cast records of affected patients (Larsen MJ, Westergaard J, *et al.* 2000). The authors stated that the classification system has the ability to index initial erosion is suitable for monitoring progression of erosion over time. They report a high degree of inter-examiner reliability. This index is very extensive and would be time consuming and resource intensive if used in epidemiological studies. Its use in an individual dentist clinic can be

recommended but each clinician must allocate sufficient time for learning and implementation of the index.

The most recent clinical index for erosion measurement was developed by O'Sullivan (2000). She designed the index to include the site of erosion, severity and area of tooth surface affected by modification of the best and most appropriate aspect of previous published indices. Her pilot study using the index (Table 1.7) verified its ease of use and reliability on 102 children attending a community dental clinic.

There is a lack of longitudinal data on erosion and there are many reasons for this. The amount of tissue lost in erosion is small and therefore quantitative measurement of this *in vivo*, over time is difficult. Within the oral cavity there are modifying factors, such as fluoride exposure, saliva (Tandon S, Paul ST, *et al.* 1995, Meurman JH & ten Cate JM 1996) and patient dependant factors that make keeping a stable environment *in vivo* difficult over a long period of time. Because of these difficulties, *in vitro* methods of measuring erosion have been developed.

| Site of erosion on each tooth                                    |                                                               |
|------------------------------------------------------------------|---------------------------------------------------------------|
| Code A                                                           | Labial or buccal only                                         |
| Code B                                                           | Lingual or palatal only                                       |
| Code C                                                           | Occlusal or incisal only                                      |
| Code D                                                           | Labial and incisal/occlusal                                   |
| Code E                                                           | Lingual and incisal/occlusal                                  |
| Code F                                                           | Multi-surface                                                 |
| Grade of severity (worst score for an individual tooth recorded) |                                                               |
| Code 0                                                           | Normal enamel                                                 |
| Code 1                                                           | Matt appearance of the enamel surface with no loss of contour |
| Code 2                                                           | Loss of enamel only (loss of surface contour)                 |
| Code 3                                                           | Loss of enamel with exposure of dentine (ADJ visible)         |
| Code 4                                                           | Loss of enamel and dentine beyond ADJ                         |
| Code 5                                                           | Loss of enamel and dentine with exposure of the pulp          |
| Code 9                                                           | Unable to assess (eg tooth crowned or large restoration)      |
| Area of surface affected by erosion                              |                                                               |
| Code -                                                           | Less than half of surface affected                            |
| Code +                                                           | More than half code affected                                  |

**Table 1.7:** Index for measurement of erosion.  
Developed by Shaw and O'Sullivan (2000).

## *In vitro* Assessment of Erosion

A number of methods of assessing tooth wear has been described (Greenby TH 1996). These include:

- General assessment of extracted teeth
- Chemical analysis
- Transverse microradiography
- Digital image analysis
- Scanning electron microscopy (SEM)
- Physical methods
  - Polarised light
  - Profilometry
  - Iodine permeability
  - Other
  - Indentation technique

These methods are used successfully in the laboratory although their relevance to the clinical setting has been questioned (Shaw L 1997). An advantage of *in vitro* assessment of erosion, is that variables such as exposure time and nature of the erosive agents, can be controlled (Greenby TH 1996). A difficulty in using extracted teeth *in vitro* assessment of erosion is that their history is unknown. Surface characteristics of teeth may be influenced by the extracted tooth's fluoride history or past exposure to erosive beverages.

## **Examination of extracted teeth**

A large study that investigated the prevalence of erosion on 10,000 extracted teeth showed that the prevalence of erosion was 18%. This study used direct examination of the teeth but gave no indication of the sites examined or the criteria used (Sognnaes RF, Wolcott RB, *et al.* 1972).

Robb and colleagues (1991) examined 151 adult skulls, from an pre-Conquest 'Anglo-Saxon' and 'Romano-British' population and found that approximately 71% of the extensive tooth wear seen on these skulls was due to erosion. This study utilised the Tooth Wear Index of Smith and Knight but did not attempt to define the relative importance of erosion over other processes. This makes comparison with other studies difficult. However it did compare modern controls with the skulls and noted that there were similarities in the patterns of overall wear in both groups (Robb ND, Cruwys E, *et al.* 1991).

## **Chemical Analysis**

Although not widely used, methods have been developed to measure the small changes in calcium (Ca) and/or Phosphorus (P) dissolved from teeth under the influence of potentially erosive acids or other erosive factors. Initial Ca and P are required so that these values can be subtracted from the final Ca and P levels. A variety of scales and micro-methods exist for the determination of Ca and P in solution (Greenby TH 1996, Greenby TH, Phillips A, *et al.* 1989).

## **Transverse Microradiography (TMR)**

Transverse microradiography is a system for measuring the amount of mineral lost from the tooth surface and the depth of the erosive lesion. Once the dental tissue has been exposed for a period of time to an erosive substance a microradiograph is then taken and the image is developed. A microscope and computer programme analyse the lesion depth and the integrated mineral loss (vol% $\mu$ m) (Amaechi BT, Higham SM, *et al.* 1998a). The integrated mineral loss is defined as the difference in volume percent of mineral between sound and eroded demineralised tissue integrated over the lesion (Ruben J & Arends J 1993). This system has been used to show mineral loss from erosive lesions on extracted bovine (Amaechi BT, Higham SM, *et al.* 1998a) and human teeth (Hall AF, Sadler JP, *et al.* 1997, Larsen MJ & Nyvad B 1999). Hall and colleagues (1997) investigated the similarity between profilometry and TMR for measuring early mineral loss *in vitro*. Due to the high correlation coefficients the authors concluded that the application of TMR was useful and acceptable for the measurement of early mineral loss associated with acid erosion.

## **Digital Image Analysis**

Digital image analysis is similar to TMR as it is a computer-aided, quantitative and sensitive technique but digital image analysis measures the area and depth of the erosive lesion, where as stated before the TMR measures the amount of mineral loss and depth of lesion (Amaechi BT, Higham SM, *et al.* 1998a). Digital image analysis uses a video camera attached to a microscope from which data on the geometric characteristics of teeth can be acquired. The

signal denoted from an intensity of the selected parts of the image is transformed into a series of numbers, which can then be directly compared (Mistry M & Grenby TH 1991).

### **Scanning Electron Microscopy (SEM)**

This method of assessing erosive potential of different substance has been widely used by itself or in combination with other methods. The scanning electron microscope (SEM) is usually carried out after a tooth has been immersed in an eroding substance. The etched surface is then examined under SEM after sputter coating the surface with gold (Greenby TH 1996).

SEM examination can be used to visualise directly changes in the dental hard tissue but can also be used to visualise an impression of an eroded surface (Rugg-Gunn AJ, Maquire A, *et al.* 1998). It has the advantage of allowing direct visual comparison of the effects of different erosive substances on the dental hard tissue. Meurman and Frank (1991) used SEM to show that in enamel prism sheaths are eroded first then, with increasing acid challenge, the prism cores and finally the interprismatic areas were affected. In dentine erosive substances have been shown to affect the interface between peritubular and intertubular dentine firstly, with total disruption and dissolution of peritubular dentine following until the intertubular dentine is ultimately attacked (Meurman JH, Drysdale T, *et al.* 1991).

A disadvantage of SEM is that there can be no actual quantitative measurement of the amount of softening of the dental hard tissue or the amount of mineral that has been lost, without the help of other *in vitro* assessment tools.

## **Physical Methods**

### **Polarised Light**

Polarised light microscopy has been used in deciduous teeth to observe erosive destruction in the enamel. Smith and Shaw (1987) describe the surface of the eroded tooth as white and opaque and the polarised light revealed appreciable destruction of enamel with complete loss of the surface enamel. Whilst information like this does add to the completeness, it provides little quantitative data.

### **Profilometry**

Profilometry is the measurement of the change in the depth of an eroded surface in comparison to a known set reference point. A profilometer uses either a stylus (Hall AF, Sadler JP, *et al.* 1997) or laser (Bartlett DW & Sognnaes RF 1997) to record digitally the depth change along an eroded surface. The information is then analysed by a computer programme.

This method is commonly used for assessment of erosion of extracted human or non human teeth after exposure to an acidic substance (Amaechi BT, Higham SM, *et al.* 1998a, Hall AF, Sadler JP, *et al.* 1997). Rugg-Gunn and colleagues (1997) used surface profilometry on enamel slabs that were worn

intra orally in removable appliances and found correlation between SEM and surface profilometry.

Profilometry has also been used to measure the erosive depth change of teeth *in vivo*. Bartlett and Smith (1997) cemented discs to the palatal surface of incisor teeth of 20 patients to act as reference points for profilometry studies. They took impressions and compared the surface depth change with profilometry around the disc in the impressions of the teeth 6 months later. Using the laser profilometry they were able to measure the very small erosive changes of a range from 0.5-108.2  $\mu\text{m}$  accurately and reproducibly.

Hunter and colleagues (2000a) recently utilised profilometry changes over time in an *in vivo* study, where volunteers wore specially constructed appliances to measure the differences in erosion on permanent and deciduous teeth when exposed to an erosive orange drink over time. Profilometry utilised in this *in vivo* technique has the advantage of allowing quantitative measurements of the dental hard tissue lost and appears to be a reliable and reproducible method of assessing erosion.

### **Iodine Permeability**

Iodine permeability has been used to detect early demineralisation changes in enamel and was evaluated as a method for measuring erosion. A specimen of either enamel or dentine is covered with a known concentration of iodide (commonly in the form of potassium iodine (KI)) and then after a set period of time, washed away. The specimen is then blotted with deionised water,

allowing back diffusion and this solution is collected and transferred by blotting paper and a micro-sample dish. While under constant agitation, the iodide content is analysed using a iodide electrode. The change in the amount of iodide present before and after exposure to an erosive substance indicates the change in porosity of a surface (Zero DT, Rahbek I, *et al.* 1990).

Lussi *et al.*, reported that as the iodine permeability measures the changes in porosity, it has the advantage that it does not alter the surface of the dental tissue being tested. Iodine permeability tests however have a high degree of operator variability and require fully intact surfaces, as they are very sensitive to surface irregularities. (Lussi A, Jaggi T, *et al.* 1993, Greenby TH 1996).

Greenby (1996) in his review article on measurements of assessing erosion and erosive potential suggest that other sophisticated and innovative techniques such as 3-D tomography, laser scanning and nuclear magnetic resonance could be used in the future for further research into erosion and erosive potential but to date there have been no reports.

### **Other Techniques**

Ultrasonic measurement of enamel thickness has recently been investigated for its diagnostic ability to measure enamel thickness changes over time. This non invasive technique involves placement of a ultrasonic pulse-echo system to a tooth which a layer thickness is calculated using a value of sound velocity that can be programmed as appropriate for the material being investigated. Huysmans and Thijssen *et al.*, (2000) reported the measurement of enamel on

extracted teeth and concluded that the ultrasonic measurement of the enamel thickness is feasible with out enamel preparation. This study was an *in vitro* study and they made many assumptions of how this technique will perform clinically. However continued research and development work is required on this technique before it is reliable for clinical usage.

### **Indentation Technique**

An indentation technique is a method whereby the change in the surface hardness is measured. Hardness is the change in the ability of a material to resist permanent indentation and therefore a decrease in hardness is a decrease in the resistance to permanent indentation.

Although no one indentation method has proven to be superior to another in measuring dental hard tissue hardness a number of authors has used the indentation technique to assess the changes in the physical properties of the dental hard tissues after exposure to an erosive substances (Maupome G, Aguilar-Avila M, *et al.* 1999, Maupome G, Diez-de-Bonilla J, *et al.* 1998, Attin T, Koidl U, *et al.* 1997, West NX, Maxwell A, *et al.* 1998). The essential theory is the same: the dental hard tissue is tested using an indentation method, the tissue is then immersed in the erosive beverage for a set time. The tooth tissue is then retested with the identical indentation test and the difference is calculated and compared statistically. This method relies on monitoring changes in two fundamental properties of tooth tissue: hardness and elastic modulus.

There is increasing use of hardness testing in the assessment of erosion (Larsen MJ & Nyvad B 1999, Lussi A, Jaggi T & Scharer S 1993, Lussi A, Jaggi T, & Scharer S 1995, Lussi A, Kohler N, Zer S, Sch M, & Magert B 2000) although there is very little information on the basic mechanical properties of dental hard tissue. Therefore the next section of this literature review will summarise the measurement of the hardness and modulus of elasticity by the indentation technique.

## Hardness Testing in Erosion

A material's susceptibility to wear can be determined by observing the material's resistance to penetration (Marshall DB & Lawn BR 1986). Indentation damage is known to simulate individual events in a range of cumulative surface removal processes, such as abrasive wear (Marshall DB & Lawn BR 1986). Change in microhardness has been used in the study of erosion by a number of authors (Grobler SR, Du Toit IJ, *et al.* 1994, Larsen MJ & Nyvad B 1999, Lussi A, Jaggi T, *et al.* 1993, Lussi A, Jaeggi T, *et al.* 1995, Maupome G, Diez-de-Bonilla J, *et al.* 1998, Maupome G, Aguilar-Avila M, *et al.* 1999). The change in surface microhardness has also been used by Lussi *et al.*, (1995) to try and predict the erosive potential of some beverages.

In common with all *in vitro* tests, probably the most important deficiency of the indentation tests is in the lack of direct clinical relevance. Hardness tests, although relatively simple do require expensive equipment that only a well equipped dental laboratory would have. There are no chairside hardness tests available to date.

There is a variety of types and methods of conducting indentation/hardness tests and there are also many different indenter shapes, making the results of these tests either not directly comparable to one another or requiring a complex mathematical formulae to convert them. Fortunately when testing erosion and erosive potential of foodstuffs with the microhardness tests, as long as the experiment protocol remains the same throughout the experiment, what is important is the microhardness difference after immersion in the potentially erosive substance, not the actual hardness itself. This means that the change in hardness as a percentage rather than a exact value can be reported and directly compared with other studies on erosion.

Besides being able to compare different experiments, there is a number of advantages of measuring erosion by measuring the change in the microhardness. Hardness tests are relatively simple to conduct and allow variables such as length of time a dental tissue to be immersed in an erosive substance or the temperature of the material to be tested, to be change to suit the experiment design. Hardness test are able to report other mechanical properties such as the elastic modulus and the change, if any can also be examined as part of the same test.

### Part 3. Mechanical Properties of Dental Hard Tissue

The data available on the mechanical properties of tooth tissue have mostly been generated by conventional compressive, tensile and bending tests (Poolthong S 1998). Conventional hardness testing methods are associated with some general limitations related to the size and quality of test specimens obtainable from a tooth. For example tensile strength testing is associated with difficulties in specimen preparation and alignment which may give rise to misleading data (Askeland DR 1992, Craig RG 1993, Mencik J 1992, Xu HHK, Smith DT, *et al.* 1998). The very small dimensions required to obtain homogeneous specimens of tissue such as enamel further compound such difficulties (Waters NE 1980, Bowen RL & Rodriques MS 1962). Preparations for these tests become difficult and time consuming in order to achieve a few ideal specimens. As a consequence of these problems results reported demonstrate a wide variation.

Hardness measurements are relatively non-destructive and the preparation time of the specimens is less time consuming than compressive, tensile and bending tests (Waters NE 1980). Hardness is the ability of a material to resist permanent indentation and is widely used to determine the mechanical properties of teeth (Craig RG 1993). Most hardness tests involve making an indentation into the surface of the material to be tested. This is done at a specified force in a controlled and reproducible manner and for conventional hardness tests the results are determined by the size of the indent made into the material once the load is removed (O'Brien WJ 1997). The resulting indent is the product of the load and the elastic recovery of the material. Hardness tests

are divided into two categories; macro (loads of over 1 kg, e.g. Brinell and Rockwell) and micro hardness (loads less than 1 kg, e.g. Knoop and Vickers) (Boyer HE 1987, Phillips RW 1991, Poolthong S 1998). These tests utilise a static indentation method where a load is applied to a material by a conical or pyramidal indenter. The relationship of the load to the area or depth of indentation is then calculated and a hardness value is given (Poolthong S 1998).

The most commonly used hardness tests used are the Brinell, Rockwell, Knoop and Vickers hardness (Craig RG 1993, Meredith N, Sherriff M, *et al.* 1996, Ryge G, Foley DE, *et al.* 1961). Each of these employs a different scale for the calculation of the hardness of a material, making direct comparison difficult. Each of the commonly used hardness tests also utilises a number of different shaped indenters and the hardness for each test is calculated in different ways depending on the shape of the indenter and the shape of indentation it leaves in the material it is testing. For example the Brinell hardness test utilises a small steel ball and the hardness is calculated by measuring the diameter of the indentation left on the material. Both the Vickers and Knoop hardness tests utilise a diamond shape indenter but the Vickers hardness number is calculated by measuring the diagonals of the square and taking an average of the two, whereas the Knoop hardness number is calculated by measuring only the long diagonal of the non equal diamond (O'Brien WJ 1997).

Hardness tests can be correlated with a number of different mechanical properties. For example, indentation hardness has been used to study elastic

modulus, creep and fracture of brittle materials (Askeland DR 1992, Ashby MF and Jones DRH. 1980). The modulus of elasticity (stiffness) is the ratio of stress to corresponding strain below the proportional limit. It is an indication of the amount of deformation that will occur in the dental tissue when a load is applied to it.

Besides a very recent publication (Lussi A, Kohler N, *et al.* 2000), very little work has been done on the mechanical properties of primary teeth. A summary of the hardness and elastic modulus of human primary and permanent teeth is shown in Table 1.8 and 1.9.

There is a number of factors which influence the hardness test results. Hegdahl and Hagebo (1972) investigated the load dependence of micro indentation hardness testing in enamel and dentine and found that a slight but significant variation in hardness was obtained when the load was varied. These authors utilised relatively large loads (8 g to 64 g) but a load dependant relationship has also been noted by other authors (Ryge G, Foley DE, *et al.* 1961, Salama SN & Kinawi NA 1989).

The structure of the test material may also influence the hardness. Few materials are completely homogeneous and minute cracks or particles of different composition may affect the hardness recorded. Knowing the size of the indenter is crucial as different results may be found for each material tested with a different indenter. Small indenters can measure individual regions whereas large indenters tend to average over a large area. As a result it is

virtually impossible to compare accurately one hardness test to another (Poolthong S 1998)

Meredith and co-workers (1996) utilising a Knoop indenter, measured the micro hardness and Young's Modulus of Elasticity of enamel and dentine of permanent teeth at 4.9 N and 0.98 N loads respectively. These authors only represented their results graphically, making comparison with other studies difficult. The mean micro hardness of enamel from the graph was approximately 273 KHN. For dentine the average micro hardness was 60.7 KHN and the average modulus of elasticity was  $60.7 \text{ GNm}^{-2}$ . The modulus for enamel was  $299.5 \text{ GNm}^{-2}$ . These authors required a load of 4.9N and 0.98N in enamel and dentine respectively to enable accurate measurements of the short diagonal that was measured by sight down a microscope. In enamel, even at this relatively light load, surface damage occurred during indentation, which resulted in cracking and subsurface chipping. They admitted that the surface damage increased the difficulty of making an accurate measurement of the short diagonal. Given the small distances being measured with traditional hardness tests, there are often difficulties in visualising both ends of the indents simultaneously, leading to large errors. Other problems encountered include tester error in loading, rate of load application, duration of a contact period, and impact. To overcome these problems, ultra micro-indentation hardness tests have been developed.

| Author and Date               | Dental tissue testing | Hardness                                                       | Modulus of Elasticity | Test method and scale                                        |
|-------------------------------|-----------------------|----------------------------------------------------------------|-----------------------|--------------------------------------------------------------|
| Lussi <i>et al.</i> , 2000    | Enamel                | 313.4 +/- 18.6 to 331.4 +/- 16.2 KHN                           | NM                    | Knoop diamond indenter, 500 gm, Knoop hardness number (KHN)  |
| Caldwell <i>et al.</i> , 1957 | Enamel                | 272 +/- 26 to 399 +/- 39 KHN                                   | NM                    | Knoop diamond indenter, 500 gm, Knoop hardness number (KHN)  |
| Atkinson and Saunbury (1953)  | Enamel                | 917 (immature deciduous teeth)<br>986 (mature deciduous teeth) | NM                    | Relative Hardness number with Diamond shaped indenter 30 gm. |

**Table 1.8:** Hardness and modulus of elasticity of primary teeth.  
(NM= not measured)

| Author and Date               | Dental tissue testing | Hardness                                                                | Modulus of Elasticity              | Test method and scale                                                 |
|-------------------------------|-----------------------|-------------------------------------------------------------------------|------------------------------------|-----------------------------------------------------------------------|
| Lussi <i>et al.</i> , 2000    | Enamel                | 346 +/- 64 to 3314 +/- 139                                              | NM                                 | Knoop diamond indenter, 500 gm; Knoop hardness number (KHN)           |
| Kotaka <i>et al.</i> , 1992   | Enamel                | 462.0 +/- 28.9 VHN<br>outer enamel<br>373.9 +/- 27.1 VHN inner enamel   | NM                                 | Vickers Hardness Number (VHN) Vickers shaped diamond indenter 25 gm   |
| Salamata and Kinawi 1989      | Enamel                | 229.32 to 238.5 VHN                                                     | NM                                 | Vickers Hardness Number (VHN) Vickers shaped diamond indenter 5-20 gm |
| Nishinara 1986                | Enamel                | 206.7 +/- 24 to 337.9 +/- 62.5 VHN<br>44.5 +/- 9.9 to 76.9 +/- 20.4 VHN | NM                                 | Vickers Hardness Number (VHN) Vickers shaped diamond indenter 200 gm  |
| Fosse <i>et al.</i> , 1986    | Dentine<br>Enamel     | 111 +/- 43 to 376 +/- 109 VHN<br>(depending on surface of tooth tested) | NM                                 | Vickers Hardness Number (VHN) Vickers shaped diamond indenter         |
| Davidson <i>et al.</i> , 1974 | Enamel                | 327 +/- 34 to 367 +/- 17 KHN                                            | NM                                 | Knoop diamond indenter, 50 or 100 gm; Knoop hardness number (KHN)     |
| Bowen <i>et al.</i> , 1962    | Dentine               | NM                                                                      | 2.8 +/- 0.79 x 10 <sup>6</sup> psi | Tensile strength measurements psi                                     |

Table 1.9: Hardness and modulus of elasticity of permanent teeth (NM= not measured).

| Author and Date               | Dental tissue testing | Hardness                                                 | Modulus of Elasticity            | Test method and scale                                                                                                                        |
|-------------------------------|-----------------------|----------------------------------------------------------|----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| Pyge et al.<br>1961           | Enamel                | 123-355 KHN<br>242-348 VHN                               | NM                               | Knoop diamond indenter, 1-10000 gm; Knoop hardness number (KHN)<br>Vickers Hardness Number (VHN); Vickers shaped diamond indenter, 1-10000gm |
| Wickesley 1959                | Dentine               | 30-72 KHN<br>65-80 VHN                                   | 1.79 x 10 <sup>6</sup> psi       | Four point bend test<br>Psi                                                                                                                  |
| Graig and Payton 1958b        | Dentine               | NM                                                       | 2.4 to 2.7 x 10 <sup>6</sup> psi | Compressive strength measurement, psi                                                                                                        |
| Graig and Payton 1958a        | Enamel                | 343-723 KHN                                              | NM                               | Knoop diamond indenter, 50 gm; Knoop hardness number (KHN)                                                                                   |
|                               | Dentine               | 68-73 KHN                                                |                                  |                                                                                                                                              |
| Stanford et al.<br>1953       | Enamel                | NM                                                       | 1.3 to 6.9 x 10 <sup>6</sup>     | Compressive strength measurement                                                                                                             |
| Galavell et al.<br>1957       | Enamel                | 365-735 KHN                                              | NM                               | Knoop diamond indenter, 500 gm; Knoop hardness number (KHN)                                                                                  |
| Alchopland Sansbury<br>(1953) | Enamel                | 751 (old permanent teeth)<br>303 (young permanent teeth) | NM                               | Relative Hardness number with Diamond shaped indenter 30 gm                                                                                  |
| Payton et al.<br>1952         | Dentine               | 63 KHN                                                   | 1 670 000 psi                    | Knoop diamond indenter, 500 gm; Knoop hardness number (KHN)                                                                                  |

Table 1.9: Hardness and modulus of elasticity of permanent teeth (NM= not measured).

## Ultra Micro-Indentation Hardness Test (UMIS)

Over the years there has been a need for a technique capable of measuring the mechanical properties of a very small area, for example when testing a thin film developed for surface coating. As a result a new generation of ultra micro-indentation system (UMIS) or nano indentation instruments has been developed. A nano-indentation technique involves very small indentations ( $< 1 \mu\text{m}$ ) being produced using loads of a few millinewtons (Doerner & Nix 1986). This technique involves recording the depth rather than the area of the indentation that other micro-indentation techniques utilise.

The operating principle is as follows: a member carrying a specially developed elastic element is driven downwards by a long-range piezo driver. The elastic element supports a shaft with a diamond pyramid or spherical indenter attached. A linear variable differential transformer (LVDT) secured to the frame of the instrument records the position of the shaft. When the indenter contacts the surface, the elastic element is deflected relative to the member carrying the elastic element and this deflection is monitored by a second LVDT. The deflection of the elastic member generates the indentation force. The depth LVDT output is zeroed when the indenter contacts the surface, establishing a datum for the measurement of the indentation depth. The depth-sensing device continuously records changes in depth of an indentation during both the loading and unloading of the indenter (Bell TJ, Bendeli A, *et al.* 1992).

The two main mechanical properties determined by the UMIS are hardness and modulus of elasticity. The hardness is calculated from force and projected area. Unlike conventional micro-indentation hardness tests, the force displacement response is also measurable and the elastic modulus is determined from the relationship between force and depth of penetration, rather than from visual measurement of the indentation impression (Poolthong S 1998).

## The Indentation Model

To make reliable estimation of the hardness it is necessary to relate the depth of penetration, the force encountered and the indenter geometry. Each material indented, whether it is a dental material or not, will have a degree of plastic and a degree of elastic deformation of its surface as a consequence of the contact force. The magnitude the deformation depends on:

- The sharpness of the indenter tip
- The hardness of the material surface
- The elastic modulus
- The contact force

The relationship between the depth of penetration and load is determined by the elastic deformation plus the behaviour of the material and typically results in a load displacement curve of the type illustrated in Figure 1.1.

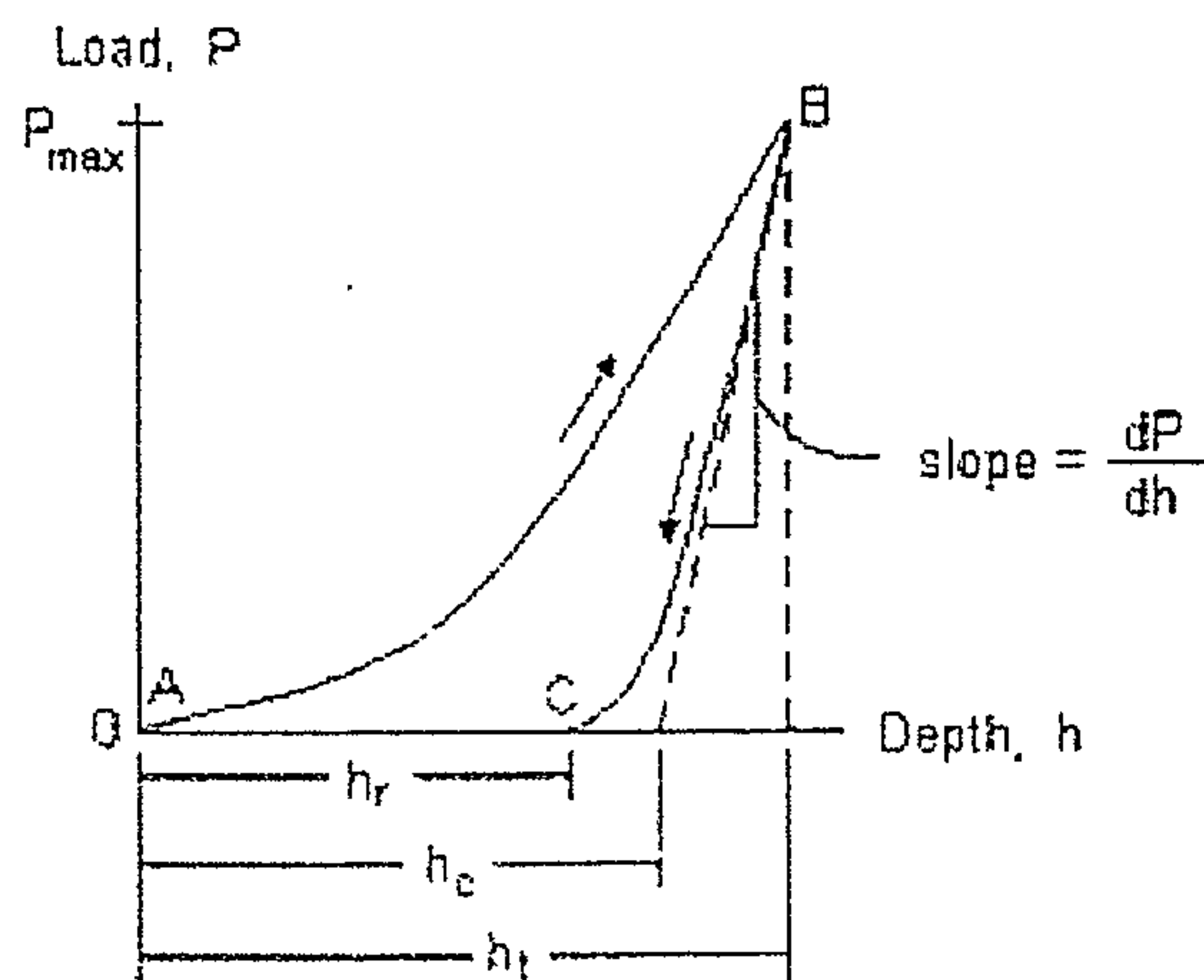


**Figure 1.1:** A typical load-displacement curve.

The quantities shown are:

- AB = loading curve
- BC = unloading curve
- Point A = first contact point between the indenter and the specimen surface
- Point B = last contact between the indenter and the specimen
- $P_{\max}$  = maximum load of the test
- $h_t$  = maximum penetration depth of the indenter
- $h_r$  = remnant depth of the indentation (length AC)

From the tangent of the unloading curve, the contact distance  $h_c$  can be determined, which is used in the determination of Young's modulus of elasticity. The depths are shown in diagrammatic form in Figure 1.2.

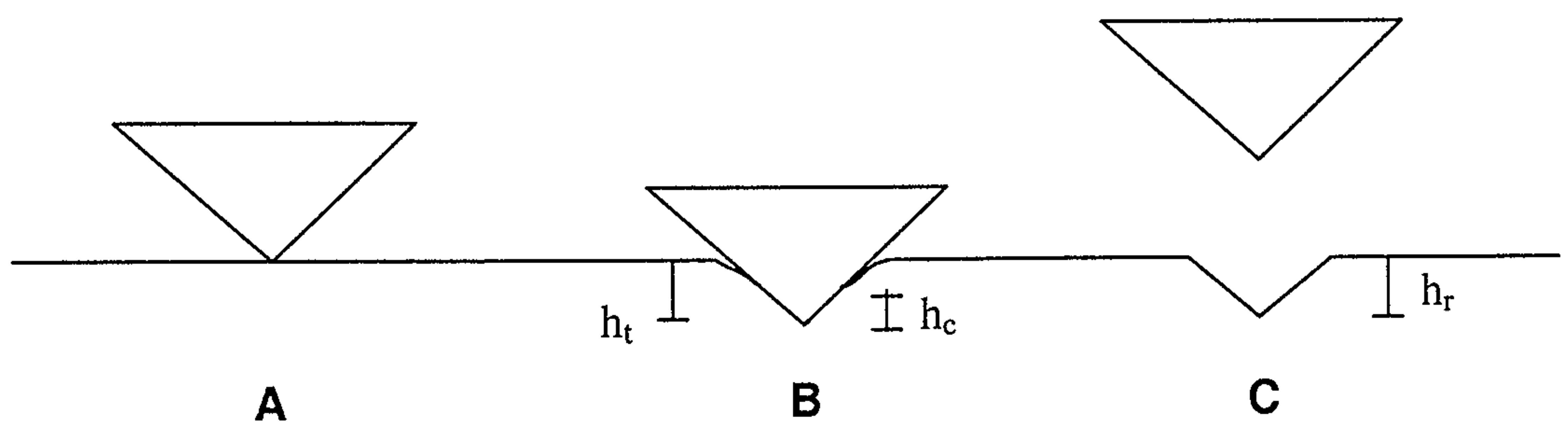


**Figure 1.2:** Schematic representation of the indenting process illustrating the total depth:  $h_t$ , contact depth:  $h_c$ , and the residual depth:  $h_r$ .

By knowing the contact force, the UMIS calculates the hardness by the force or load placed on a surface divided by the surface area of the indentation.

$$H (\text{hardness}) = \frac{\text{force}}{\text{Projected surface area}}$$

Or

$$H = \frac{P}{A}$$

where A is the area of contact and is defined as

$$A = kh_c^2$$

where k is a geometric constant which for a Berkovich indenter is 24.5

The contact depth,  $h_r$  can be obtained from the load-displacement curve by fitting a line tangent to the unloading curve at maximum load and extrapolating to zero. The hardness is deduced from the value of  $h_c$ , the maximum load and knowledge of the indenter geometry.

Both the Knoop and Vickers hardness tests utilise known force and the surface area to calculate the hardness. The UMIS on the other hand utilises a known load and the projected area of indentation in three dimensions to calculate the hardness. As long as the indenter tip dimensions are known then any type of tip can be utilised. The UMIS has the advantage of being able to calculate the Vickers hardness number as well as the unit of the UMIS (GPa) because the geometry of the indentation tip is known.

Young's modulus of elasticity of the indented material can be obtained from the unloading curve, provided the area of contact, Poisson's ratio and the elastic modulus of the indenter are known.

Xu and co workers (1998) employed a Vickers diamond indenter in a modified microhardness tester but utilising smaller forces (2, 3, 5, 10, 20 and 50 newtons) than conventional Vickers hardness tests, to measure the mechanical properties of teeth. Using a similar technique as the UMIS, the hardness and modulus of elasticity of the teeth tested were calculated from graphs of the loading and unloading cycles. The displacement was measured between the base of the Vickers indenter and the sample mounting plate, with a pair of capacitive transducers, whereas the load was measured with a strain gauge. The results from this test for enamel was  $(3.26 \pm 0.21)$  to  $(3.62 \pm 0.26)$  GPa, depending on the individual specimen and whether the measurement was on the occlusal or axial enamel. For dentine the hardness was  $0.53 \pm 0.02$  to  $0.60 \pm 0.02$  GPa. The modulus of elasticity for enamel also varied depending if it was measured from the axial or occlusal enamel from between  $78 \pm 1$  to  $98 \pm 4$  GPa. Dentine's modulus of elasticity was reported to vary between 18 to  $22 \pm 1$  GPa.

Kinney *et al.*, (1996a and b) measured the hardness of peritubular and intertubular permanent dentine using an atomic force microscope, and the modulus of elasticity using a Berkovich diamond indenter. The low load forces utilised by a UMIS enabled different parts of the dentine tested. The hardness

of fully hydrated peritubular dentine was independent of location and ranged from 2.23 to 2.54 GPa. The intertubular dentine hardness did vary depending on location of indentation and the values for it was  $0.49 \pm 0.02$  to  $0.52 \pm 0.03$  GPa near the amelodentinal junction and  $0.12 \pm 0.02$  to  $0.18 \pm 0.02$  GPa near the pulp. The modulus of elasticity for peritubular dentine reported was  $29.8 \pm 8.9$  GPa and  $17.7 \pm 0.03$  to  $21.1 \pm 1.13$  GPa for the intertubular dentine.

It is known that unlike a carious lesion, in the process of erosion the subsurface of the enamel and dentine are more or less intact and it is the outer layer of the dental hard tissue that has its mineral dissolved layer by layer (Larsen MJ & Nyvad B 1999). Microhardness testing with an instrument such as the UMIS offers a great advantage as it examines the change hardness in the surface layers.

## Part 4. Predicting Erosive Potential

With respect to erosion the largest amount of literature produced is on the topic of potentially erosive beverages. There is no doubt in the literature that many beverages have the ability to cause erosion in both the primary and permanent dentition due to their acidic nature. The difficulty has been finding a causal relationship between a particular beverage, the erosion seen clinically and the property of the beverage that is responsible for the amount of erosion observed.

The factors that have been identified in the literature to be potentially important in determining the erosive potential of beverages are the pH, titratable acidity, fluoride, calcium and phosphate concentration and the temperature of the beverage consumed. For *in vivo* studies there also appears to be a great variation in the erosive capacity of beverages depending on the subject that is tested. This can be due to the differing oral anatomical and physiological variation between individuals (Hughes JA, West NX, *et al.* 2000).

Due to the fact that multiple interrelated factors that affect erosive potential, the relative importance of each factor is difficult to determine.

### pH

It is known that from early work in cariology that dental mineral begins to dissolve below pH 5.5 (Stephan RM 1940). It is therefore not surprising that pH is most widely studied property in relation to erosion both in *in vitro* or *in vivo*. The pH of a liquid is the negative logarithmic of the hydrogen free ion concentration (Stedman TL 1995).

Commonly consumed beverages such as fruit juices and soft drinks have all been reported to have a pH below the 'critical pH' for dental mineral of 5.5 (Touyz LZG 1994, Smith AJ & Shaw L 1987, Greenby TH, Mistry M, *et al.* 1990, Larsen MJ & Nyvad B 1999, Lussi A, Jaggi T, *et al.* 1993). From the literature, most research indicates that as the pH of a beverage decreases, becoming more acidic, there is an increased amount of erosion noted independent of the way in which erosion is measured (Hughes JA, West NX, *et al.* 2000, Meurman JH, Harkonen M, *et al.* 1989).

Unfortunately most studies into the effect of pH have not measured the amount and effect of the other factors believed to be responsible for erosion such as titratable acidity, content of fluoride and calcium and phosphate content. Furthermore across many studies a wide variety of methods has been used as summarised in the preceding section in this literature review. This makes comparison between different research difficult. Various researchers have tried to overcome these problems to determine if the pH is the critical factor in the erosive potential of various beverages. Hughes and colleagues (2000), using profilometry investigated the effects of varying the pH (by addition of sodium hydroxide or hydrochloric) of experimental beverages while the acid concentration (g/100mL) remained the same. These authors clearly showed that as the pH increased to become more alkaline, the mean amount of enamel lost decreased. Interestingly the type of acid that was important in the amount of erosion observed. Maleic acid produced significantly less effect on enamel at a higher pH than citric or lactic acid.

Although there appears to be a relationship between the pH and the degree of erosion noted experimentally, a number of authors have noted that this is not always straightforward (Greenby TH, Mistry M, *et al.* 1990, Grobler SR, Senekal PJC, *et al.* 1990, Grobler SR, Senekal PJC, *et al.* 1989, Dodds AP, Gragg PP, *et al.* 1997). Grobler and colleagues (1990) studied the amount of calcium lost when enamel was exposed to a variety of cola and fruit juices. These authors reported that over a 40-minute period, the rate of enamel demineralisation was not a direct function of the pH of the drinks. These findings have led researchers to investigate not only the pH of various beverages but also to test and relate the titratable acidity, buffering capacity and fluoride and other mineral content of the beverages to the experimental erosion observed.

### Titrateable Acidity

Titrateable acidity is the total amount of ionised and un-ionised acid present in a solution (Shaw L 2000b). It is measured as millilitres of sodium hydroxide at a known concentration required to neutralise a given amount of the beverage and is therefore also a measure of the buffering capacity. Titrateable acidity can correlate directly with the pH of the liquid tested but also has been shown to *not* have a high degree of correlation at low pH values, when a significant amount of the  $H^+$  is bound to other ions in solution (Greenby TH, Mistry M *et al.* 1990, Edwards M, Crenor SL, *et al.* 1999).

It has been suggested that titratable acidity may be a better indicator of the erosive potential of a beverage than the pH alone (Greenby TH, Phillips A, *et al.* 1989, Grobler SR, Senekal PJC, *et al.* 1990, Milosevic A 1997, Lussi A, Kohler N, *et al.* 2000). Greenby and colleagues (1989) measured the calcium and phosphate dissolution in fruit juices, cola and cordial beverages. They found that demineralisation potential of the drinks generally increased as their total acidity levels rose (ie. free  $H^+$  and bound  $H^+$ ). They demonstrated graphically that titratable acidity was a more accurate measurement of the erosive potential than the pH by showing that demineralisation potential of the drinks generally increased with the rise in their total acidity level (Greenby TH, Phillips A, *et al.* 1989).

Titratable acidity is rarely investigated in isolation with most authors reporting it in combination with at least one of the following other properties; pH, calcium, phosphate and fluoride concentration.

### Calcium, Phosphate and Fluoride Ion Concentration

It is not surprising that beverages that are known to cause erosion are reported to be undersaturated with respect to hydroxyapatite and fluoroapatite (Larsen MJ 1975, Meurman JH & ten Cate JM 1996). If there is a high enough concentration of calcium, phosphate and fluoride in an acidic beverage, it is believed that these ions would help to counter the erosive process (Larsen MJ & Nyvad B 1999).

Greenby and colleagues (1990) investigated the amount of *in vitro* erosion of 18 infants drinks. They found that the drinks that dissolved the least amount of hydroxyapatite were amongst the richest in calcium and phosphorus. These drinks also had the higher pH.

Lussi and colleagues when investigating the change in surface microhardness found that all drinks investigated (n=12) significantly reduced the microhardness except orange flavoured yogurt and orange yogurt drink. The orange yogurt drink with a pH of 4.25 did not cause any significant decrease in the surface microhardness of either primary or permanent teeth and orange flavoured yogurt caused an increase in surface microhardness over the 3 minutes the teeth were immersed in the yogurt. The orange flavoured yogurt and orange yogurt drink had significantly higher phosphate and calcium levels than any other beverage. It was postulated that this was the reason for the increase in surface microhardness even though the pH values both were below the critical pH for enamel dissolution (orange yogurt drink pH=4.25 and orange flavoured yogurt pH=4.08) (Lussi A, Kohler N, *et al.* 2000).

Larsen and Nyvad (1999) investigated enamel erosion by various beverages and related it to pH, buffering capacity and concentration of calcium and phosphate. Although the exact amount of experimental erosion observed by the researchers was only given graphically the authors reported that the calcium and phosphate rich orange juice (the orange juice was manufactured as a source of these ions to combat osteoporosis) had a strong preventive effect on erosion. The authors state that the addition of the calcium and phosphate appeared to

prevent erosion by the juice. It is difficult to make direct comparisons between the different orange juices that were investigated as no numerical evidence for the amount of dissolution/erosion was reported in the paper.

Hughes, West and colleagues (1999a, 1999b, 2000) have published a series of articles on the development of a low erosive blackcurrant juice drink. These authors have shown through their *in vitro* studies and a series of *in vivo* studies, where volunteers wore removable acrylic appliances over 15 days, that a blackcurrant drink with added calcium caused significantly less erosion than the negative controls. The negative controls were orange juice of various brands and a non modified apple and blackcurrant concentrate. Although the pH of the negative controls were slightly less than the modified beverages, this could in no way explain the large differences in the erosion seen between the negative controls and the modified beverages. These authors concluded that there was indisputable evidence that the low pH blackcurrant drink modified with the addition of calcium causes minimal dental erosion which even in long term usage would be clinically insignificant. These articles highlight how product modification can aid in decreasing the erosive potential of a beverage, even if it has a low pH, and also indicates that the calcium concentration is an when investigating the erosive potential (Baker NJ, Parker DM, *et al.* 1998, Hughes JA, West NX, *et al.* 1999a, Hughes JA, West NX, *et al.* 1999b, West NX, Hughes JA, *et al.* 1999).

Like the amount of calcium and phosphate in beverages the available literature reports the protective effect of fluoride in these beverages to be very variable.

Levels of between 0.00 to 1.78 ppm have been reported in various studies (Lussi A, Jaggi T, *et al.* 1993, Larsen MJ & Nyvad B 1999). The fluoride level will vary depending on where the research is carried out, if the community is fluoridated and whether the beverage is meant to be drunk diluted with tap water which may contain varying amounts of fluoride.

There have been various conclusions in the literature as to whether the fluoride concentration of beverages has an effect on its erosive potential. Larsen and Nyvad (1999) concluded in their *in vitro* study that none of the inorganic ions, including fluoride in the drinks tested, had any observable effect on the erosive capabilities except for calcium and phosphate when present in very high amounts. These authors concurred with Larsen *et al.*, (1976) in suggesting that because fluoride is supposed to act in concert with the other ions of the apatite lattice (calcium and phosphate) and in concentrations that saturate the solution with respect to fluoroapatite, it does not seem likely that fluoride alone has the ability to suppress erosion (Larsen MJ, Vd Fehr FR, *et al.* 1976). Other researchers do not support this view (Lussi A, Kohler N, *et al.* 2000, Lussi A, Jaggi T, *et al.* 1993). Lussi and colleagues (1993) investigated the influence of different factors on *in vitro* enamel erosion using multiple regression analysis. After measuring the change in microhardness and iodine permeability they showed that the erosive capacity of different drinks was significantly associated with their fluoride content ( $p < 0.001$ ).

Sorvari *et al.*, (1988) supplemented a sports drink mixture with 15 ppm fluoride and found that the rats who had this supplemented mixture had

significantly less erosion than controls. These researchers reported that at the end of the 42-day period that the rats who consumed the supplemented sports drink had significantly less dentine exposure to their molar teeth and there were significantly more areas of intact surfaces than the rats who were made to consume the sports drink without supplementation (Sorvari R, Kiviranta I, *et al.* 1988). Sorvari and colleagues have published other reports with a very similar study design that has confirmed these findings (Sorvari R 1989, Sorvari R, Pelttari A, *et al.* 1996). These studies suggest that fluoride can have a protective effect against erosion although it is not recommended that such drinks be consumed by very young children due to the risk of fluorosis.

## Permanent versus Primary Teeth

Multiple epidemiological and case reports in the literature have highlighted that erosion is not only a problem for permanent teeth but it also occurs with a high frequency in primary teeth (Shaw L & Smith A 1994, O'Brien M 1994, Millward A, Shaw L, *et al.* 1994). There is also *in vitro* literature showing the harmful effects of erosive beverages on deciduous enamel and dentine (Grando LJ, Tames DR, *et al.* 1996). This has led researchers to try and determine if there is a difference in the susceptibility of deciduous teeth in comparison to permanent teeth. Unfortunately there is very little research into this question at present and the literature is conflicting.

Hunter and colleagues investigated the differences in the susceptibility of deciduous versus permanent teeth in an *in vitro* study using a low pH fruit drink (Hunter ML, West NX, *et al.* 2000b). The authors suggested that the

deciduous enamel was more severely affected than permanent enamel, whereas deciduous dentine showed less erosion than permanent dentine. There were no statistically significant differences in the differences between the deciduous and permanent enamel except at day 15 (the last day of testing). It is interesting therefore that these authors concluded that 'though not of consistent statistical significant, the increased susceptibility of deciduous enamel to erosion is of clinical significance'. There is no doubt that the thickness of enamel in deciduous teeth is less than permanent teeth and any erosion will mean that dentine is exposed at a quicker rate than in permanent teeth, but this research has failed to statistically prove their conclusions. An increased number of teeth will need to be tested if their conclusions are to be supported.

Amaechi and colleagues (1999) investigated the enamel type and its susceptibility to erosion while investigating the influence of temperature on erosion. When human deciduous enamel was exposed, *in vitro* to orange juice, eroded lesions progressed 1.5 times more rapidly than in permanent enamel. They attributed this to differences in both the structure and chemical composition of permanent and deciduous enamel. Jenkins (1978) reported that the fluoride concentration in deciduous enamel is lower than permanent enamel formed under the same conditions. However it should be remembered that permanent and deciduous enamel do not form in the same environment. Deciduous teeth begin formation of enamel in utero, whereas the permanent teeth enamel formation varies from in utero to well after birth. Stack (1953) observed a slightly higher proportion of organic matter in deciduous enamel than in permanent enamel.

Lussi and colleagues (2000) also have investigated the relative susceptibility of permanent and deciduous teeth in their *in vitro* study. This study utilised the indentation method and Knoop hardness measurements taken before and after the extracted permanent and deciduous teeth were subjected to variety of different beverages. These researchers concluded that although the Knoop hardness measurements were initially lower for the deciduous teeth, the deciduous teeth were not more susceptible to erosion in comparison to permanent teeth as the hardness difference between deciduous and permanent enamel were not statistically significant (Lussi A, Kohler N, *et al.* 2000).

### Other Properties or Modifying Factors

Researchers have investigated other *in vitro* factors that appear to have an effect on the erosive potential of beverages.

The rate of enamel dissolution has been shown to be temperature dependant and therefore Amaechi and colleagues (1999) carried out an *in vitro* experiments on human permanent and deciduous teeth as well as bovine enamel to investigate the influence of temperature of orange juice on erosion. Their experiments showed that as the temperature of the beverage that the teeth were exposed to increased from 4 degrees to 37 degrees Celsius, the amount of erosion also significantly increased. These authors concluded that the greater degree of erosion observed at higher temperatures strongly suggested that warm fruit juices including those at room temperature could be more erosive than ice-cold fruit juices.

Dental erosion has a complex aetiology. The *in vivo* measurement of erosion is complicated by the lack of a single valid index that can be reliably used for epidemiological studies that will enable aetiological agents to be accurately identified. In the absence of accurate, reliable *in vivo* information, *in vitro* research must continue to determine the erosive potential of commonly consumed beverages. Knowledge of the erosive potential of beverages and the potential modifiers of the erosive potential of a beverage, such as the fluoride, calcium and phosphate content, will allow recommendations to be given to beverage manufacturers and clinicians who daily are faced with the growing problem of dental erosion.

## Chapter 2. AIMS

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The aims of this thesis are three fold.

1. To determine the mechanical properties of primary tooth enamel and dentine using the Ultra Micro-Indentation System.
2. To establish the erosive potential of a number of different orange flavoured beverages.
3. To define the relationship between chemical composition and erosive potential.

## Chapter 3. MATERIALS AND METHODS

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The teeth used for this research were obtained from Westmead Children's Hospital. They were placed in 1% Milton (disinfectant) immediately after extraction and stored at 4°C for at least 24 hours. After the disinfection procedure the teeth were stored in distilled water at 4°C until required for preparation. All teeth were 1st deciduous maxillary molars. As was noted by Saunbury and Atkinson (1953), caries free teeth are difficult to obtain for testing. For these experiments a large number of teeth were obtained and the teeth with the smallest infected regions were chosen. When carious teeth were used, the testing was performed on non-carious regions. For the initial experiment which was to determine the mechanical properties of primary teeth, eight teeth were finally selected. For the second set of experiments 20 teeth were selected and for all teeth the donor's age ranged from 5 to 10 years.

### Preparation

#### Resin Setting

The teeth were entirely set in a cold-curing epoxy resin [Epofix, Struers, Copenhagen, Denmark] for manageability and to allow the polishing and grinding system to obtain a better grip for holding the samples. The resin used comprised of two constituents; resin and hardener. The required ratio for mixing these constituents was 25 parts of resin to 3 parts of hardener by weight. The resin and hardener were mixed in a plastic cup for 2 minutes using a wooden stirrer. The teeth were placed in a rubber mould with the roots of the

teeth resting on a small piece of re-useable adhesive (Blue Tack). The sides of the rubber mould were lined with a thin plastic film to allow easy removal. The resin mixture was slowly poured around the teeth in the mould so that the liquid sufficiently covered the tooth crown. The samples were given at least 24 hours to set.

## Sectioning

Once the resin had set, the embedded teeth were removed from their moulds and the region to be removed was determined. Since the hardness of both enamel and dentine was being investigated the crown of the tooth needed to be removed to expose the inner dentine. To enable the mechanical properties of paediatric teeth to be comparable, it was required to standardise the sectioned distance (ie - the amount removed). It was decided that 50% of the crown of the tooth, measured from the top most point to the CEJ, was to be removed. This meant that for teeth of different sizes, the *same region* was being investigated, rather than it being a similar distance and *different region*. It also meant that the prism orientation in the enamel was virtually horizontal to the plane chosen.

The teeth were sectioned using a low speed saw [Isomet, Buehler Ltd., Lake Bluff, USA] under tap water irrigation to expose the testing surface (See Figure 3.1).

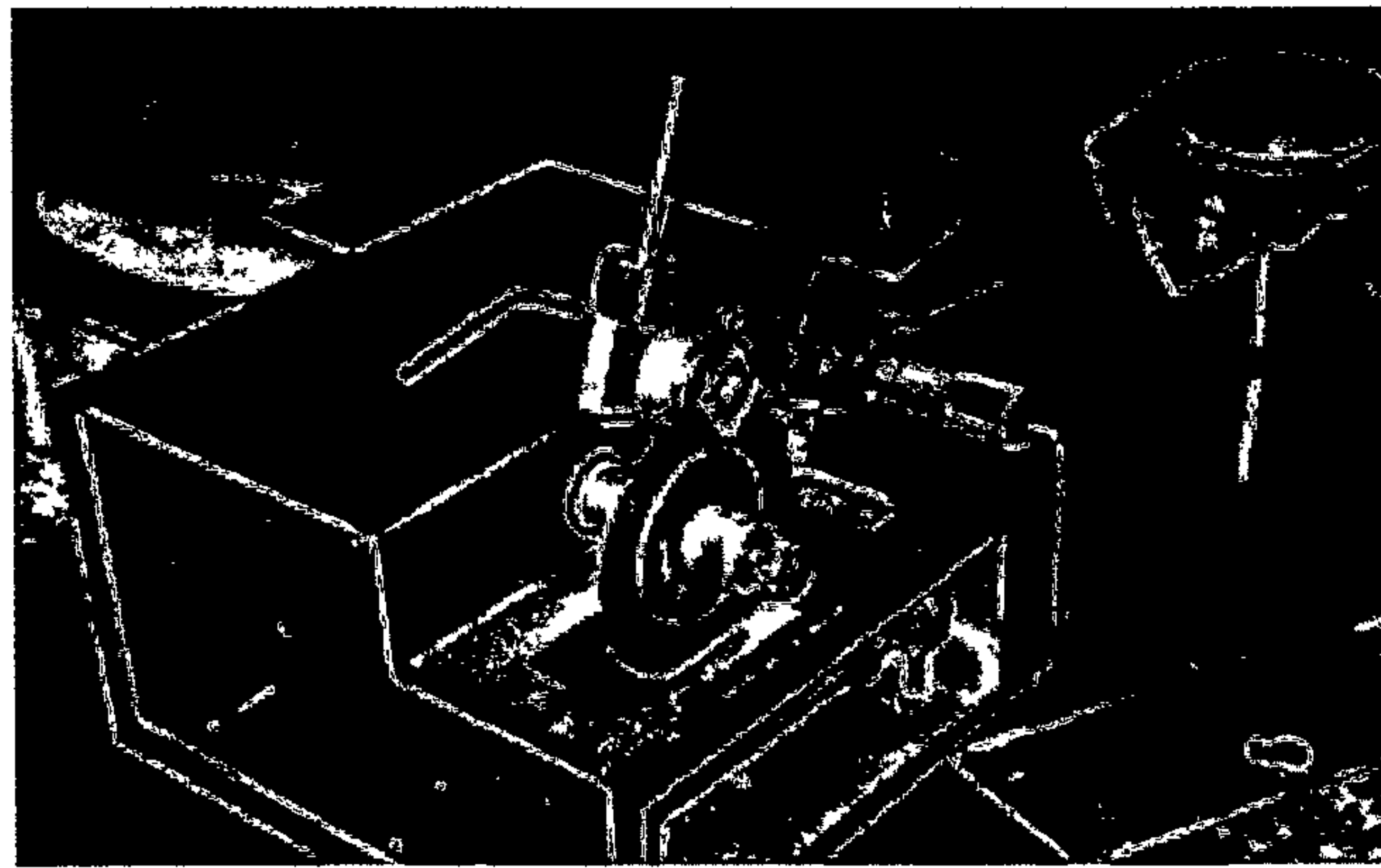


**Figure 3.1:** Low speed saw used in cutting samples.

## Grinding and Polishing

To ensure the surface of the exposed teeth were free from scratches and deformation the specimens were polished prior to the indenting. To achieve this result, surface material was removed by means of successively finer sizes of abrasive particles.

The grinding and polishing procedure involved four very similar steps undertaken in succession and was very similar to that used by Poolthong (1998) and Willems *et al.* (1993). See Table 3.1.

### Step One

The newly exposed surface was ground using grit #500 silicon-carbide paper in conjunction with a water lubricant/coolant. This was undertaken using hand motion on a specially designed polishing apparatus. The grinding paper was attached to the polishing apparatus with the adhesive back. The sample was polished for 5 minutes in one orientation and then polished for a further 5 minutes in an orientation 180 degrees to the original. The embedded teeth were

cleaned in the ultrasonic cleanser for 10 minutes to dislodge any silicon sediment from the tooth surface.

## Step Two

The ground surface of the embedded tooth was polished with a 9  $\mu\text{m}$  diamond suspension [DP-Suspension P, Struers], mixed with lubricant [DP-Lubricant Green, Struers]. This was undertaken using an automatic polishing machine [Rotoforce-4 and Multidoser, Struers]. The grinding paper was attached to the polishing disc with the adhesive back. A polishing cloth<sup>#</sup> was attached to the polishing disc [DP-Pan, Struers] with rotational speed of 150 rpm (see Figure 3.2). The force applied to the samples was 5 N. The polishing disc and specimen holder rotated in the opposite direction for five minutes and then in the same direction for another five minutes - the force was increased to 10 N. The sample was cleaned in the ultrasonic cleanser for 10 minutes as before.

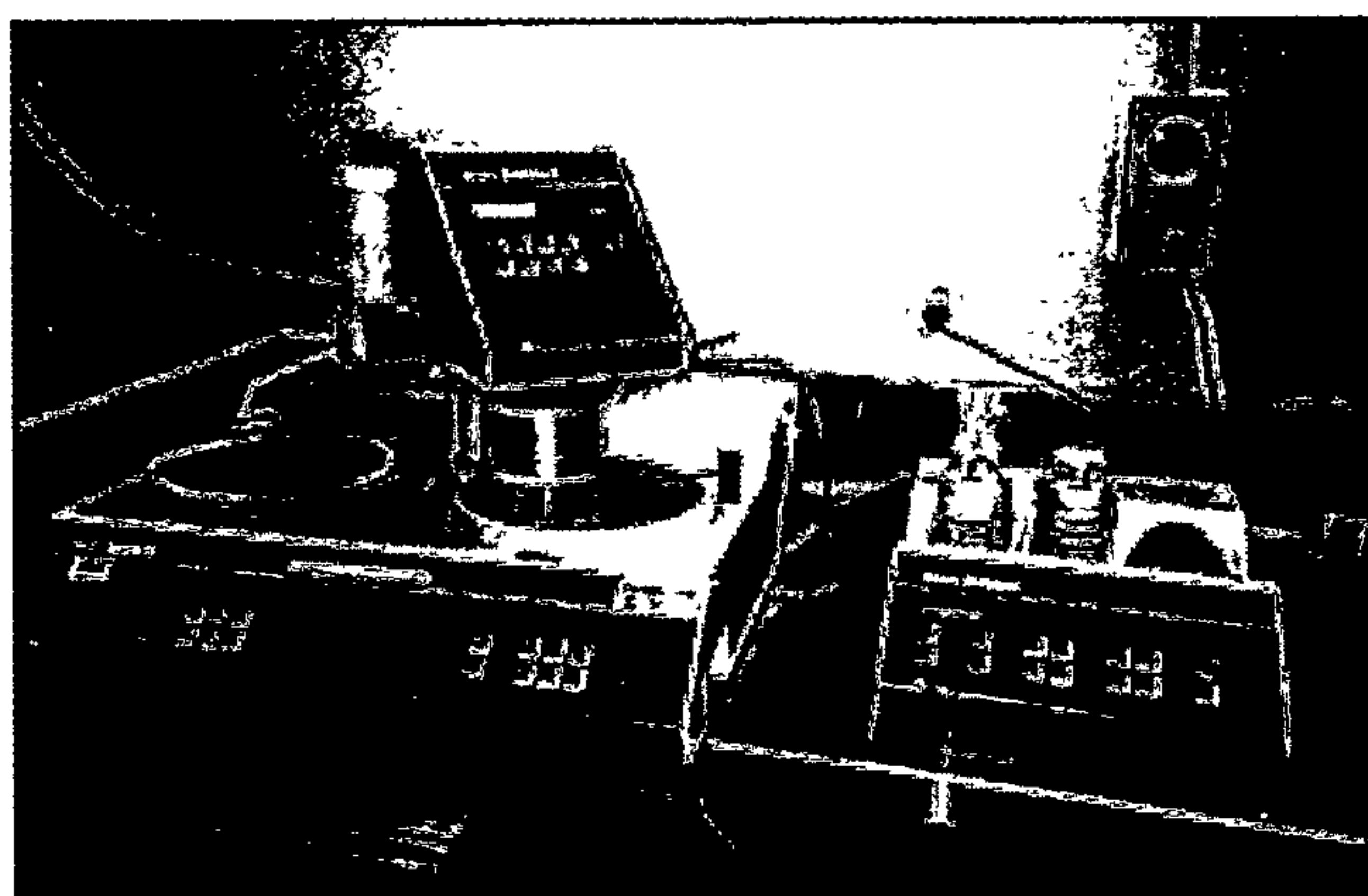


**Figure 3.2:** Automatic polishing machine used to polish samples.

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<sup>#</sup> The polishing cloth contains an intermediate layer that prevents the diamond particles from embedding into the next layer

Step Three

The exposed surface was polished with a 1 µm diamond suspension. A new polishing cloth was attached to the rotating disc and a different lubricant was administered [DP-Lubricant Red, Struers].

The four grinding and polishing steps are summarised in Table 3.1.

| Step                                     | 1                                | 2                                   | 3                                 |
|------------------------------------------|----------------------------------|-------------------------------------|-----------------------------------|
| Abrasive<br>Grit/Grain size<br>Lubricant | Silicon-carbide<br>#500<br>Water | Diamond particle<br>9µm<br>DP-Green | Diamond particle<br>1µm<br>DP-Red |
| Rotational speed<br>[rpm]                | -                                | 150                                 | 150                               |
| Force [N]                                | -                                | 10                                  | 10                                |
| Time [min]                               | 5 in ea.<br>direction            | 5 in ea.<br>direction               | 5 in ea.<br>direction             |
| Cleanser<br>Time [min]                   | 10                               | 10                                  | 10                                |

Table 3.1: Summary of the grinding and polishing steps

Wax Mounting

The finished teeth specimens were mounted on metal bases with wax by pressing with a paralleling machine [Leitz, Wetzlar, Germany] (Figure 3.3 Shows two teeth mounted on the metal bases, ready for testing). The mounting base contained a strong magnet to ensure adequate contact is obtained with the test base in the UMIS. The wax was melted by heating with a hot plate prior to the pressing stage.



**Figure 3.3:** Two test teeth mounted on metal bases prior to indentation.

### Intermediate Storage

Once the teeth had been prepared they were stored at room temperature in a separate container in distilled water. The metal base attached to the tooth was placed end-up to prevent it entering the water and corroding.

### Testing - UMIS

The UMIS apparatus is enclosed in a cabinet and is installed in a small isothermal room (see Figure 3.4). The temperature controller was started and ambient conditions of  $(23 \pm 1)^\circ\text{C}$  and  $(50 \pm 10)\%$  relative humidity were set throughout the indenting tests, similar to those used by Mencík and Swain (1994) and Poolthong (1998). Before use the UMIS was allowed at least 30 minutes to stabilise to the conditions. Prior to testing the samples were removed from the incubator, dried with an optical lens tissue and also given 30 minutes to reach a stable temperature in the UMIS cabinet.

The UMIS instrument consists of three major components, the triangular pyramidal diamond indenter or Berkovich indenter [Synton BA, Switzerland], an optical microscope, and an X-Y transport table. The transport table moves

the specimen from the viewing position to the indenting position with a precision of approximately  $\pm 1 \mu\text{m}$  [UMIS 2000 Operating Manual 1995]. The complete device is installed on an anti-vibration table to exclude environmental vibrations.

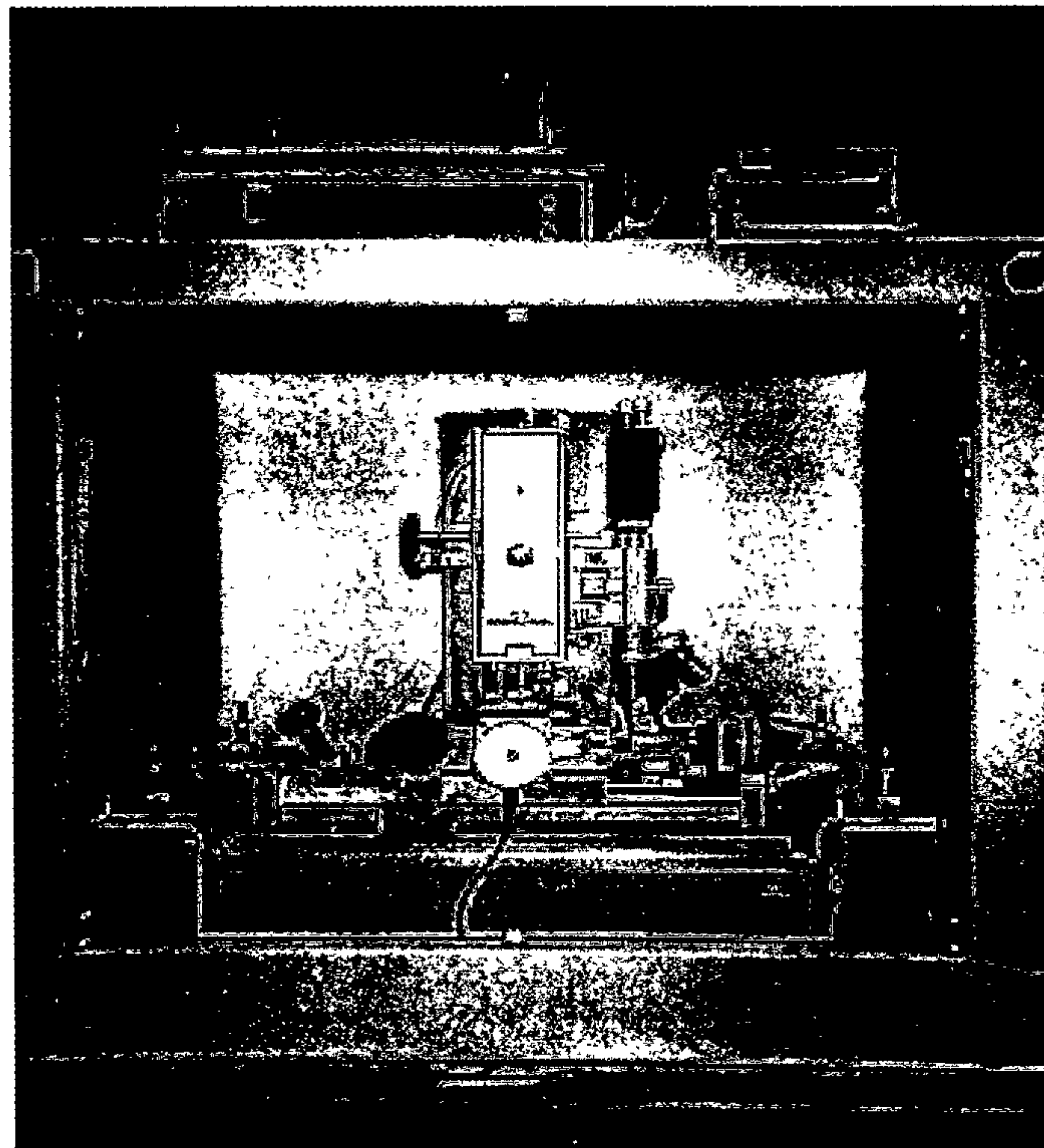


**Figure 3.4:** The Ultra Micro Indentation System.

### Positioning and Array Layout

Specimens were placed on the 50 mm diameter work-table and the regions to be tested was positioned under the microscope (Figure 3.5). The region was chosen so that it did not include any enamel imperfections, particularly lamellae. The selected region was displayed on the monitor connected to the UMIS and the location of the first indentation was set with an X-Y co-ordinate of 0-0. A series of indentation positions were then individually selected and the X-Y co-ordinates of each selected position were programmed into the computer following an array layout. The layout for each test run was

individually programmed prior to testing, yet the basic layout was similar to that shown in the following diagram (Figure 3.6).

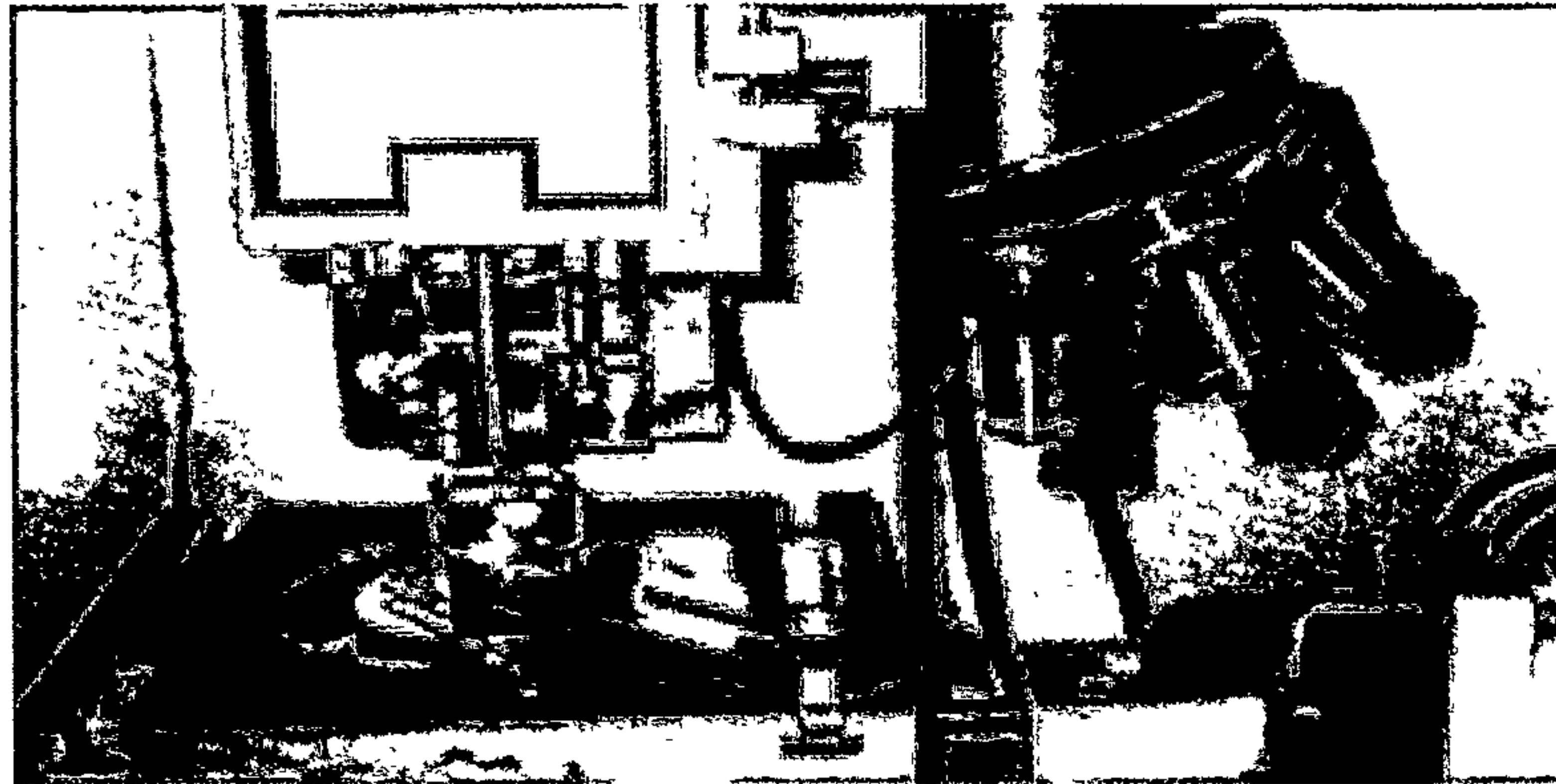


**Figure 3.5:** Figure showing the sample at the indenting position on the left and viewing microscope on the right.

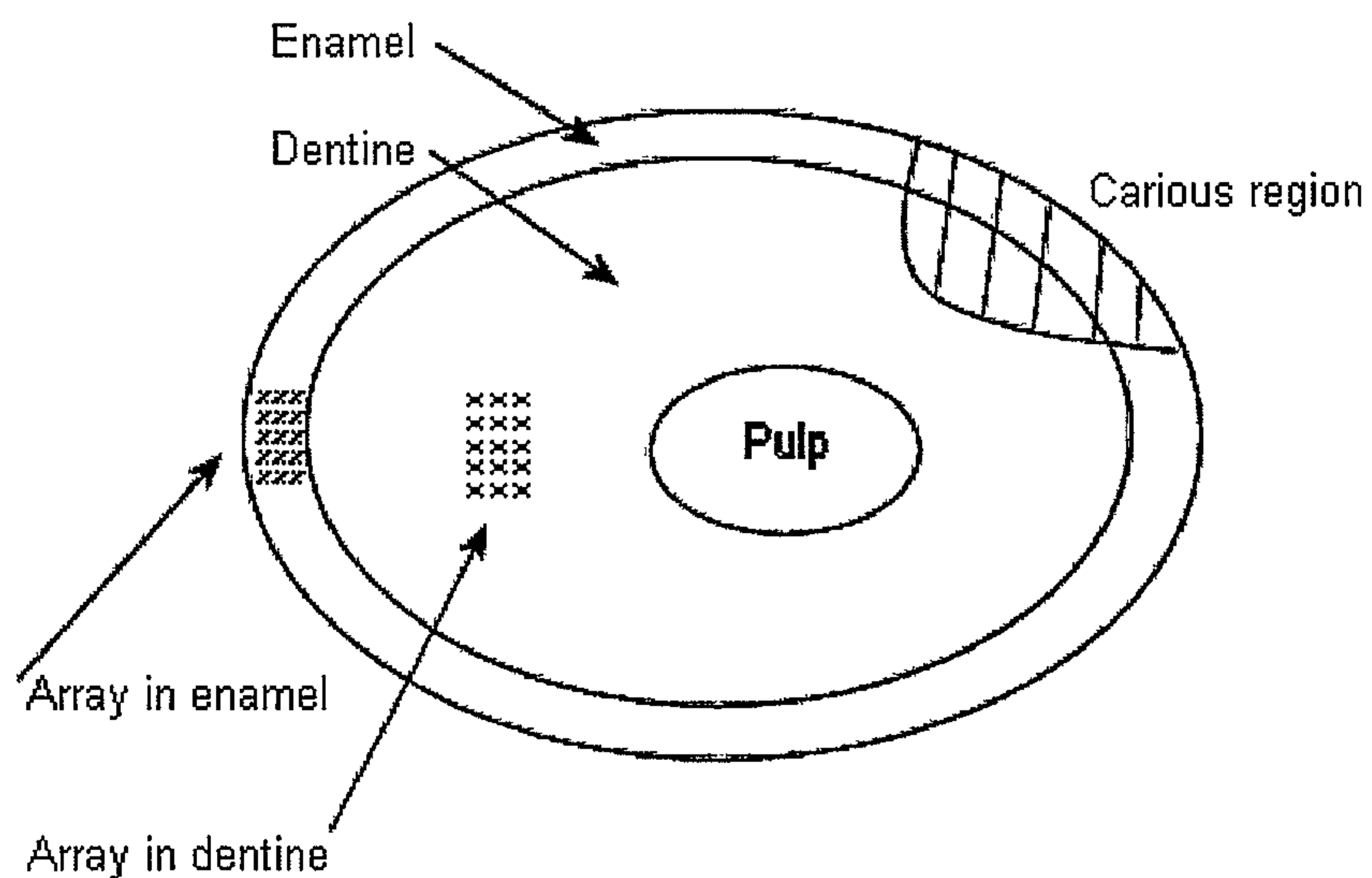


**Figure 3.6:** Layout of indents in enamel and dentine.

### Setting the Working Distance

After the positioning was complete, the specimen was translated from the viewing position to the indenting position. At the indenting position, the indenter was lowered until the tip was approximately 1 mm from the specimen surface. The option 'set working distance' was chosen from the software

associated with the UMIS and the indenter was automatically lowered until a contact force of 0.2 mN was encountered. If this contact force was not met then the graphic display indicated whether the indenter head needed to be raised or lowered. When the setting was correct it was known that the indenter had located the surface and this was set as the start position.

A thermal-stability monitoring mode was available and the computer continuously measured the depth of the indenter as a function of time. This gave an indication of the stability of the system as changes in the contact position were measured in nm.

The instrument was very sensitive to vibration and temperature changes and any alteration on the monitor indicated:

1. A drift due to environmental temperature instability or,
2. A drift due to specimen temperature instability or,
3. A fluctuation due to vibration and residual system noise.

When stability was acceptable (drift rate less than 0.02 nm/sec) the specimen was ready for testing. A hold segment of 30 seconds was inserted at the maximum load to monitor possible creep and/or mechanical stabilisation. A hold segment was also used in the research undertaken by Willems *et al.* (1993).

The test parameters were set as shown in Table 3.2.

| TEST PARAMETERS                |                                         |
|--------------------------------|-----------------------------------------|
| Indenter type                  | <b>Berkovich (pyramidal triangular)</b> |
| Contact Force [mN]             | <b>0.2</b>                              |
| Max. Indentation Force [mN]    | <b>50 or 150</b>                        |
| No. of increments              | <b>25</b>                               |
| Increment progression          | <b>Square root</b>                      |
| Dwell at Load/Max/Unload [sec] | <b>0.1 / 30.0 / 0.1</b>                 |
| Array row size                 | <b>3</b>                                |
| Array column size              | <b>17 – 35</b>                          |
| Delay between locations [s]    | <b>30.0</b>                             |
| Testing conditions             | <b>(23±1)°C and (50±10) %RH</b>         |

**Table 3.2:** The parameters assigned to the testing procedure.

## Experiment One

Experiment one was designed to determine the mechanical properties of eight primary teeth. For each tooth, 30 indentations were conducted on both enamel and dentine. Half of the 30 indentations in enamel were conducted at a loading force up to 50 mN and the other half were up to a loading force of 150 mN; this was to observe if there were changes in the mechanical properties over the tooth surface at different forces. Table 3.3 summarises the layout and forces used for the arrays in this experiment.

The indentations in enamel for all eight teeth began approximately 100 µm from the dento-enamel junction on the buccal aspect of the tooth. In enamel the distance between indentations was 50µm in the X and Y axis.

To standardise the position of the impressions in dentine, the indentations began half way across the buccal aspect of the cut dentine surface. In dentine, the indentations needed to be further apart than those across the enamel as at the higher (150 mN) force used there was a possibility of overlap of adjacent indentations at 50  $\mu\text{m}$  in dentine (Figure 3.7). The distance of 75  $\mu\text{m}$  between rows was found to be sufficiently large. For each tooth the testing procedure took approximately 6 hours. There was some concern as to the possible drying effect during this period however earlier work showed that teeth can be dry for up to two days without influencing the hardness and modulus of elasticity of either dentine or enamel (Poolthong S 1998).

| Tissue  | Force of indentation (mN) | Array | Number of indentations | Distance between individual indentations |        |
|---------|---------------------------|-------|------------------------|------------------------------------------|--------|
|         |                           |       |                        | X axis                                   | Y axis |
| Enamel  | 50                        | 3 X 5 | 15                     | 50                                       | 50     |
|         | 150                       | 3 X 5 | 15                     | 50                                       | 50     |
| Dentine | 50                        | 5 X 3 | 15                     | 100                                      | 75     |
|         | 150                       | 5 X 3 | 15                     | 100                                      | 75     |

**Table 3.3:** Layout and indent force used for Experiment one.

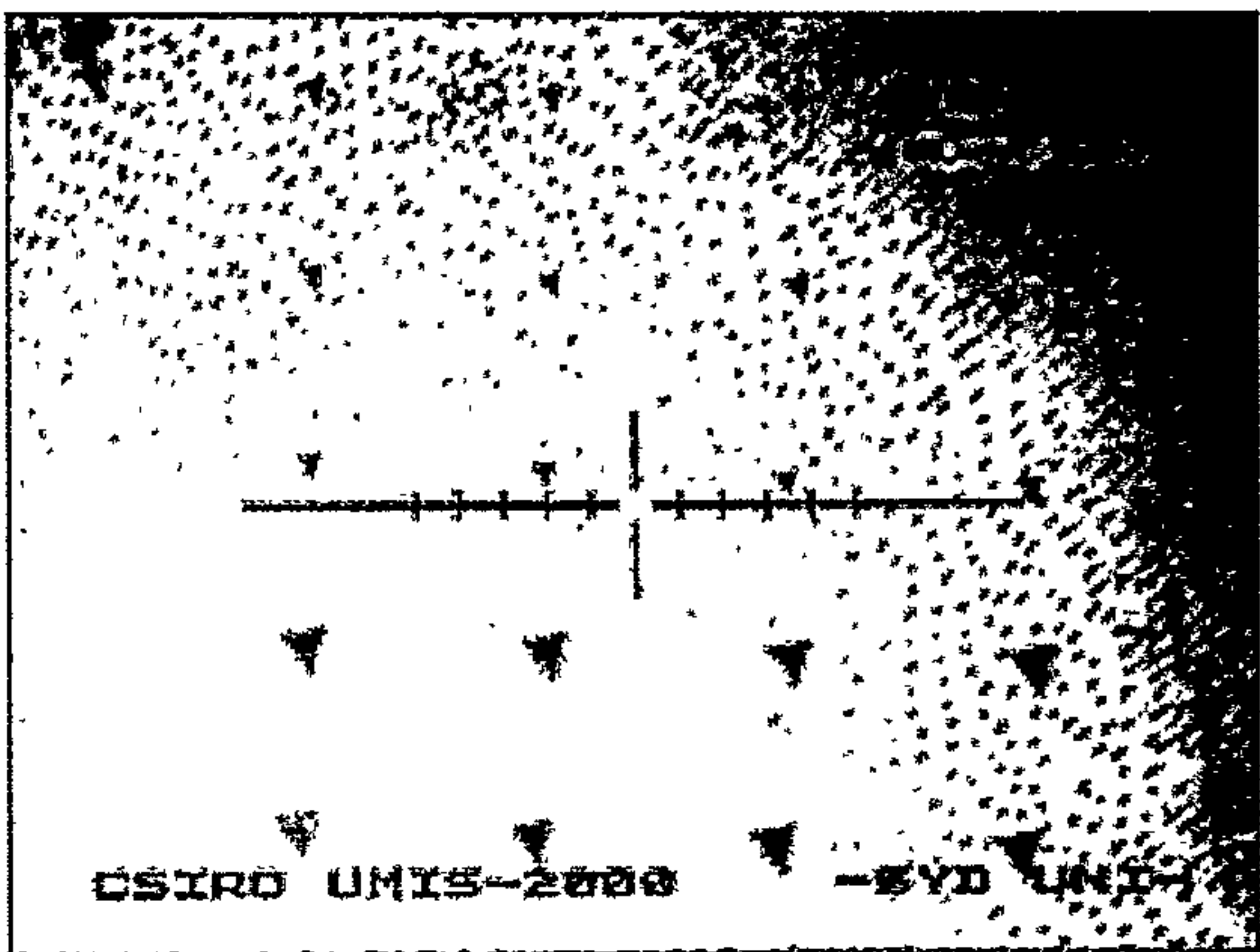


**Figure 3.7:** Picture showing a typical example of the indents made in dentine. Top of figure: indents made at 50 mN, bottom of figure: indents made at 150 mN.

# Experiment Two

There were 20 teeth chosen for experiment 2. Each of these teeth was randomly assigned to either a control group (distilled water) or one of four erosive beverage groups. The groups and numbers of teeth assigned to each group are summarised in Table 3.4.

| Group   | Number of teeth | Beverage                           |
|---------|-----------------|------------------------------------|
| Control | 4               | Distilled water                    |
| 1       | 4               | Orange Juice                       |
| 2       | 4               | Orange Cordial                     |
| 3       | 4               | Fanta (soft drink)                 |
| 4       | 4               | Ribena Toothkind (orange tropical) |

**Table 3.4:** Number of teeth in each of the test beverage groups.  
Orange Juice: Just Juice, Orange Cordial: Orange Crush, Cottees, Fanta: Coca Cola Company, Ribena Toothkind; GlaxoSmithKline Ltd.

## Preparation of the Beverages

As there are no instructions from the manufacturer to alter the product in any way, distilled water, Orange Juice and Fanta were used in this experiment as purchased. Ribena Toothkind and Orange Cordial were diluted as per manufactures instructions, which were for both products; 1 part product to 4 parts water. It was decided that tap water from the Sydney city water supply was to be used as the diluent, as this is the water that most people in Sydney will be using for the dilution. All beverages and tap water used in this experiment were used at room temperature.

One hundred millilitres of the experimental beverage was placed in a new plastic cup. If the beverage needed dilution then the mixture was made up to a total of 100 mL. For each individual tooth a new 100 mL sample of each liquid was used and for Orange Juice and Fanta a new carton or bottle respectively was used for each tooth.

## Indentation and Exposure Procedure

For each tooth 50 mN was chosen as the indentation force for experiment 2. Each tooth was positioned and 15 indentations in enamel and dentine were carried out. The overall process of indentation was identical to experiment 1. The procedure is summarised in Table 3.5. The tooth was then removed from the UMIS apparatus.

| Tissue                             | Force of indentation (mN) | Array | Number of indentations | Distance between individual indentations |        |
|------------------------------------|---------------------------|-------|------------------------|------------------------------------------|--------|
|                                    |                           |       |                        | X axis                                   | Y axis |
| Enamel                             | 50                        | 3 X 5 | 15                     | 50                                       | 50     |
| Enamel after exposure to beverage  | 50                        | 3 X 5 | 15                     | 50                                       | 50     |
| Dentine                            | 50                        | 5 X 3 | 15                     | 100                                      | 75     |
| Dentine after exposure to beverage | 50                        | 5 X 3 | 15                     | 100                                      | 75     |

**Table 3.5:** Layout and indentation force for Experiment 2.

Immediately the metallic base used in the indenting procedure was removed by slightly heating the base. There was very little transference of heat to the resin and tooth due to the shortness and low temperature of the heat used to remove the metallic base. The tooth was allowed 10 minutes to return to room temperature (22 degrees Celsius). The tooth was placed in the 100mL of experimental liquid with the prepared surface facing upwards. No agitation was performed. The tooth was left undisturbed in the liquid for exactly 10 minutes, removed and washed continuously with distilled water from a slow running squeeze bottle for two minutes.

The tooth was remounted as before onto the metallic base plate and repositioned on to the 50 mm diameter work-table. The area previously indented was identified under the microscope. A coin was then tossed to randomly determine the position of the second set of indentations in enamel and dentine. If the coin was a head then the second set of indentations was

carried out directly above the previous indentations, and if the coin was a tail then the indentations were carried out below the previous indentations.

Once the indentations were completed for experiment 2, the teeth were stored in distilled water to be used for electron microscopy examination.

## Data Analysis

It was important to evaluate the quality of the indentations and determine whether any values needed to be discarded. For both experiments this was done immediately after testing by viewing under microscope each indentation in the array allowing inspection of their geometry and position (Figure 3.7). The software associated with the UMIS also allowed elimination of unsuitable indentation values by production of individual load-unload curves recorded for each indentation. By observing these graphs extraneous data could be deleted as required. Figure 3.8 shows a force versus penetration graph for a normal and acceptable indentation in enamel and dentine. Figure 3.9 shows that not only does the rejected indent not reach the appropriate penetration depth for dentine, but also the shape of the curve is abnormal. The reason for this could be a surface imperfection or cracking of the tissue being tested.

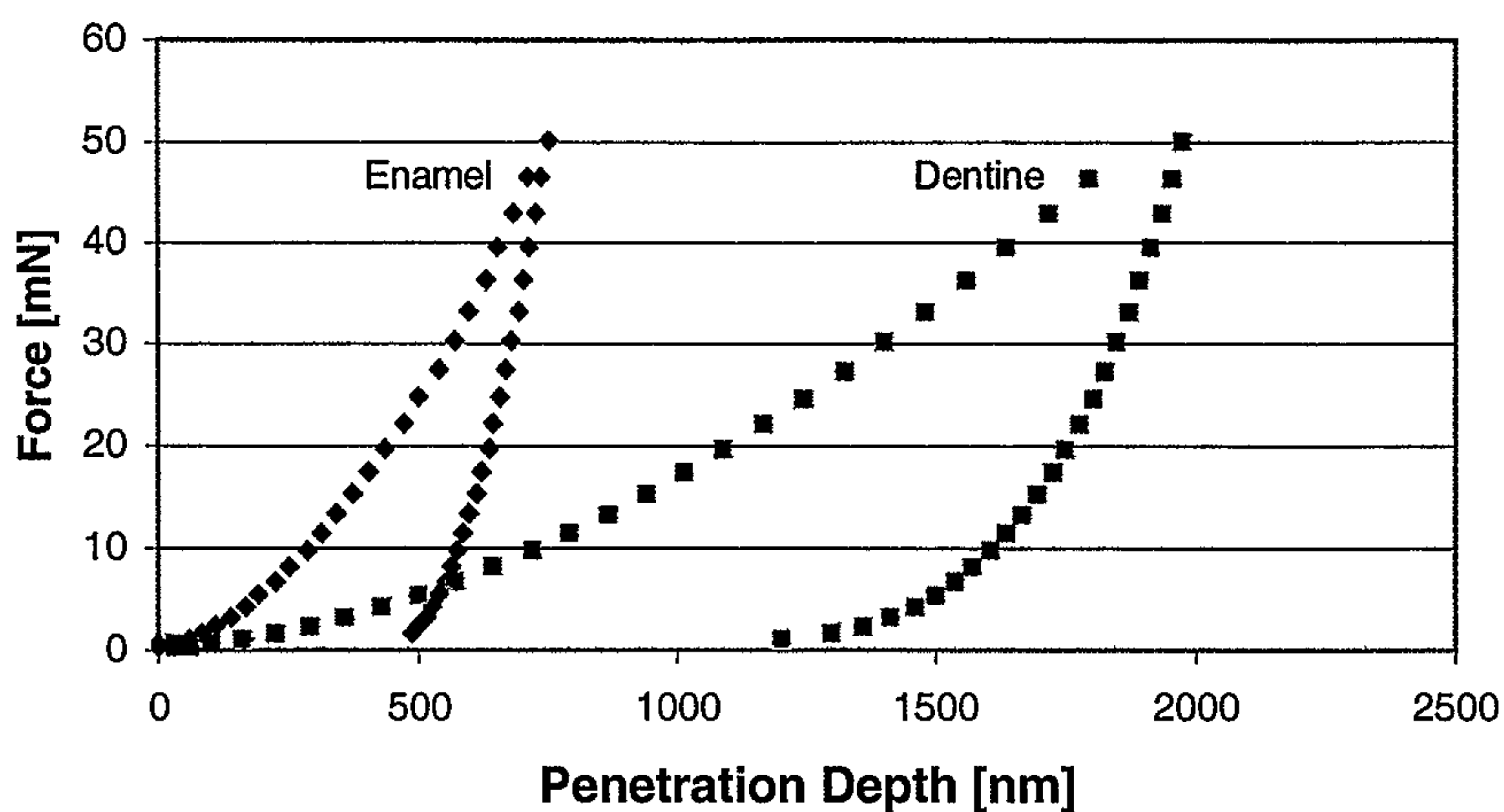


**Figure 3.8:** Force vs penetration depth curve for enamel and dentine.

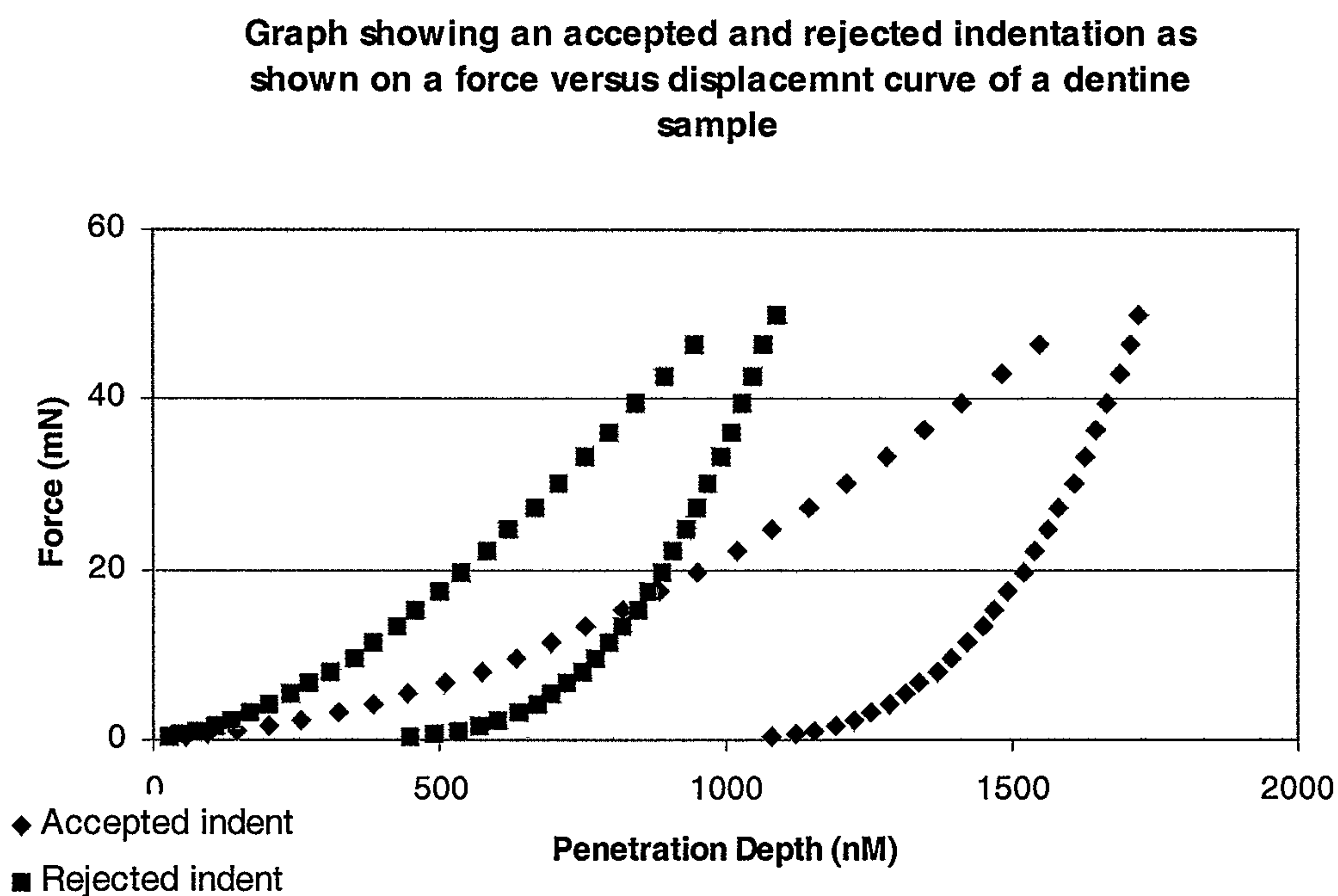


**Figure 3.9:** Force vs penetration graph showing an acceptable and an unacceptable indentation for dentine on a force vs displacement curve.

The unacceptable indent did not reach the adequate depth of penetration.

The software associated with the UMIS also allows analysis of the test data.

This program adjusts the data for initial penetration under the contact force and makes corrections for indenter geometry. Each indentation was individually analysed using a curvilinear fit for the upper fifth portion of the unloading

curve. This information was then calculated and displayed as required. The two main outputs retrieved were;

- Hardness as a function of plastic penetration depth
- Elastic modulus of the material determined at the maximum penetration depth in terms of  $E_m/(1-\nu_m^2)$ .

A typical hardness as a function of penetration depth graph is shown in Figure 3.10. The mean hardness for each indent was reported by averaging all data points except the first three due to the surface roughness and tip irregularity, and the last one which is the creep induced hardness. This method of deleting the first three data points is similar to that used by Poolthong [1998]. Increasing the dwell time leads to an increase in the size of the indentation for a given load and results in an apparent reduction in the hardness. This effect is called indentation creep and the related hardness value was deleted.

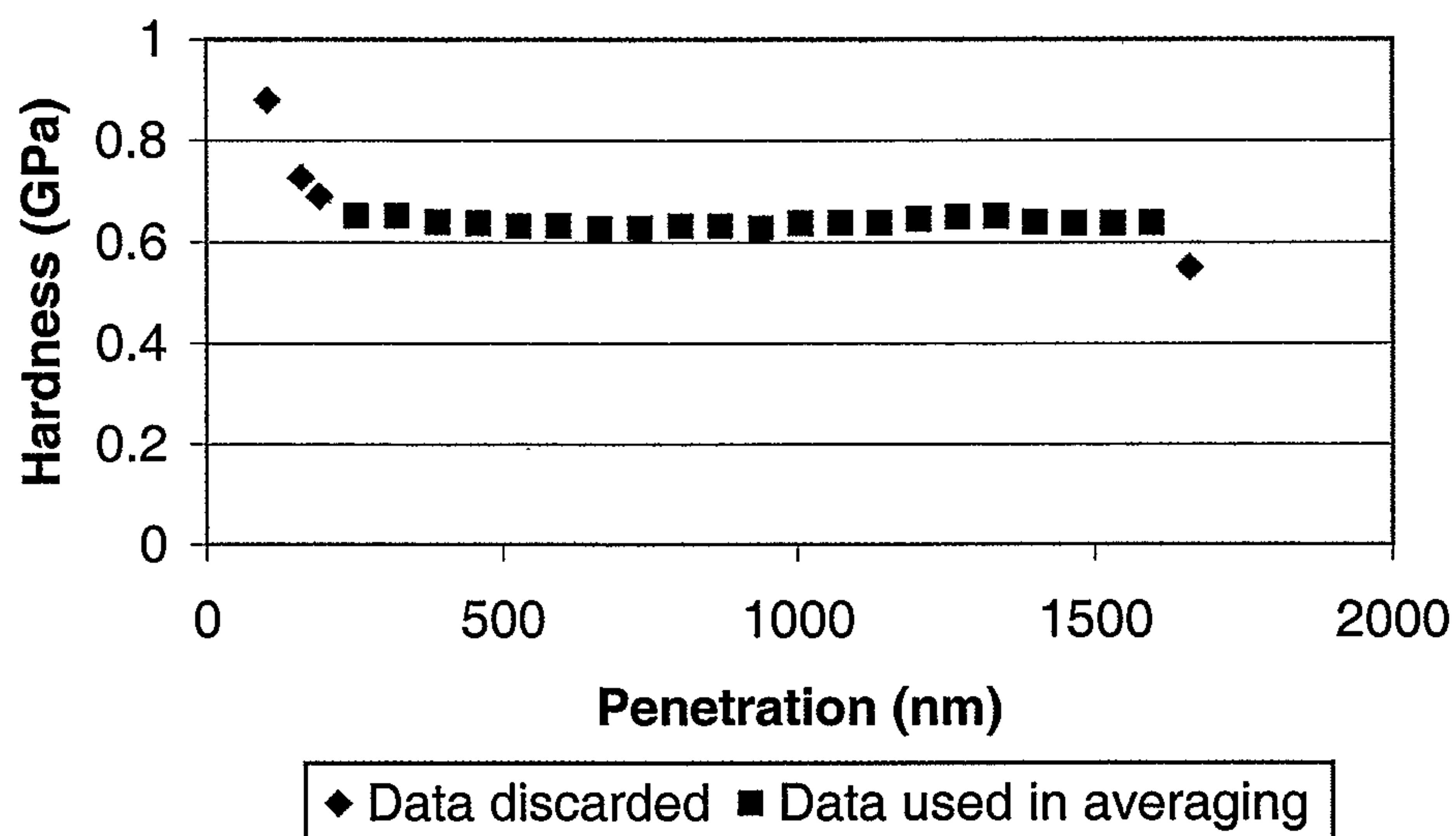


**Figure 3.10:** Data used for averaging from hardness vs penetration graphs.

## Statistical Analysis

### Experiment One

Once the hardness values as a function of penetration depth were determined for each indentation of each array, the values were analysed in a Microsoft Excell spreadsheet and statistics programme. For each tooth each indent's mean hardness values were averaged to give an overall hardness for enamel and dentine for that tooth.

A value for the elastic modulus for both enamel and dentine was determined at the maximum penetration depth for each indentation in an array. The mean value was calculated for enamel and dentine for each tooth by averaging the means of all indents (maximum 15) in an array for each tooth.

By combining all data for each indentation on all teeth mean value was calculated to give an overall hardness and modulus of elasticity for enamel and dentine.

Finally linear regression analysis was carried out to evaluate the relationship between hardness and elastic modulus of both enamel and dentine.

## Drink Analysis

For experiment two, three commonly consumed orange beverages on the Australian market were chosen and one beverage from United Kingdom which had been marked as being 'Toothfriendly' (Ribena; GlaxoSmithKline Ltd). The beverages were Fanta (Coca-Cola Company), Orange Juice (Just juice), Orange Crush Cordial (Cottees) and Ribena Toothkind (tropical flavour) (Figure 3.11).

Chemical analyses of these drinks were conducted at Westmead Hospital and the School of Chemistry at the University of Sydney.



**Figure 3.11:** Test beverages used in Experiment 2.

### pH and Titratable Acidity

The pH and titratable acidity were determined for the four test beverages as well as distilled water. All samples were tested at room temperature. The Ribena Toothfriendly beverage and Cordial were freshly prepared with a five fold dilution with tap water prior to testing as per manufacturers instructions. Fresh, undiluted samples were obtained for Fanta and Orange Juice.

Measurement of the pH was done using a PHM 83 Autocal pH meter (Radiometer, Copenhagen). The mean pH of two samples was taken to give the final pH reading for each beverage.

Aliquots of 100 mL of each drink were titrated to neutrality (pH 7.0) against 0.1 M of sodium hydroxide (NaOH) solution and the volume of NaOH required was recorded in mEq/L.

### **Wet Ashing**

Wet ashing is an important preliminary step in metal determination. It is used to decompose organic matter to bring the metals into solution in a form which is suitable for analysis in a matrix comparable to the standards used in the determination of Ca.

Three 20 mL samples each of Orange Juice (OJ) and Fanta (FA) were pipetted into 100 mL conical flasks and a small glass funnel was placed on the top of each to prevent splashing. To compensate for the four-fold dilution as per the manufacturers instructions, only 5 mL samples of Ribena Toothfriendly (RT) and Orange Cordial (OC) were used. All 12 samples were placed onto a hot plate in a fume hood (Figure 3.12). Approximately 2 mL of 18 M sulphuric acid was added to each of the samples. This acted to aid in dehydration of the excess water from the samples. To oxidise the organic matter varying amounts of 15 M nitric acid were added to each of the samples (Figure 3.13). The occasional addition of nitric acid continued until each of the samples no longer

had dark particles in solution, there was no further evolution of brown nitrogen oxide fumes from each sample and most of the water was evolved, indicated by the distillation of oily sulphuric acid up the walls of the conical flask and by white vapour evolution of  $\text{SO}_3$ . The samples were then cooled and stored covered in their flasks until needed.



**Figure 3.12:** Wet ashing of test beverages.

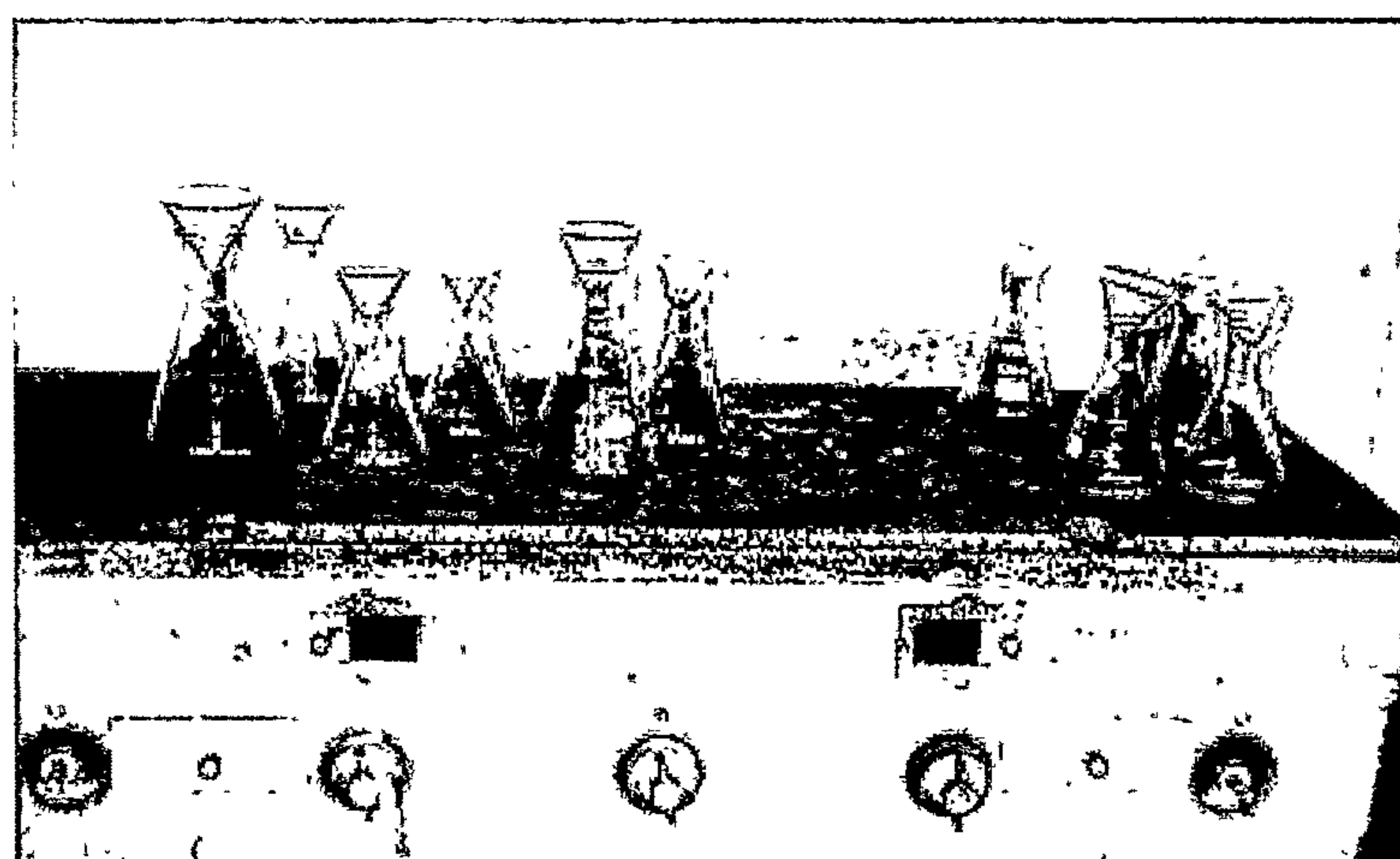


**Figure 3.13:** Wet ashing of test beverages.

## Calcium Determination by Atomic Absorption

*Appendix B for method and standards.*

Two samples of each beverage were obtained (Figure 3.14). The first sample was a pure sample of each beverage which was filtered through Grade 1 and 5 filter paper. For these samples, Ribena Toothfriendly and Orange Cordial were diluted as per to manufactures instructions. Each of these first samples were diluted for atomic absorption as shown in Table 3.6. A second sample of each of the wet ashed test beverages was diluted for atomic absorption as shown in Table 3.6. The dilution was calculated from previously published data (Larsen MJ & Nyvad B 1999, Lussi A, Jaggi T, *et al.* 1993) on the probable concentration of the Ca in each beverage. The spectrometers were calibrated to detect a maximum of 5 ppm with the use of five known standards (see Figure 3.15). The samples were diluted to ensure the final diluted concentrations were within the range of 0 to 5 ppm.

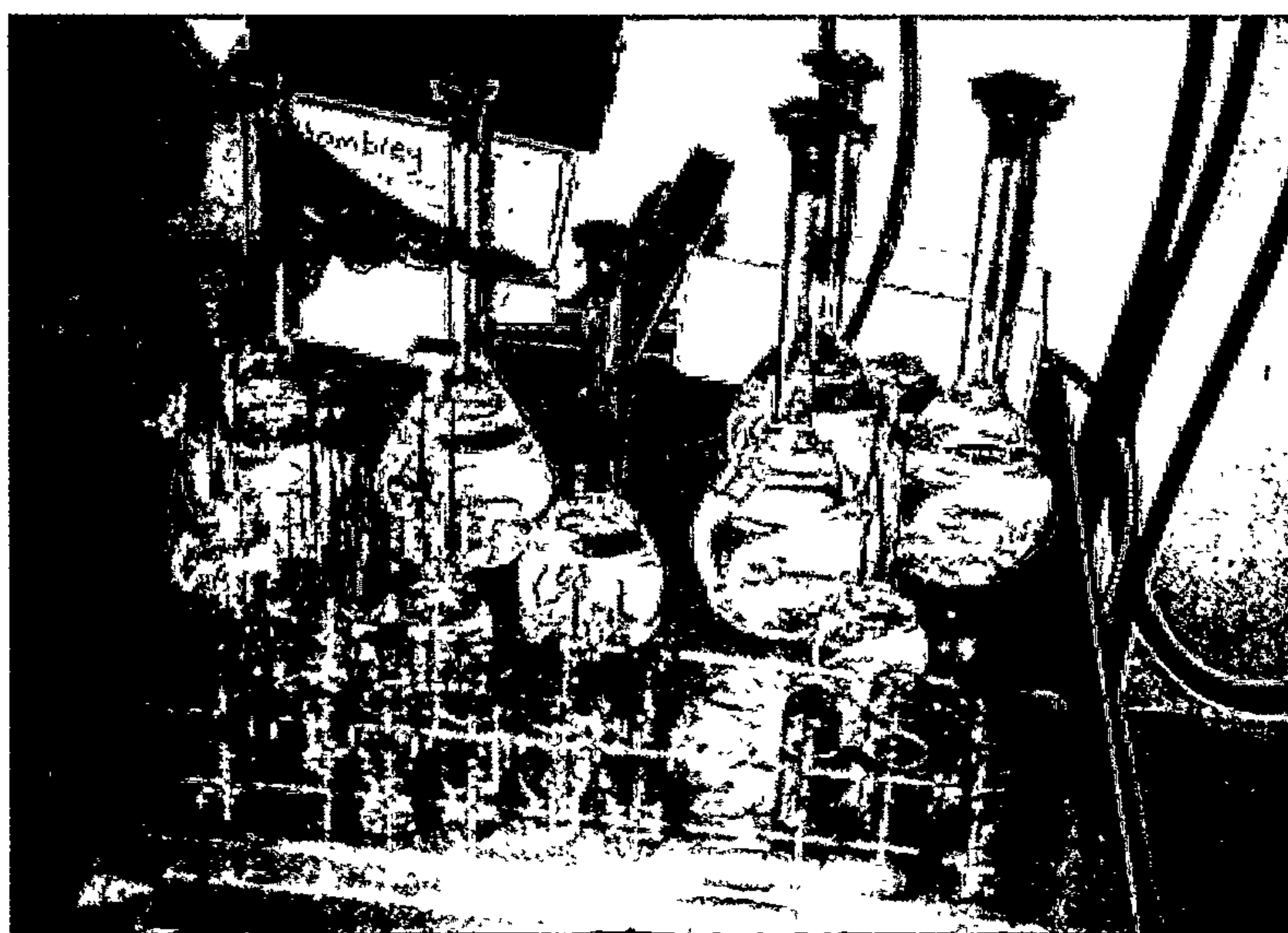


**Figure 3.14:** The samples of test beverages used in calcium determination.

Two spectrometers were used in the determination of the calcium concentration - the Varian Spectra AA220 and AA800 (Figure 3.16 and 3.17). For the older of the two machines, the Spectra AA800, EDTA was added to the samples before analysis. The standards used to determine the calibration also had the

addition of EDTA. The EDTA was used for the samples run on this apparatus to solubilise the calcium which may be bound to other ions such as phosphate. The addition of EDTA to calcium solutions before analysis may also increase the sensitivity of the subsequent flame spectrophotometric determination; this is possibly due to the formation of an EDTA complex with calcium which is readily dissociated in the flame (Vogel 2001). After addition of the EDTA, sodium hydroxide (NaOH) was added to the samples to adjust the samples to a neutral pH to avoid precipitation of EDTA.. The Spectra AA220 did not require the addition of the EDTA as it is a newer machine and has previously been tested in experimental situations without any evidence that EDTA affected the result for calcium analyses.

| Beverage     | Wet ashed volume (mL) | Dilution |
|--------------|-----------------------|----------|
| Fanta        | 20                    | 5        |
| Orange Juice | 20                    | 12.5     |
| Ribena       | 5                     | 500      |
| Cordial      | 5                     | 20       |

**Table 3.6:** Dilutions of test beverages.

A sample of approximately 10 mL of the diluted solutions was used for the determination of the calcium content for each test (Figure 3.17).

**Graph of Standard Solutions for Atomic Spectroscopy**

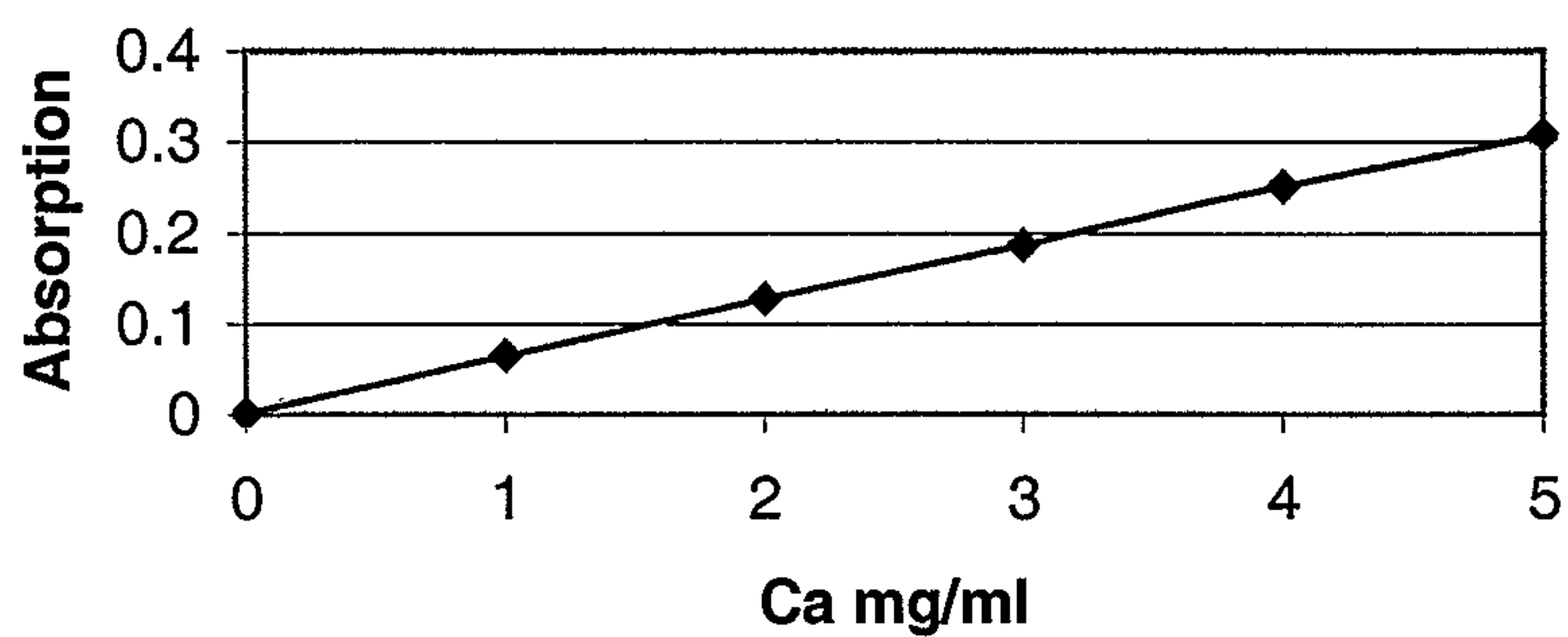


**Figure 3.15:** Calibration graph for calcium determination.

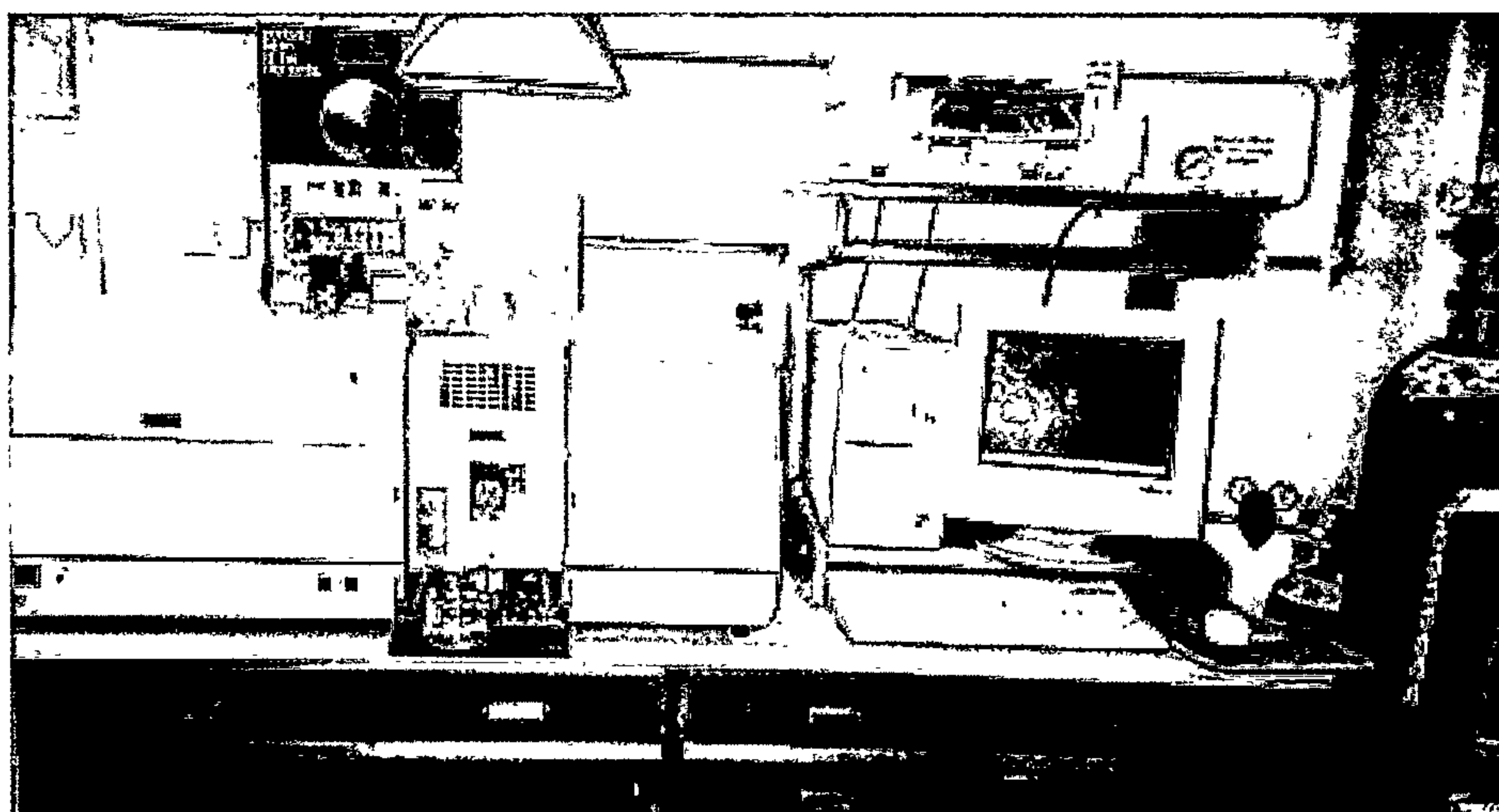


**Figure 3.16:** Atomic spectrometer AA220.

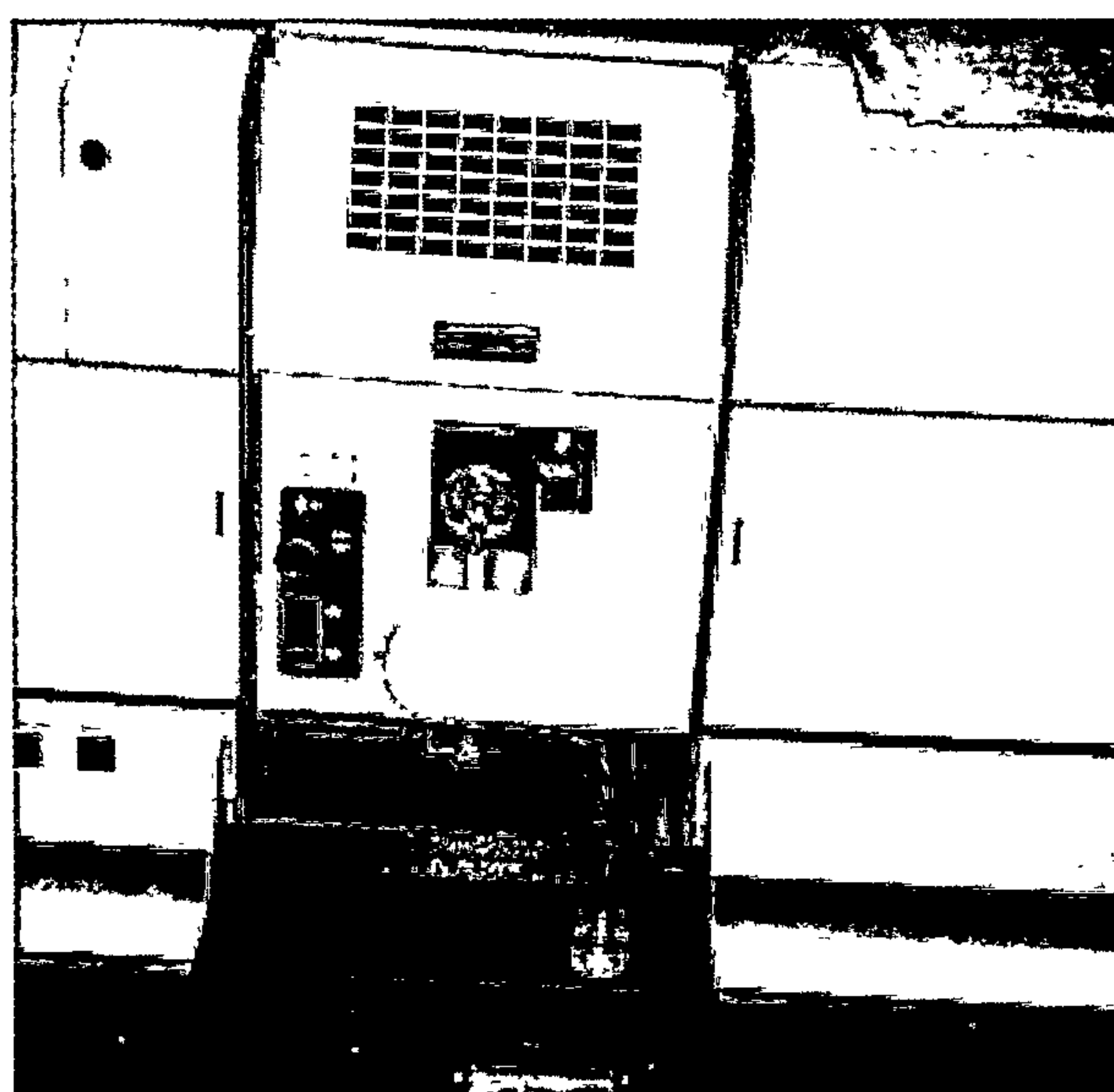


**Figure 3.17:** Test beverage sample being tested on AA220.

## Fluoride Determination using Ion Selective Electrode

### *Appendix C for method*

The fluoride ion concentration was determined by potentiometry using a fluoride ion-selective electrode (See Figure 3.18). The initial part of the experiment involved calibration of the electrode. This involved plotting  $E$  (in mV) for a series of standard fluoride solutions against  $\ln[F^-]$  and obtaining a least square estimate of the slope ( $S = -RT/F$ ). Figure 3.19 and Table 3.7 show the standard calibration and resulting fluoride concentration and the graph of results.



**Figure 3.18:** Fluoride electrode.

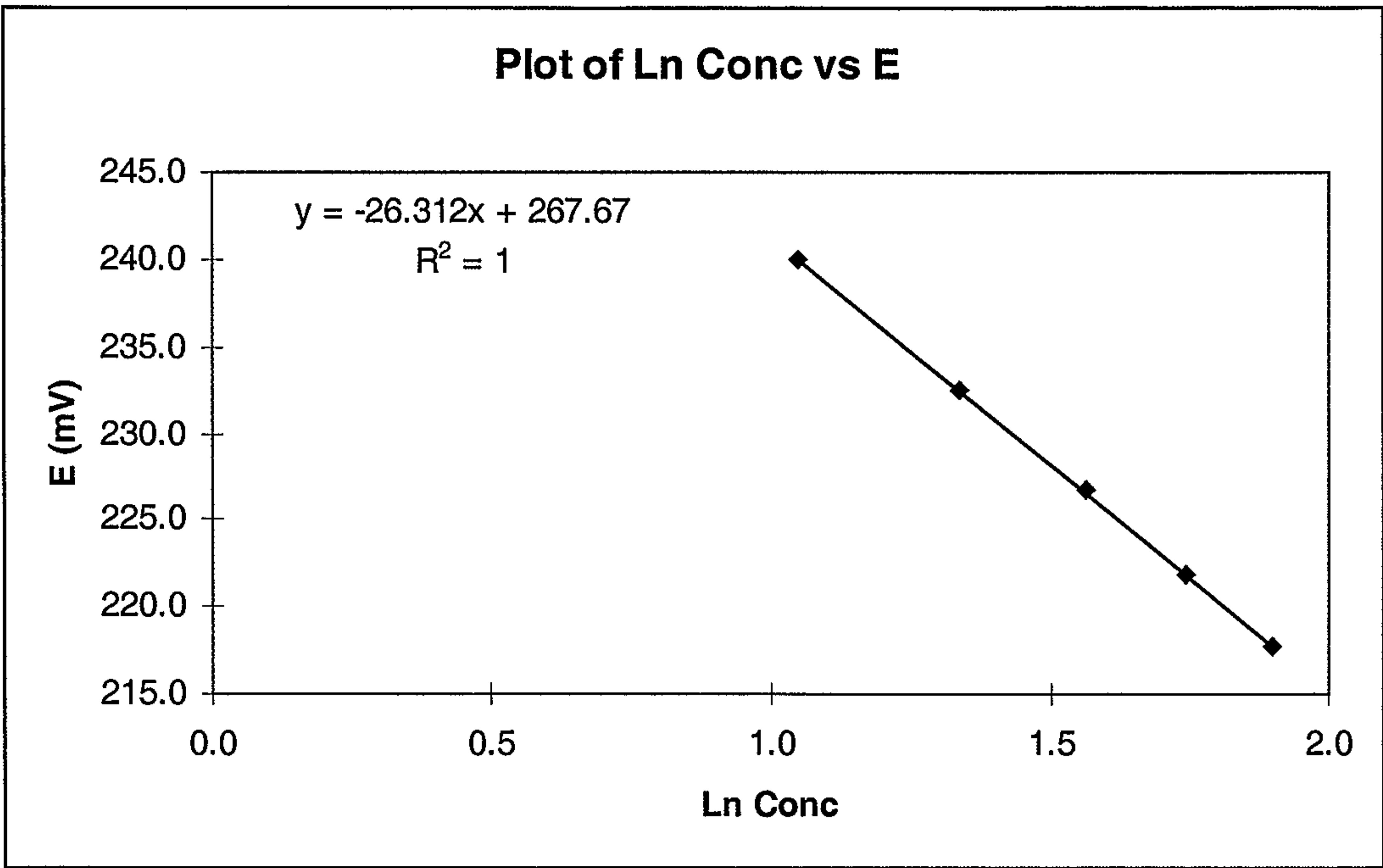


**Figure 3.19:** Calibration line of fluoride electrode.

| Solutions<br>Standard           | Calibration |        |
|---------------------------------|-------------|--------|
|                                 | ln Conc     | E(mV)  |
| 4. Solution (3) + 0.1 mL std F- | 1.05        | 240.0  |
| 5. Solution (3) + 0.1 mL std F- | 1.34        | 232.5  |
| 6. Solution (3) + 0.1 mL std F- | 1.56        | 226.7  |
| 7. Solution (3) + 0.1 mL std F- | 1.74        | 221.8  |
| 8. Solution (3) + 0.1 mL std F- | 1.9         | 2217.7 |
| Slope                           | -26.31168   |        |

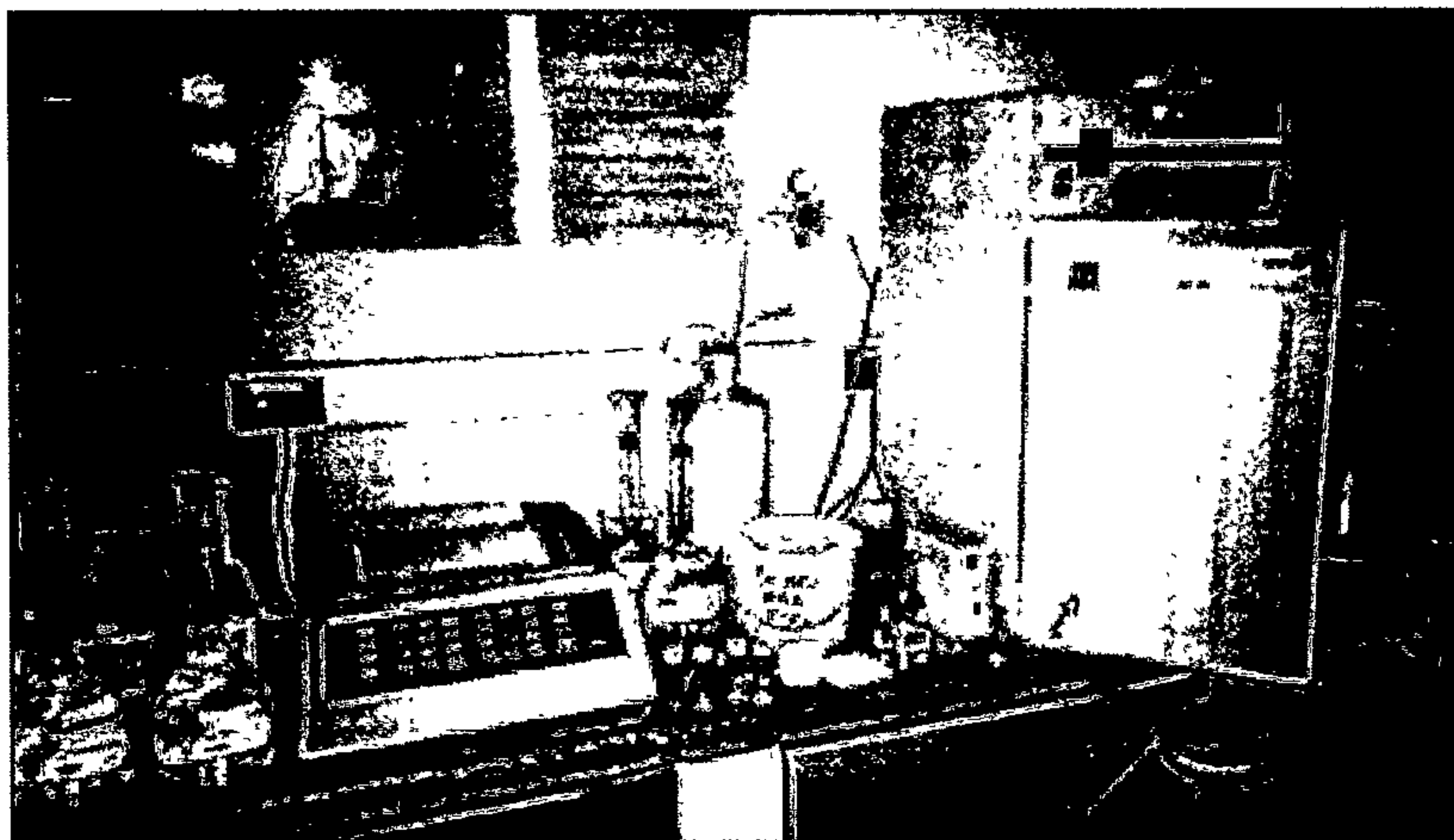
**Table 3.7:** Standard calibration and resulting fluoride concentration and the graph of results.

After calibration each of the four test drinks was measured for fluoride concentration using the method of standard additions of fluoride. Each of the test beverages was made to manufactures instructions or poured directly out of the manufacturer’s container. The concentration of added fluoride is plotted against  $e^{E/S}$ . This allows the least square estimate of the ppm of unknown fluoride in the sample. A value of  $R^2$  was required to be as close to 1 as possible.

# Phosphate Determination using DIONEX Ion Chromatography

## *Appendix D method*

To determine the phosphate concentration of the test beverages, ion chromatography was used. The equipment used was a DIONEX chromatographic system (Dionex Corporation) consisting of a peristaltic pump, chromatography module, conductivity detector, eluent organiser, phosphate standards and deionised water (Figure 3.20).



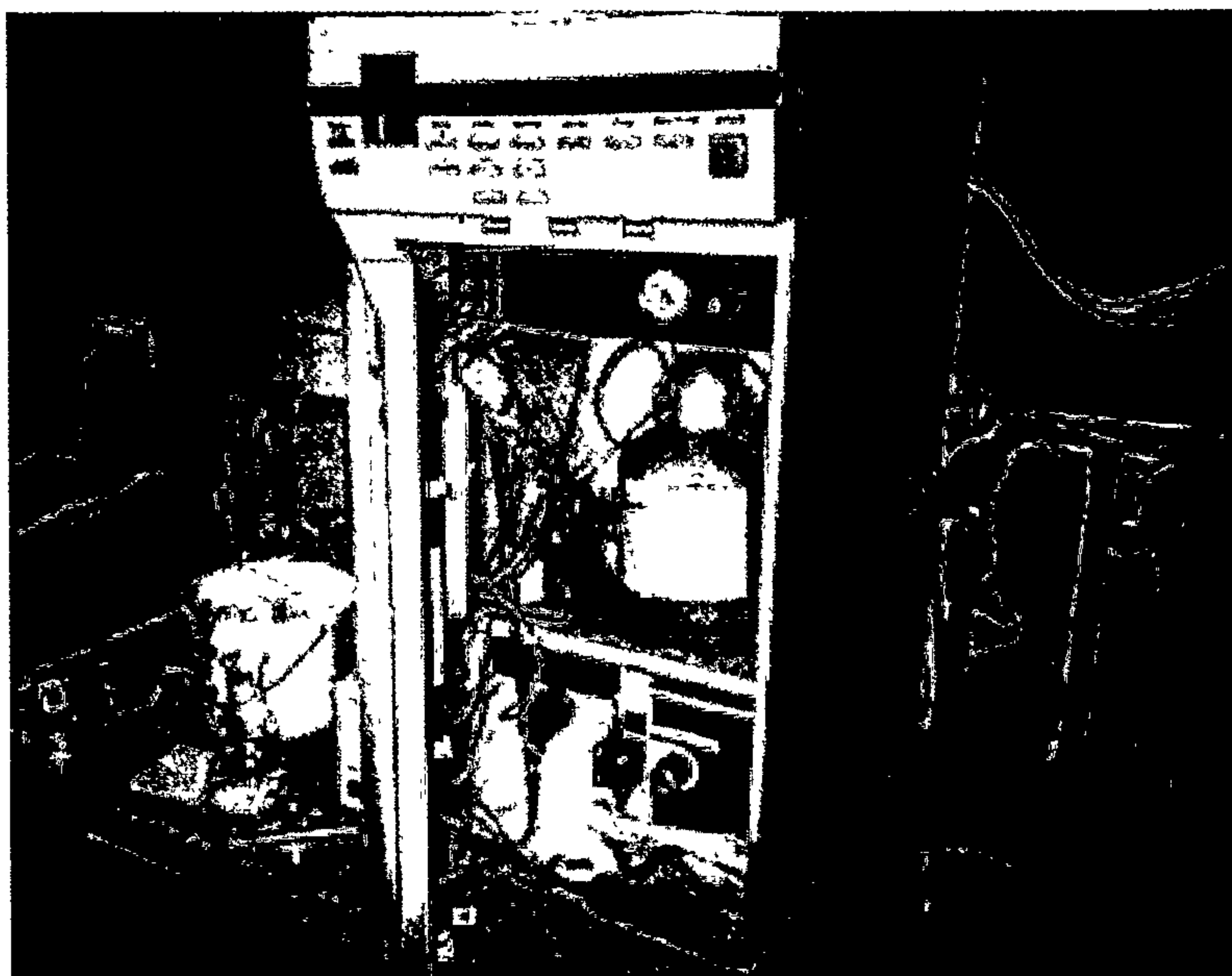


**Figure 3.20:** DIONEX chromatographic system.

Table 3.8 lists the experimental conditions.

| Column                                 | AS11 with guard column                                                          |
|----------------------------------------|---------------------------------------------------------------------------------|
| Eluent 1                               | Deionised water                                                                 |
| Eluent 2                               | 3.6 mM Na <sub>2</sub> CO <sub>3</sub> /3.4 mM Na <sub>2</sub> HCO <sub>3</sub> |
| Flow rate                              | 2 mL/minute.                                                                    |
| Injected volume                        | 1 mL                                                                            |
| Standard concentration                 | 5 mg/L<br>10 mg/L<br>15 mg/L<br>20 mg/L                                         |
| RT (reading time) of peak of phosphate |                                                                                 |
| • Standards                            | 6.94-7.03 minutes                                                               |
| • Test beverages                       | 6.36-6.94 minutes                                                               |

**Table 3.8:** List of experimental conditions of the DIONEX machine.

Initially the standards were made to the concentrations shown in Table 2.8, in 2 litre flasks. Then 100 mL of the test samples were each filtered using a vacuum filter. Approximately 10 mL the filtered test beverage was then re-filtered through a 0.2 µm filter to ensure that each of solution was clear. Any sediment would contaminate or block the ion chromatography column. Each test sample was then used without dilution except for the Orange Juice which required a 1 in 20 dilution to be able to be measured within the required concentration range chosen.

The standards were then tested by the DIONEX machine and the area produced for each known concentration was obtained for each standard (Figure 3.21 shows a typical area example for a phosphate standard). The area was determined by the DIONEX machine and a numerical value for each area was

produced by the computer associated with the DIONEX system. This graph was then plotted as shown in Figure 3.22. From the standards, the reading for phosphate was known to occur 7 minutes into the experiment. One millilitre of each test sample was tested in the DIONEX and the numerical area data produced from the DIONEX computer for each beverage were plotted and the unknown concentration of phosphate for each beverage was obtained from this graph. Figure 3.23 shows a typical example of a test beverage area print out obtained through ion chromatography. Each test was run in duplicate.

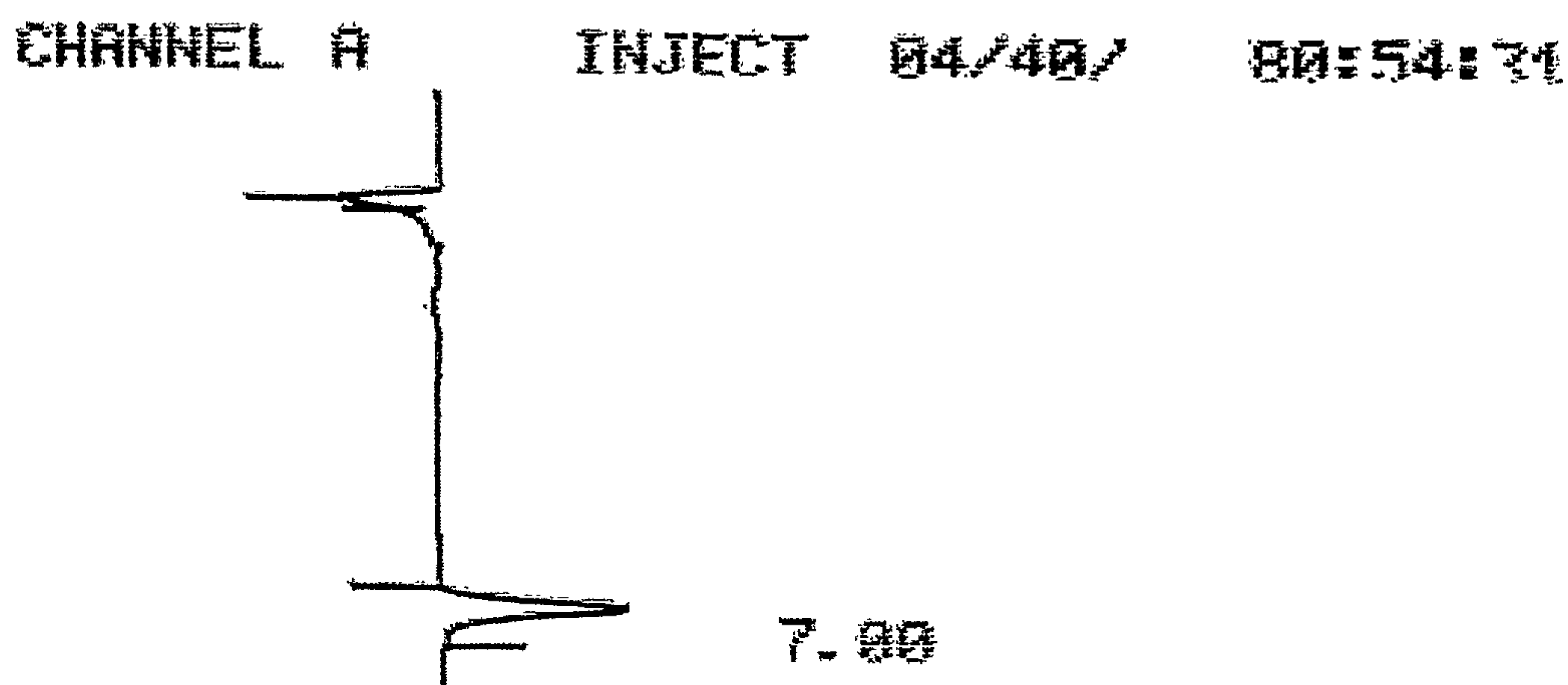


**Figure 3.21:** Peak and area generation for standard of 15mg/L.

The 7.00 on the graph corresponds to the known reading time of the DIONEX for phosphate concentration.

# Concentration of phosphate standards and resulting area using ion chromatography

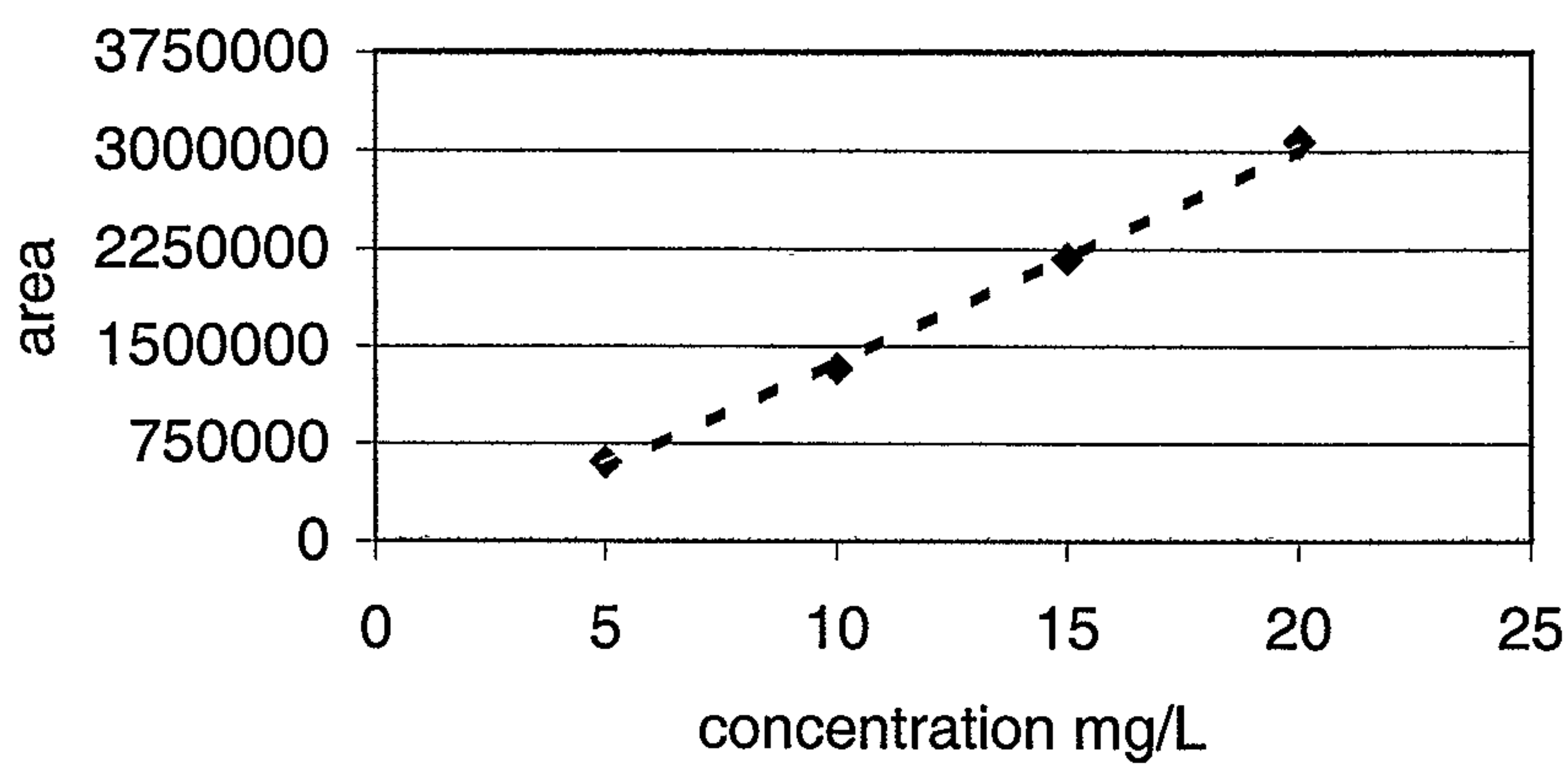


**Figure 3.22:** Phosphate standards and area produced using ion chromatography.

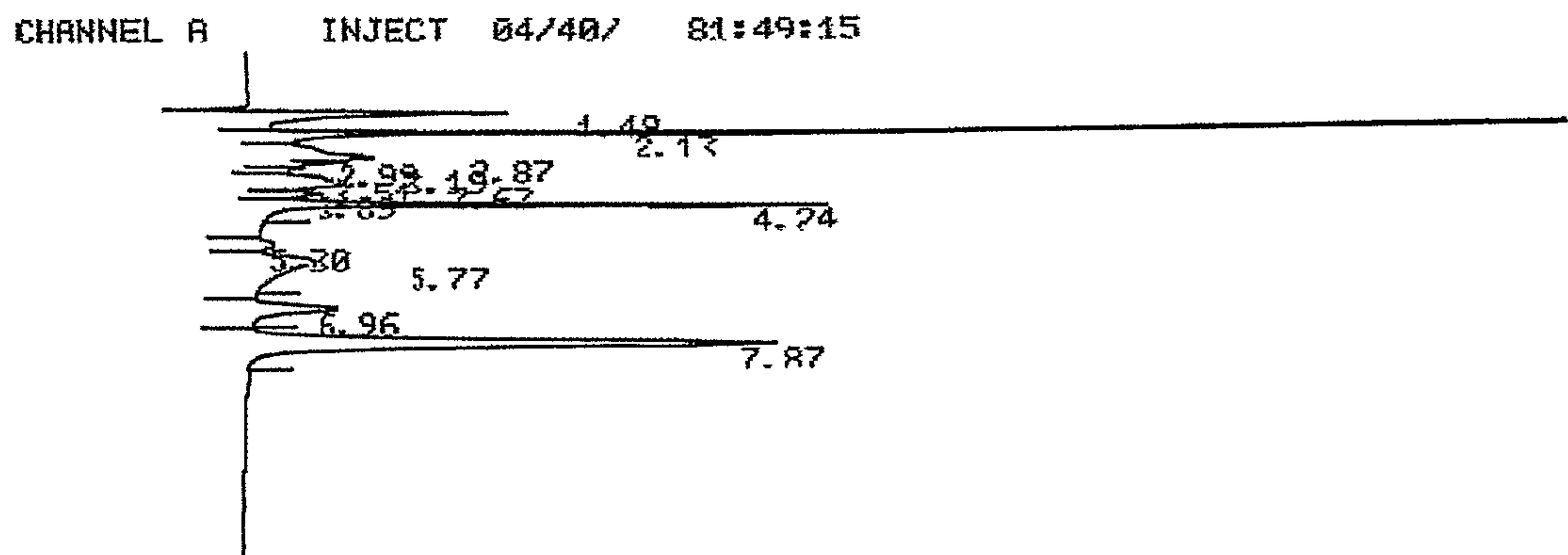


**Figure 3.23:** Typical example of test beverage (Orange Cordial).

Note that other anions such as chloride, nitrate, sulphate etc are also registering for the test beverage in comparison to the standards which have a peak for phosphate only.

## Statistical Analysis

### Experiment Two

Hardness data from the each indentation were reduced to mean hardness for each tooth. This was done by averaging the mean hardness value for each indentation (maximum 15) of each tooth before and after exposure to a test beverage or control (distilled water) (see Figure 3.10). This data were used to analyse the differences between the pre- and post-treatment control hardness values. The change in hardness was expressed as a mean percent reduction in hardness for each treatment. The same procedure was carried out for the modulus of elasticity for each tooth pre- and post-exposure values. T-tests were conducted to determine if there was a statistical significantly change in hardness and modulus of elasticity of the teeth exposed to a test beverages in comparison to the control. The P values were Bonferroni-corrected.

Degrees of significance were determined for the correlation coefficients of the beverage variables and their relationship to dentine and enamel hardness and modulus of elasticity. Stepwise multiple regression analysis and scatter plots were used to determine whether the different properties of a beverage explained the variation in enamel hardness.

Experiment one

The hardness and modulus of elasticity of enamel and dentine of each tooth were determined from 12-15 indentations carried out in the enamel and dentine of each tooth. Although a total of 15 indentations was done at 50 and 150 mN in both enamel and dentine for each tooth, between one and three indentations were discarded from each tooth. The data were discarded for three main reasons: the indentation in enamel lay on amelodentinal junction (ADJ) or outside the testing, area or when observing the individual load-unload curves or hardness graphs obscure data were deleted. Such data included those indentations in which cracking had occurred. Figure 4.1 is an example of the appearance of a hardness vs depth graph in which cracking had occurred and Figure 4.2 shows a picture of cracking in enamel.

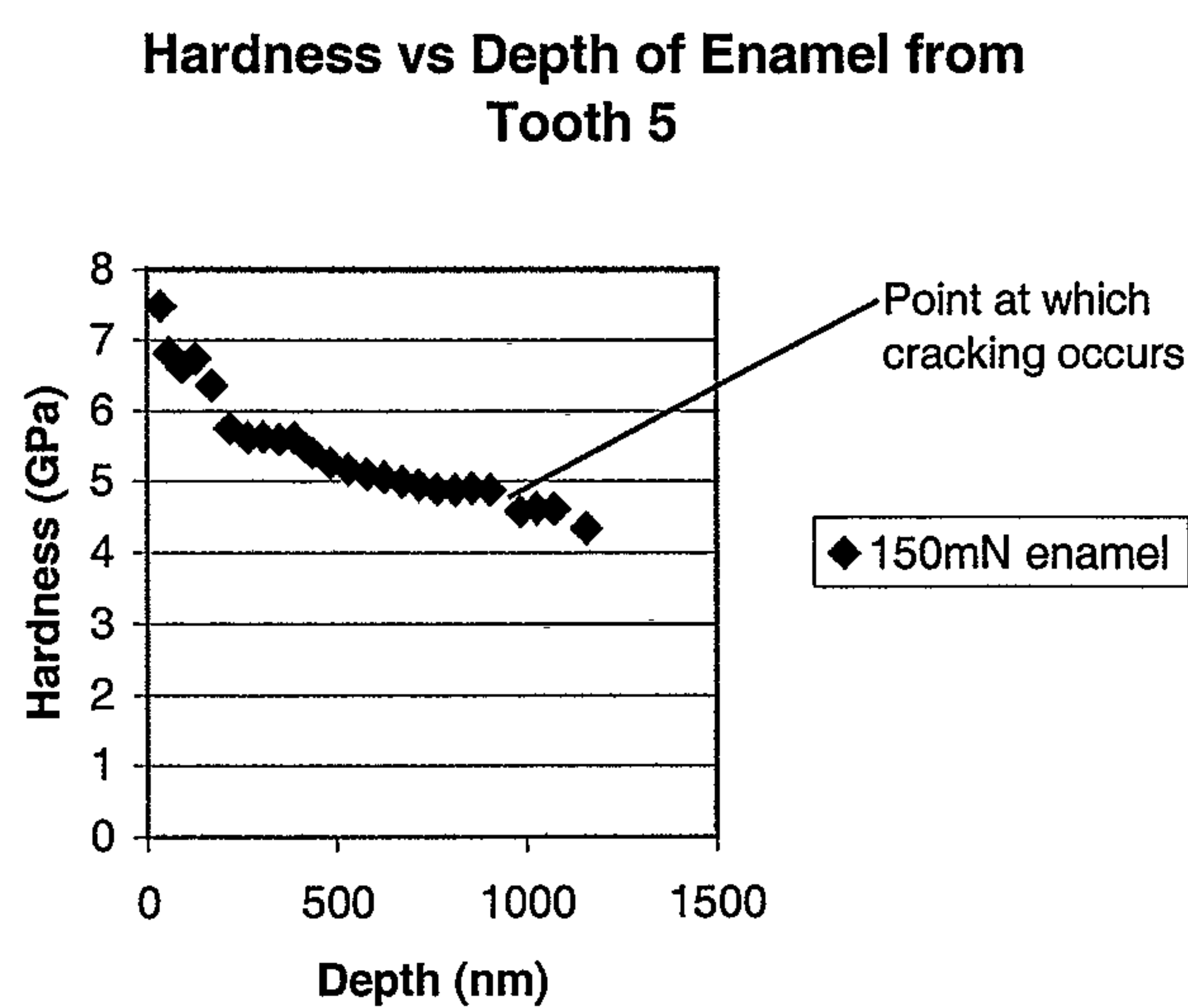


**Figure 4.1:** Hardness vs depth graph where cracking of enamel has occurred (tooth 5).

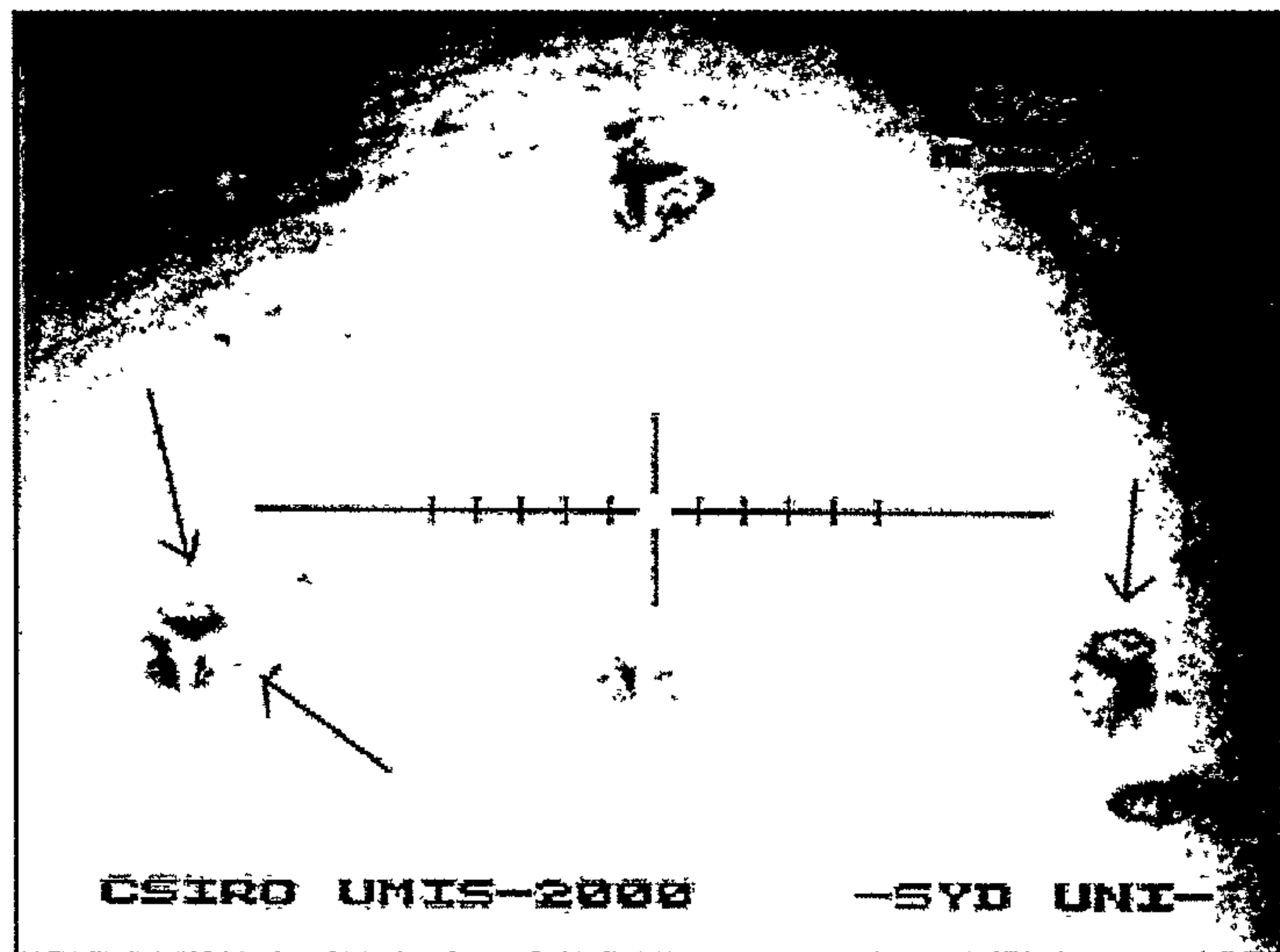


**Figure 4.2:** Cracking associated with a series of indents that were not used in data analysis. Arrows indicate areas of cracking.

As expected, tests conducted on enamel at 150 mN force had a higher frequency of cracking in comparison to tests at 50 mN. In the case of tooth 4 at an indentation force of 150 mN the number of indentations that cracking was observed was far greater than the number of indentations without cracking. No hardness or modulus of elasticity value at 150 mN for this specimen was therefore calculated.

## Hardness

The hardness of enamel and dentine for each tooth is presented in Table 4.1 and Figure 4.3. The hardness of enamel under loads of 50 mN and 150 mN ranged between teeth from  $4.66 \pm 0.23$  to  $5.15 \pm 0.53$  GPa (mean  $4.88 \pm 0.41$  GPa) and  $4.64 \pm 0.22$  to  $5.12 \pm 0.34$  GPa (mean  $4.87 \pm 0.29$  GPa) respectively. For dentine the hardness varied from  $0.78 \pm 0.07$  to  $1.07 \pm 0.07$

GPa (mean  $0.95 \pm 0.11$  GPa) under a 50 mN load and  $0.76 \pm 0.03$  to  $1.07 \pm 0.08$  GPa (mean  $0.90 \pm 0.09$  GPa) under 150 mN.

| Tooth number | 50 mN enamel (GPa) | 150 mN enamel (GPa) | 50 mN dentine (GPa) | 150 mN dentine (GPa) |
|--------------|--------------------|---------------------|---------------------|----------------------|
| 1            | $5.15 \pm 0.48$    | $5.05 \pm 0.24$     | $0.92 \pm 0.04$     | $0.91 \pm 0.05$      |
| 2            | $4.79 \pm 0.27$    | $4.70 \pm 0.19$     | $0.78 \pm 0.07$     | $0.76 \pm 0.03$      |
| 3            | $4.66 \pm 0.23$    | $4.90 \pm 0.17$     | $0.95 \pm 0.06$     | $0.92 \pm 0.06$      |
| 4            | $4.69 \pm 0.39$    |                     | $1.07 \pm 0.07$     | $0.94 \pm 0.06$      |
| 5            | $5.02 \pm 0.43$    | $4.67 \pm 0.18$     | $0.98 \pm 0.09$     | $0.94 \pm 0.04$      |
| 6            | $5.06 \pm 0.41$    | $5.00 \pm 0.22$     | $0.85 \pm 0.10$     | $0.81 \pm 0.05$      |
| 7            | $4.81 \pm 0.45$    | $4.64 \pm 0.22$     | $1.01 \pm 0.08$     | $1.03 \pm 0.08$      |
| 8            | $5.15 \pm 0.53$    | $5.12 \pm 0.34$     | $1.01 \pm 0.10$     | $0.93 \pm 0.04$      |

**Table 4.1:** The hardness for enamel and dentine averages for each tooth.

### Hardness of enamel and dentine of primary mandibular molars

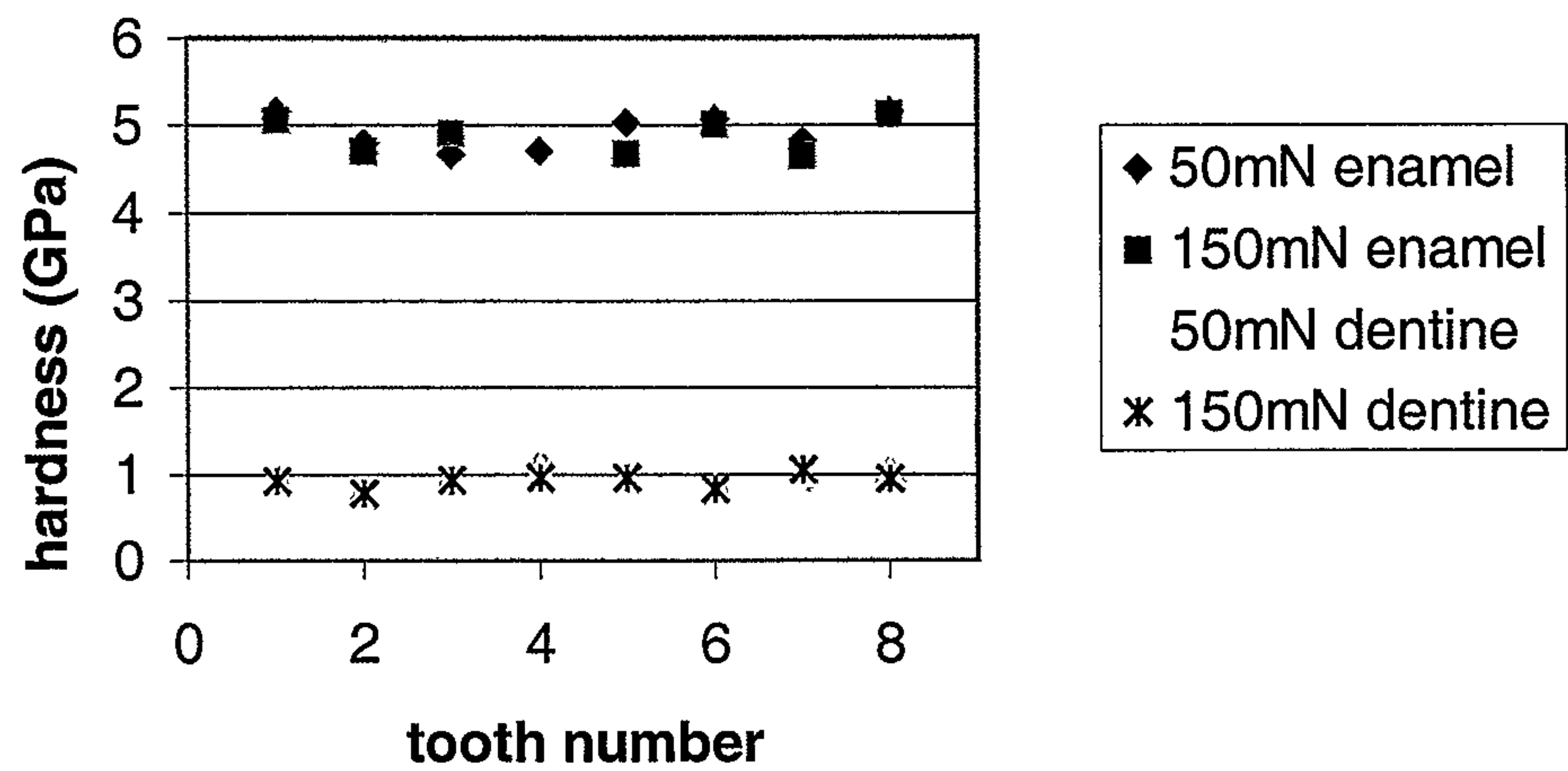


**Figure 4.3:** Hardness of enamel and dentine of the 8 test teeth.

# Modulus of Elasticity

The results for the modulus of elasticity for each tooth are presented in Table 4.2 and Figure 4.4. The modulus of elasticity for enamel ranged from  $74.47 \pm 2.85$  to  $99.94 \pm 3.95$  GPa (mean  $80.94 \pm 6.65$  GPa) at 50 mN and  $73.31 \pm 3.53$  to  $95.15 \pm 8.36$  GPa (mean  $79.77 \pm 8,86$  GPa) under the 150 mN load. For dentine under 50 mN elastic modulus ranged from  $18.23 \pm 0.81$  to  $23.97 \pm 0.72$  GPa (mean  $20.55 \pm 2.00$  GPa) whilst at 150 mN tests the elasticity was  $16.39 \pm 0.31$  to  $22.53 \pm 1.03$  GPa (mean  $19.22 \pm 1.84$  GPa).

| Tooth number | 50 mN enamel (GPa) | 150 mN enamel (GPa) | 50 mN dentine (GPa) | 150 mN dentine (GPa) |
|--------------|--------------------|---------------------|---------------------|----------------------|
| 1            | $87.62 \pm 5.00$   | $85.35 \pm 4.07$    | $20.24 \pm 1.40$    | $20.53 \pm 0.64$     |
| 2            | $82.33 \pm 5.06$   | $76.45 \pm 3.67$    | $18.28 \pm 1.15$    | $16.39 \pm 0.31$     |
| 3            | $79.78 \pm 5.97$   | $77.77 \pm 3.03$    | $21.12 \pm 0.64$    | $19.88 \pm 0.34$     |
| 4            | $74.47 \pm 2.85$   |                     | $22.70 \pm 0.80$    | $19.89 \pm 0.42$     |
| 5            | $81.90 \pm 5.81$   | $78.04 \pm 2.30$    | $20.52 \pm 0.56$    | $19.37 \pm 0.29$     |
| 6            | $80.77 \pm 2.80$   | $73.31 \pm 3.53$    | $18.23 \pm 0.81$    | $17.60 \pm 0.54$     |
| 7            | $99.94 \pm 3.95$   | $95.15 \pm 8.36$    | $23.97 \pm 0.72$    | $22.53 \pm 1.03$     |
| 8            | $79.55 \pm 4.99$   | $72.78 \pm 2.68$    | $19.84 \pm 0.82$    | $18.03 \pm 0.31$     |

**Table 4.2:** Modulus of elasticity of the primary teeth in enamel and dentine.

## Modulus of elasticity of enamel and dentine in primary first molars

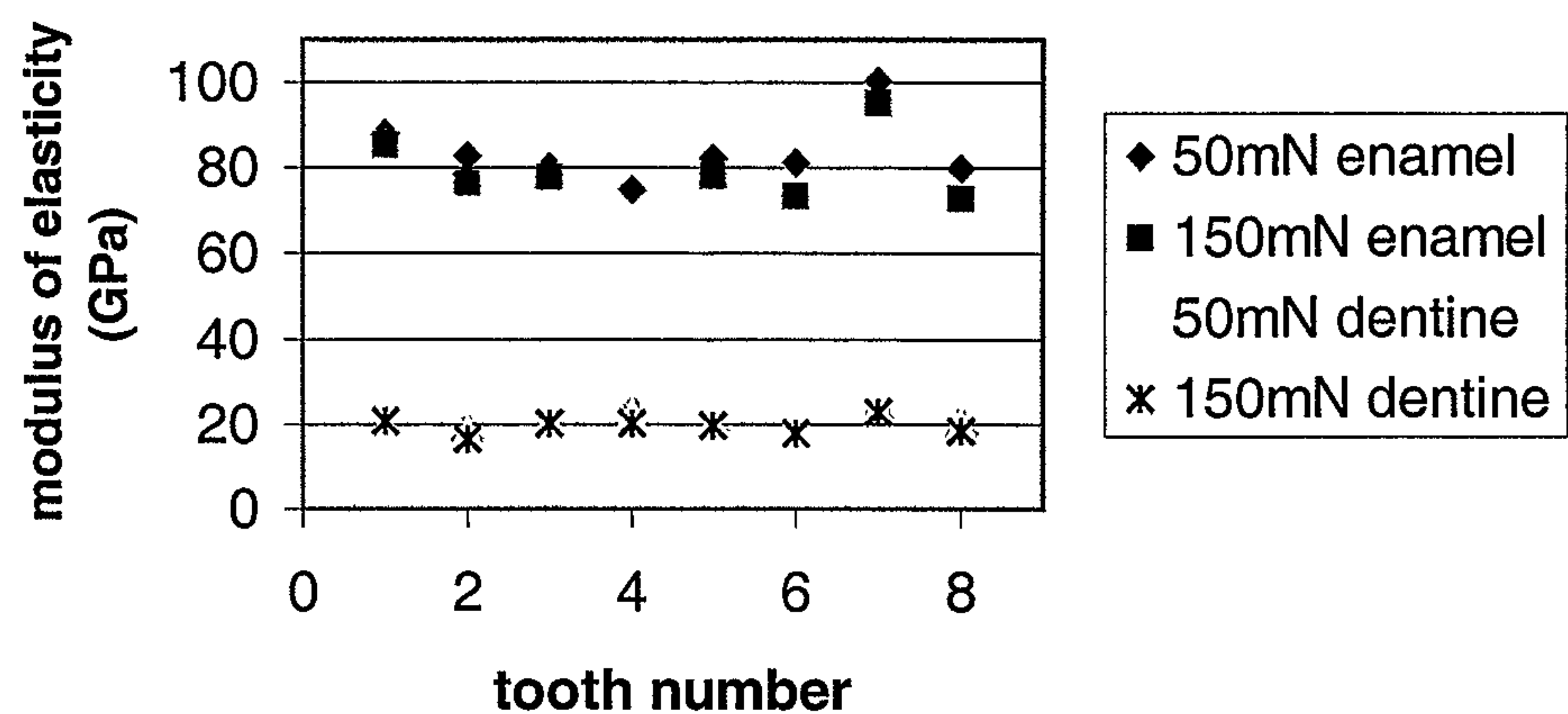


**Figure 4.4:** Modulus of elasticity for the eight test teeth.

### Overall Hardness and Modulus of Elasticity for Enamel and Dentine

When each indentations results for hardness and modulus of elasticity were combined an overall hardness and modulus of elasticity for enamel and dentine were calculated. An average of 220 indentations was used to give these results. This is shown in Table 4.3.

| Dental Tissue   | Overall         |                                 |
|-----------------|-----------------|---------------------------------|
|                 | Hardness<br>GPa | Modulus of<br>elasticity<br>GPa |
| Enamel (n~220)  | 4.88 ± 0.35     | 80.35 ± 7.71                    |
| Dentine (n~220) | 0.92 ± 0.11     | 19.89 ± 1.92                    |

**Table 4.3:** Overall hardness and modulus of elasticity of primary enamel and dentine.

## Relationship between Hardness and Modulus of Elasticity

Figures 4.5 to 4.8 demonstrate the relationship between hardness and elastic modulus for enamel and dentine under a load of 50 mN and 150 mN. There was a statistically significant relationship ( $p < 0.001$ ) between the hardness and the elastic modulus for enamel ( $R^2 = 0.117$ ) and for dentine ( $R^2 = 0.477$ ) at a load of 50 mN. However under the load of 150 mN there was a statistically significant relationship ( $p < 0.05$ ) for dentine only ( $R^2 = 0.604$ ).

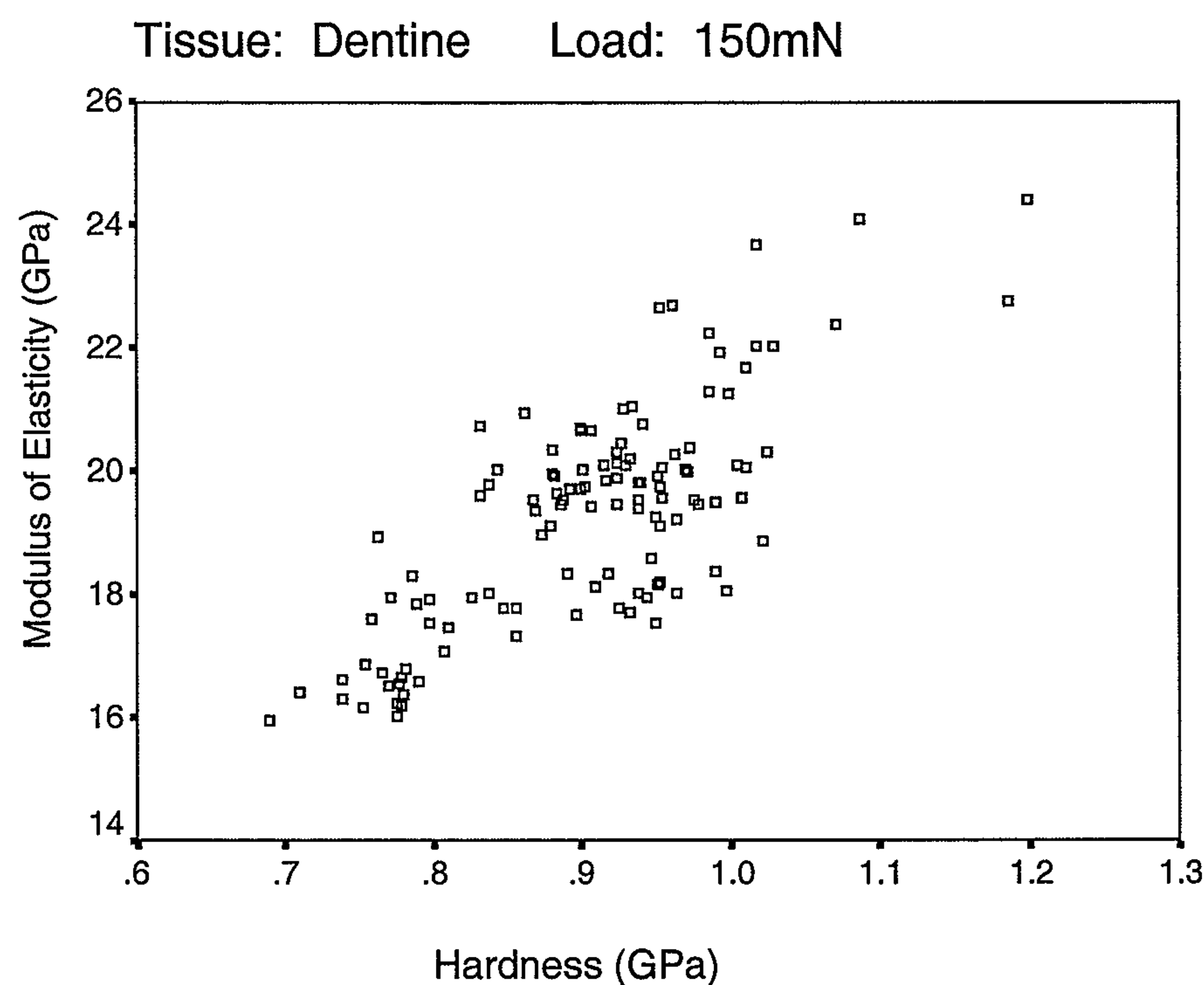


**Figure 4.5:** Relationship between dentine modulus of elasticity and hardness at 150 mN.

Modulus of Elasticity =  $5.533 + 15.186 \times \text{Hardness}$  ( $p < 0.001$ ). ( $R^2 = 0.604$  ie 60% of the variation in Modulus of Elasticity is explained by the above equation).

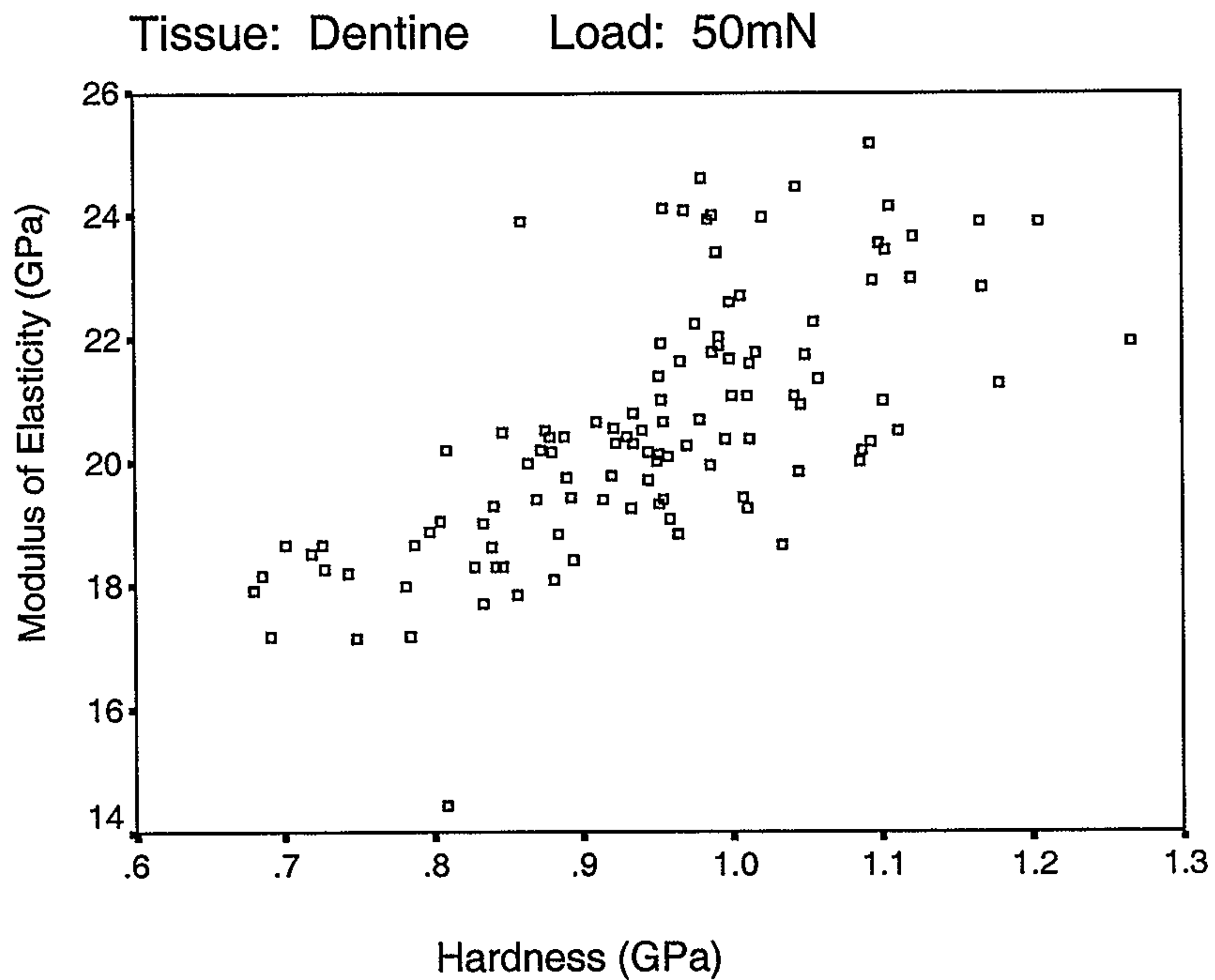


**Figure 4.6:** Relationship between dentine modulus of elasticity and hardness at 50 mN.

Modulus of Elasticity =  $9.555 + 11.630 \times \text{Hardness}$  ( $p < 0.001$ ). ( $R^2 = 0.477$  ie 48% of the variation in Modulus of Elasticity is explained by the above equation).

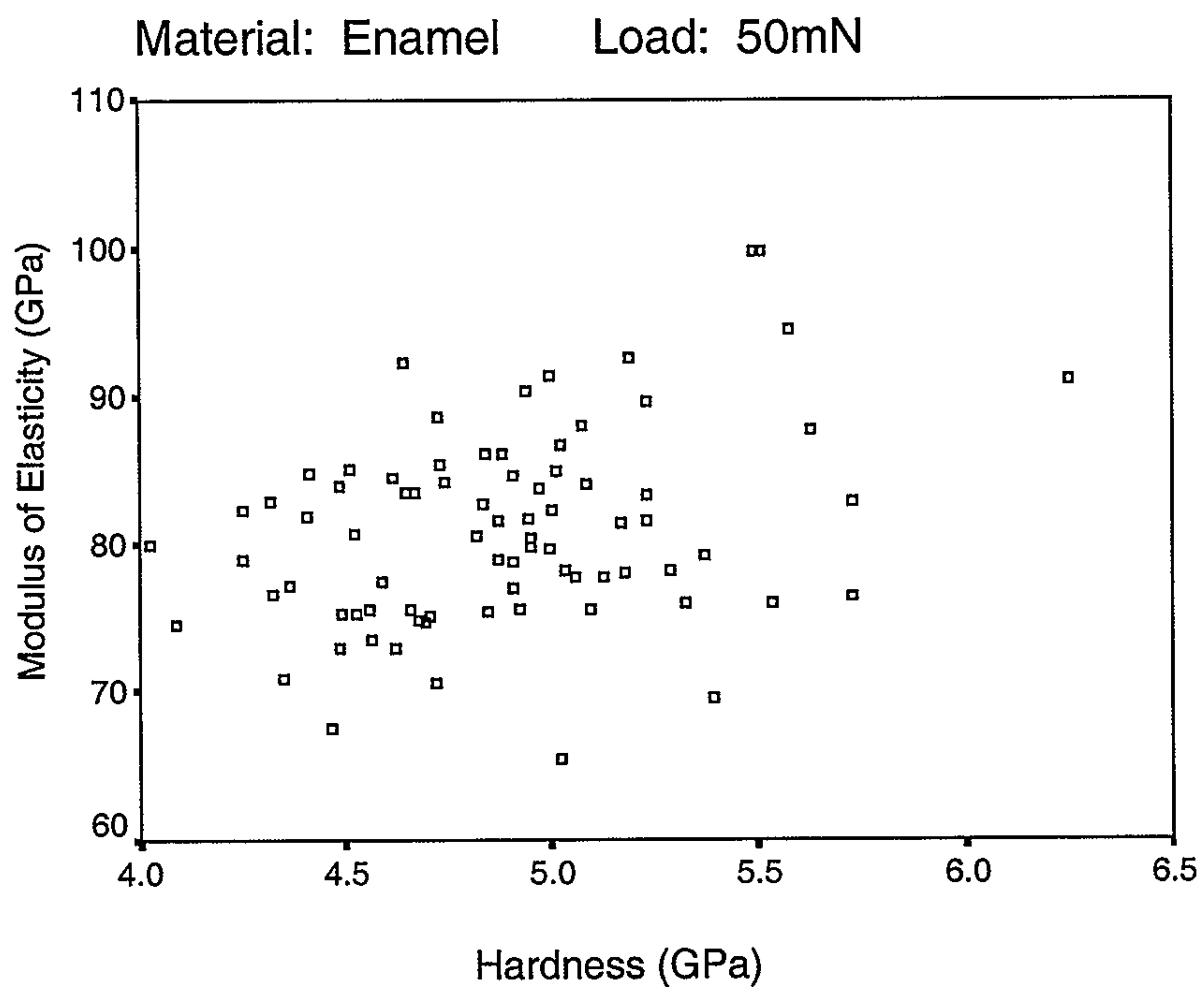


**Figure 4.7:** Relationship between enamel modulus of elasticity and hardness at 50 mN.

Modulus of Elasticity =  $53.77 + 5.563 \times \text{Hardness}$  ( $p = 0.002$ ). ( $R^2 = 0.117$  ie 12% of the variation in Modulus of Elasticity is explained by the above equation).

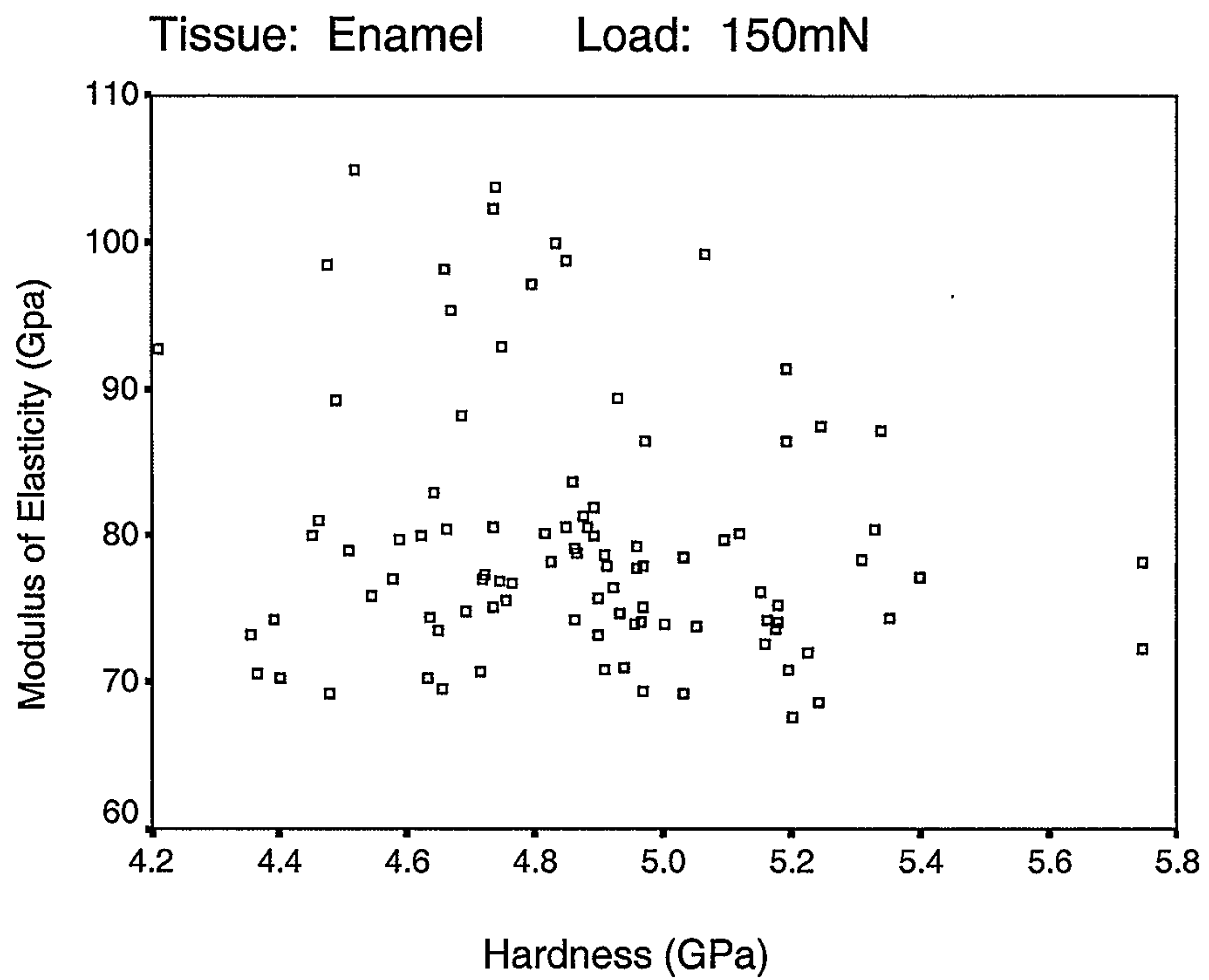


**Figure 4.8:** Relationship between enamel modulus of elasticity and hardness at 150 mN.

Relationship not significant ( $p = 0.87$ ).

# Experiment Two

## Beverage Analysis

### pH

Table 4.4 and Figure 4.9 show the mean pH determined for each of the beverages as well as the control distilled water. From these data all beverages have a pH less than the critical pH for enamel dissolution.

| Beverage        | pH   |
|-----------------|------|
| Distilled water | 6.35 |
| Ribena          | 3.84 |
| Orange Juice    | 3.80 |
| Cordial         | 2.96 |
| Fanta           | 2.72 |

Table 4.4: Showing the pH of the test beverages.

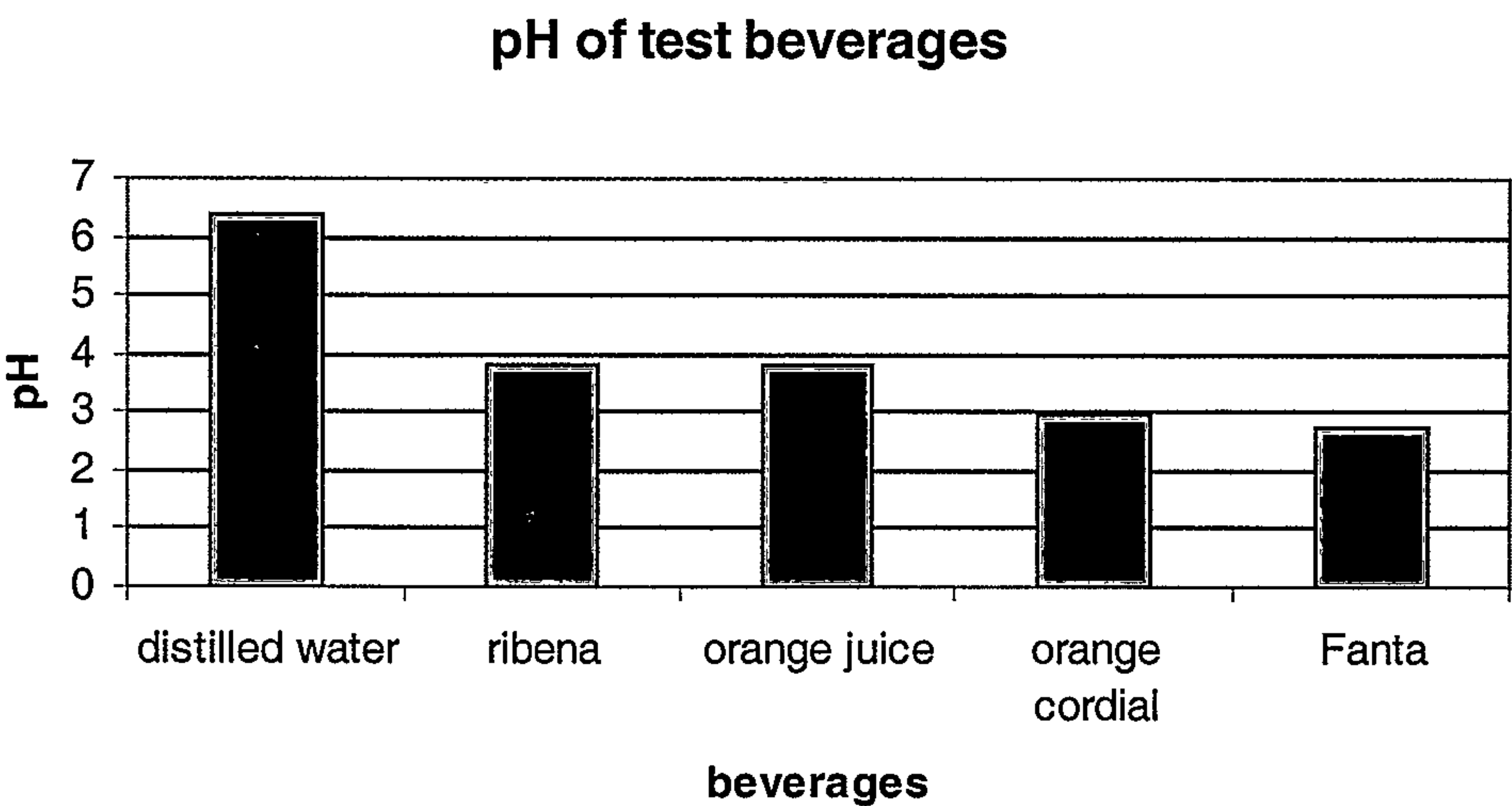


Figure 4.9: Showing the pH of the test beverages in decreasing order.

**Titrateable Acidity**

Table 4.5 and Figure 4.10 show the average titrateable acidity for the four test beverages and the distilled water control.

| Beverage        | Titrateable acidity mEq/L |
|-----------------|---------------------------|
| Distilled water | 6.85                      |
| Ribena          | 25.8                      |
| Orange Juice    | 105                       |
| Cordial         | 28.8                      |
| Fanta           | 67.0                      |

**Table 4.5:** Titrateable acidity of the test beverages.

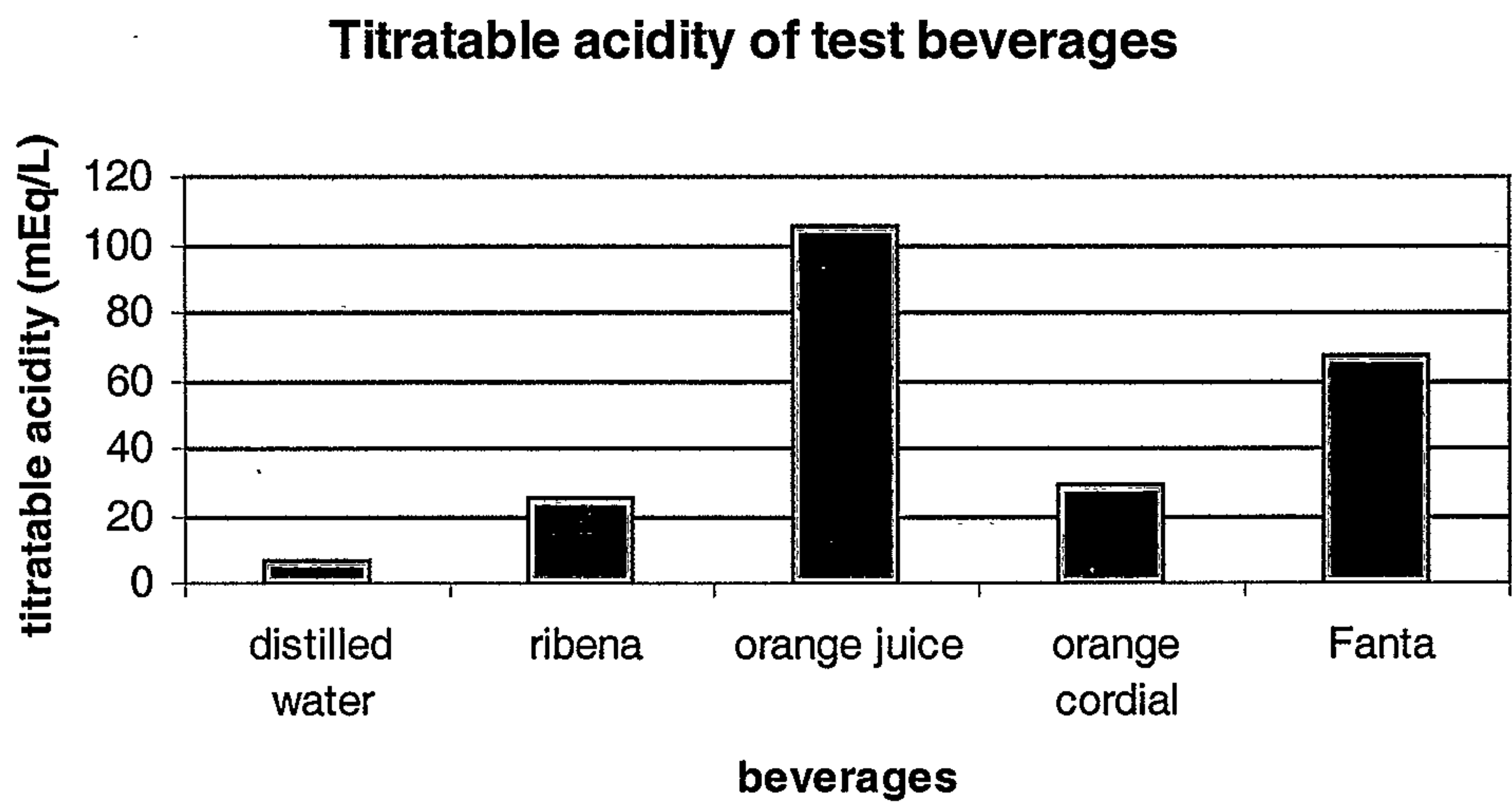


**Figure 4.10:** Showing titrateable acidity of test beverages.

**Calcium Determination**

Table 4.6a and 4.6b summarise the concentration of the diluted specimens of each test beverage, the mean absorption and the final calcium concentration calculated after dilution from each of the two machines used.

| Beverage              | Mean Absorption | %RSD | Concentration mg/L | Final calcium concentration after factoring in dilution |        |
|-----------------------|-----------------|------|--------------------|---------------------------------------------------------|--------|
|                       |                 |      |                    | mg/L                                                    | mmol/L |
| <u>Ribena</u>         |                 |      |                    |                                                         |        |
| Wet ashed             | 0.0324          | 1.3  | 2.55               | 1277.5                                                  | 31.94  |
| Pure filtered         | 0.0053          | 3.2  | 0.467              | 233.5                                                   |        |
| <u>Orange Juice</u>   |                 |      |                    |                                                         |        |
| Wet ashed             | 0.0557          | 0.9  | 4.311              | 53.89                                                   | 1.35   |
| Pure filtered         | 0.0604          | 0.5  | 4.699              | 58.74                                                   |        |
| <u>Orange Cordial</u> |                 |      |                    |                                                         |        |
| Wet ashed             | 0.0228          | 1.2  | 1.849              | 36.98                                                   | 0.93   |
| Pure filtered         | 0.0154          | 1.6  | 1.289              | 25.78                                                   |        |
| <u>Fanta</u>          |                 |      |                    |                                                         |        |
| Wet ashed             | 0.0452          | 0.4  | 3.517              | 17.59                                                   | 0.44   |
| Pure filtered         | 0.0442          | 1.4  | 3.444              | 17.22                                                   |        |

**Table 4.6a:** Showing the calcium concentration results of the test beverages from Spectra AA220 machine.

| Beverages      | Mean absorption | %RDS | Concentration mg/L | Final calcium concentration after dilution factored in |        |
|----------------|-----------------|------|--------------------|--------------------------------------------------------|--------|
|                |                 |      |                    | mg/L                                                   | mmol/L |
| Ribena         | 0.0488          | 0.5  | 0.0564             | 902.4                                                  | 22.26  |
| Orange Juice   | 0.2196          | 0.2  | 2.775              | 34.69                                                  | 0.847  |
| Orange Cordial | 0.1165          | 0.3  | 1.393              | 22.29                                                  | 0.557  |
| Fanta          | 0.1144          | 0.3  | 1.367              | 6.84                                                   | 0.171  |

**Table 4.6b:** Showing the calcium concentration for the test beverages Spectra AA880 machine.

Figure 4.11 shows the results of the calcium concentration of the test beverages in graphic form from Spectra AA220. The results from the Spectra AA220 machine were used because of the two machines the AA220 was the most reproducible.

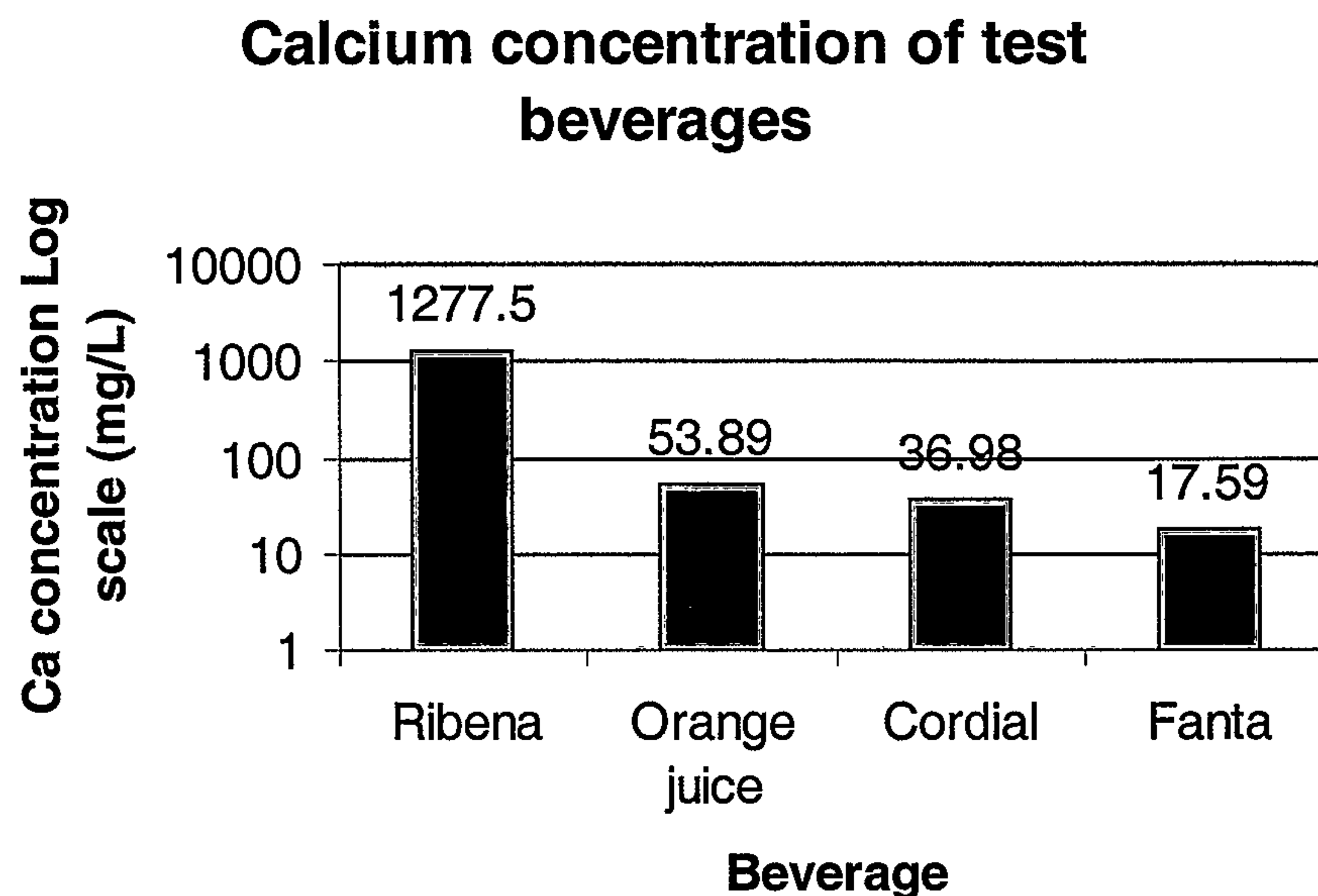


**Figure 4.11:** Calcium concentration of test beverages.

These graphs show that the test beverages on the whole have a low amount of calcium in them except for Ribena which has approximately 25 times greater calcium concentration than Orange Juice which has the next highest calcium concentration.

### **Fluoride Determination using Ion Selective Electrode**

Using the standard addition of fluoride to the unknown the concentration in parts per million were calculated and shown in Table 4.7 and Figure 4.12. These tables show that all test beverages had a low fluoride concentration of approximately 1 part per million. Fanta had the lowest fluoride concentration of 0.86 ppm.

| Solutions                                                                                                                                                                   |                           |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| 4. Solution (3) + 0.1 mL std F-<br>5. Solution (3) + 0.1 mL std F-<br>6. Solution (3) + 0.1 mL std F-<br>7. Solution (3) + 0.1 mL std F-<br>8. Solution (3) + 0.1 mL std F- | <b>Ribena E(mV)</b>       |
|                                                                                                                                                                             | <b>230.7</b>              |
|                                                                                                                                                                             | <b>224.9</b>              |
|                                                                                                                                                                             | <b>220.2</b>              |
|                                                                                                                                                                             | <b>216.2</b>              |
|                                                                                                                                                                             | <b>212.7</b>              |
| Fluoride concentration : 1.03 ppm<br>$R^2 = 1$                                                                                                                              |                           |
| 4. Solution (3) + 0.1 mL std F-<br>5. Solution (3) + 0.1 mL std F-<br>6. Solution (3) + 0.1 mL std F-<br>7. Solution (3) + 0.1 mL std F-<br>8. Solution (3) + 0.1 mL std F- | <b>Orange Juice E(mV)</b> |
|                                                                                                                                                                             | <b>227.8</b>              |
|                                                                                                                                                                             | <b>222.4</b>              |
|                                                                                                                                                                             | <b>217.8</b>              |
|                                                                                                                                                                             | <b>213.9</b>              |
|                                                                                                                                                                             | <b>210.5</b>              |
| Fluoride concentration : 1.22 ppm<br>$R^2 = 1$                                                                                                                              |                           |
| 4. Solution (3) + 0.1 mL std F-<br>5. Solution (3) + 0.1 mL std F-<br>6. Solution (3) + 0.1 mL std F-<br>7. Solution (3) + 0.1 mL std F-<br>8. Solution (3) + 0.1 mL std F- | <b>Cordial E(mV)</b>      |
|                                                                                                                                                                             | <b>229.0</b>              |
|                                                                                                                                                                             | <b>223.3</b>              |
|                                                                                                                                                                             | <b>218.7</b>              |
|                                                                                                                                                                             | <b>214.8</b>              |
|                                                                                                                                                                             | <b>211.5</b>              |
| Fluoride concentration: 1.19 ppm<br>$R^2 = 0.9999$                                                                                                                          |                           |
| 4. Solution (3) + 0.1 mL std F-<br>5. Solution (3) + 0.1 mL std F-<br>6. Solution (3) + 0.1 mL std F-<br>7. Solution (3) + 0.1 mL std F-<br>8. Solution (3) + 0.1 mL std F- | <b>Fanta E(mV)</b>        |
|                                                                                                                                                                             | <b>230.0</b>              |
|                                                                                                                                                                             | <b>223.7</b>              |
|                                                                                                                                                                             | <b>218.7</b>              |
|                                                                                                                                                                             | <b>214.8</b>              |
|                                                                                                                                                                             | <b>211.3</b>              |
| Fluoride concentration: 0.86 ppm<br>$R^2 = 0.9996$                                                                                                                          |                           |

**Table 4.7:** The voltage and resulting fluoride concentrations for each of the test beverages.

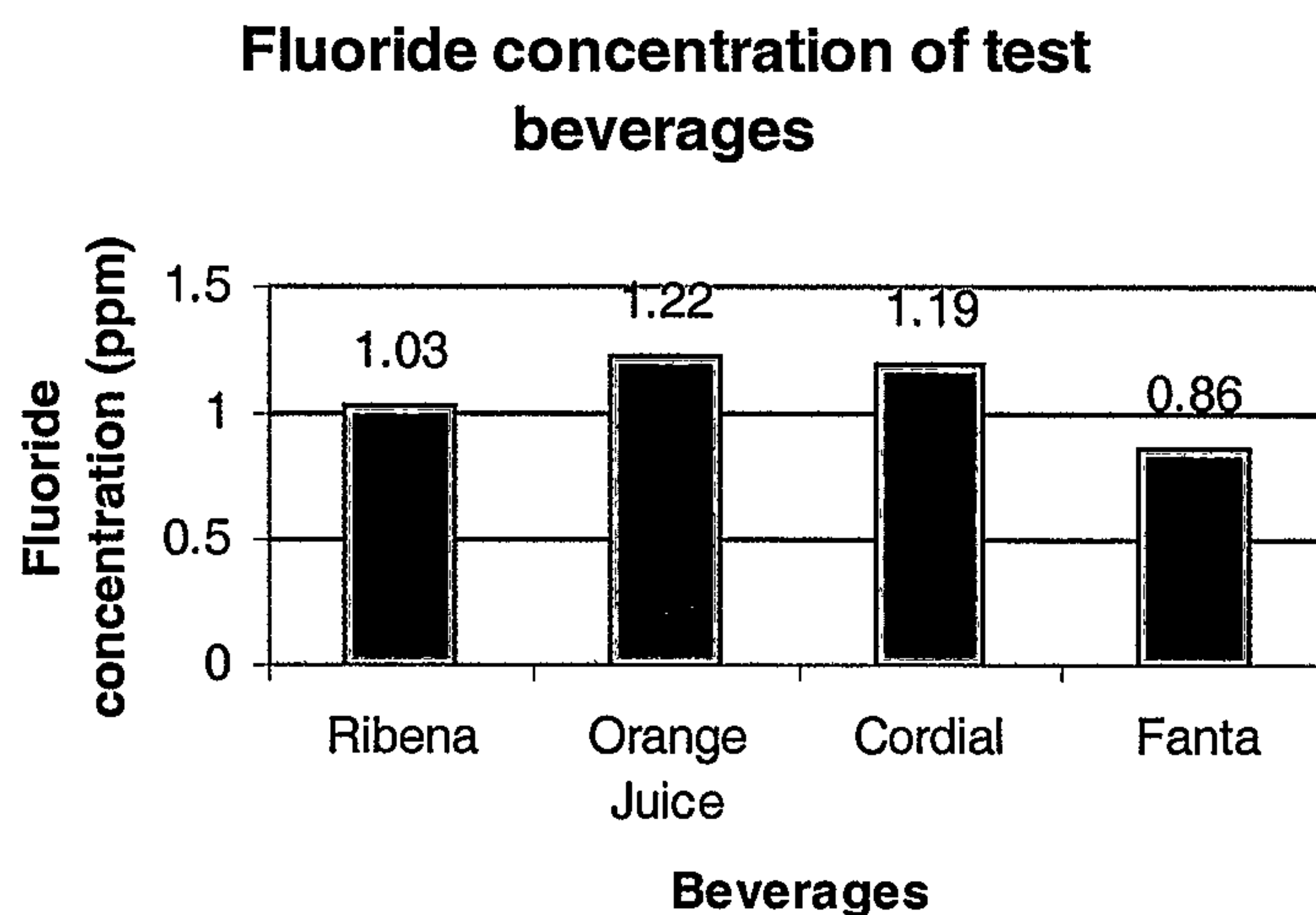


**Figure 4.12:** Fluoride concentrations of the test beverages.

### Phosphate Ion Determination Using Ion Chromatography

Two separate samples of each beverage were used for the determination of the phosphate concentration of the test beverages. For each of the test beverages an area was determined from the DIONEX ion chromatographer and the results of the average of the two tests were plotted on a graph, with the standards, to give a final concentration for each beverage. Table 4.8 shows the final area and reading time for each beverage. Figure 4.13 shows the graph of these results.

| Test substance  | Reading time | Area recorded (μS) |
|-----------------|--------------|--------------------|
| Standard 5mg/mL | 7.02         | 59.1266            |
| Standard 10mg/L | 7.03         | 1312741            |
| Standard 15mg/L | 7            | 2164912            |
| Standard 20mg/L | 6.94         | 3054705            |
| Ribena          | 6.94         | 2485781            |
| Orange Juice    | 6.96         | 1007792            |
| Orange Cordial  | 6.36         | 1692865            |
| Fanta           | -            | ND                 |

**Table 4.8:** Showing the reading times of the ion chromatography DIONEX unit and the resulting areas recorded for each of the standards and test beverages. ND= non detected.

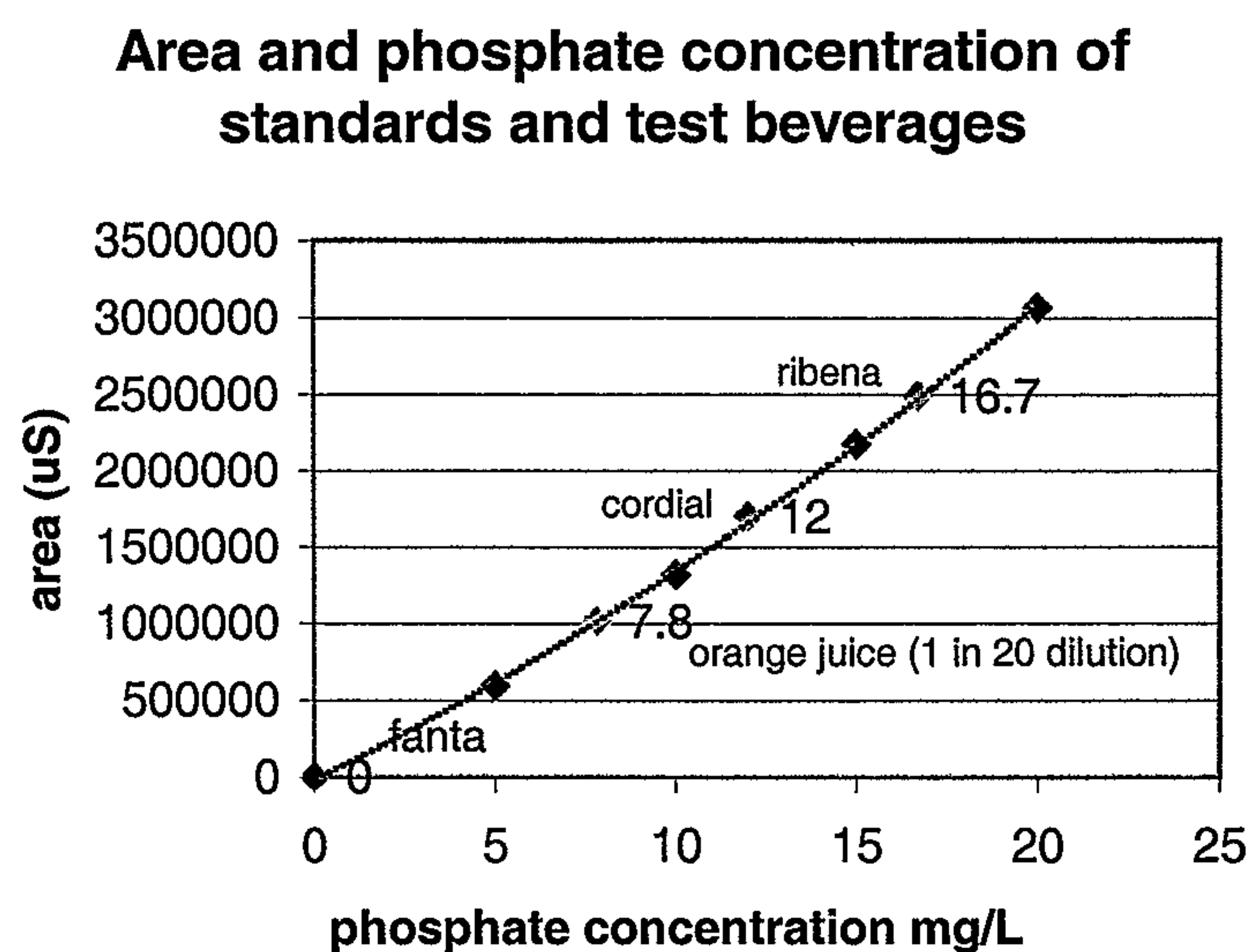


**Figure 4.13:** Standards and results of phosphate concentrations of test beverages. Standards shown as blue diamonds.

From graph in Figure 4.13 the concentration of phosphate for each beverage was calculated and is shown in Table 4.9 and Figure 4.14 below.

| Beverage     | Phosphate concentration |               |
|--------------|-------------------------|---------------|
|              | mg/L                    | mmol/L        |
| Ribena       | 16.7                    | 0.18          |
| Orange Juice | 140                     | 1.47          |
| Cordial      | 12                      | 0.13          |
| Fanta        | None detected           | None detected |

**Table 4.9:** Overall phosphate concentration of the test beverages.

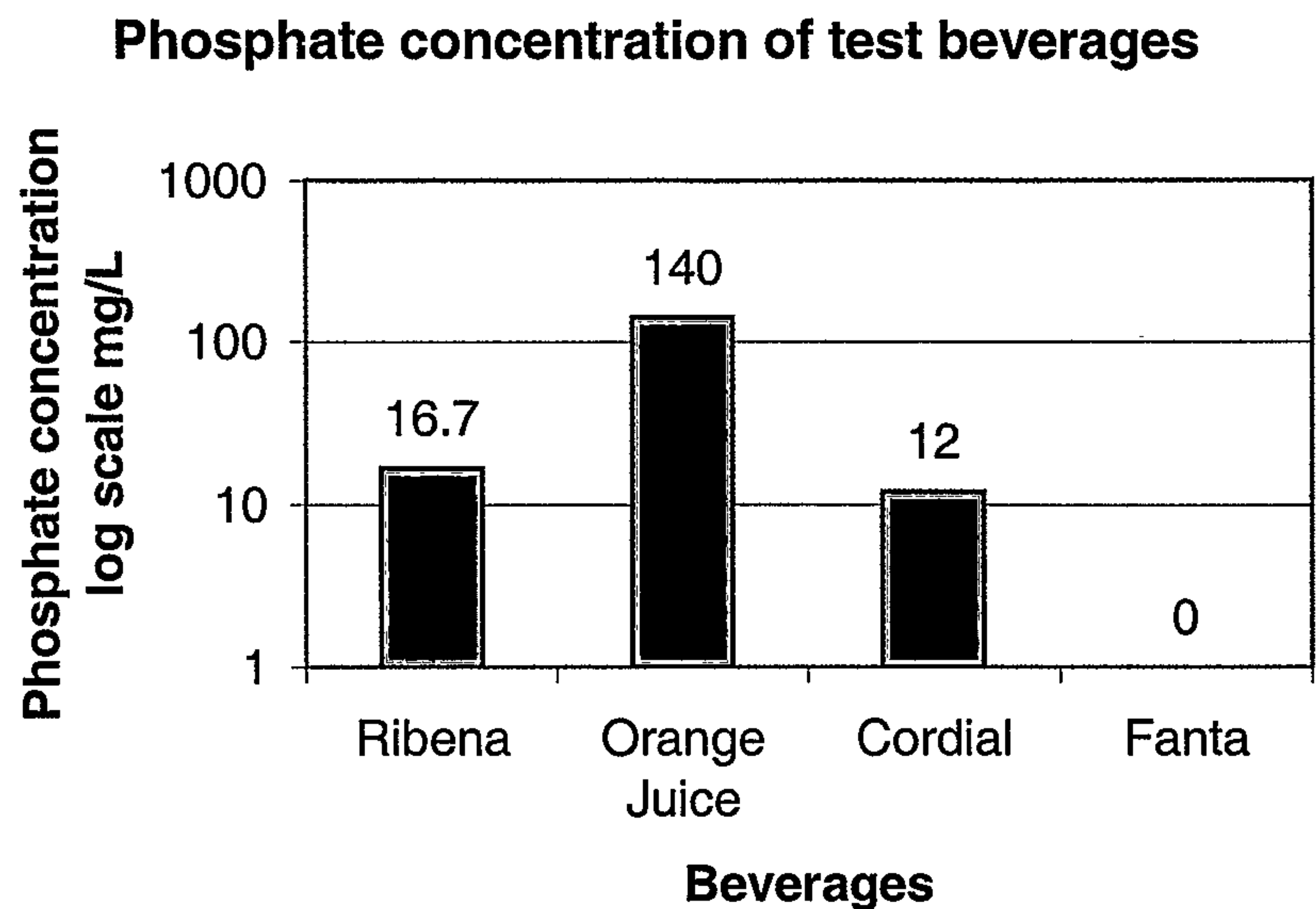


**Figure 4.14:** Phosphate concentrations of test beverages.

All test beverages were noted to have a low phosphate concentration except for Orange Juice which had a phosphate concentration of approximately eight times Ribena.

### Change in Hardness and Modulus of Elasticity after Exposure to Test Beverages

Table 4.10 and 4.11 show the hardness for both enamel and dentine of each tooth before and after exposure to each of the test beverages and the mean overall percent reduction in hardness resulting from each beverage. Figure 4.15 shows the overall % change in hardness in graphical form. Note that the change in hardness of enamel and dentine upon immersion in water is close to zero.

| Beverage<br>tooth exposed<br>to: | Tooth<br>number | Mean<br>hardness pre<br>exposure<br>Enamel<br>GPa | Mean<br>hardness<br>after<br>exposure<br>Enamel<br>GPa | Mean<br>percent %<br>change in<br>hardness<br>and 95%<br>confidence<br>interval |
|----------------------------------|-----------------|---------------------------------------------------|--------------------------------------------------------|---------------------------------------------------------------------------------|
| Control<br>distilled water       | 1               | 3.82                                              | 3.91                                                   | 1.7 (13--9)                                                                     |
|                                  | 2               | 4.46                                              | 4.78                                                   |                                                                                 |
|                                  | 3               | 4.95                                              | 4.70                                                   |                                                                                 |
|                                  | 4               | 4.30                                              | 4.38                                                   |                                                                                 |
| Ribena<br>Toothfriendly          | 5               | 4.61                                              | 5.04                                                   | -8.3 (-3--19)                                                                   |
|                                  | 6               | 3.57                                              | 3.31                                                   |                                                                                 |
|                                  | 7               | 4.57                                              | 3.46                                                   |                                                                                 |
|                                  | 8               | 5.63                                              | 5.02                                                   |                                                                                 |
| Orange Juice                     | 9               | 4.66                                              | 4.85                                                   | -2.7 (8--14)                                                                    |
|                                  | 10              | 4.68                                              | 4.46                                                   |                                                                                 |
|                                  | 11              | 5.02                                              | 4.31                                                   |                                                                                 |
|                                  | 12              | 5.06                                              | 5.29                                                   |                                                                                 |
| Orange<br>Cordial                | 13              | 3.82                                              | 2.43                                                   | -32.0 (-21--43)                                                                 |
|                                  | 14              | 5.00                                              | 2.92                                                   |                                                                                 |
|                                  | 15              | 4.30                                              | 2.86                                                   |                                                                                 |
|                                  | 16              | 4.19                                              | 3.42                                                   |                                                                                 |
| Fanta                            | 17              | 5.15                                              | 2.91                                                   | -47.7 (-37--59)                                                                 |
|                                  | 18              | 4.79                                              | 2.43                                                   |                                                                                 |
|                                  | 19              | 4.46                                              | 2.84                                                   |                                                                                 |
|                                  | 20              | 4.95                                              | 1.78                                                   |                                                                                 |

**Table 4.10:** Showing the change of enamel hardness for each tooth upon emersion in each test beverage.

NB: a positive number means an increase in hardness where as a negative number is a reduction in hardness..

This table shows that enamel hardness is reduced when submerged in all test beverages. Fanta reduced the enamel hardness the greatest followed by Orange Cordial, Ribena and finally Orange Juice. Interestingly Orange Juice reduced enamel hardness by only 2.7% reduction, which is comparable to distilled water (1.7%). Distilled water had the overall lowest change in hardness of 1.7%, although its range was large.

| Beverage tooth exposed to: | Tooth number | Mean hardness pre exposure Dentine. GPa | Mean hardness after exposure. Dentine GPa | Mean percent % change in hardness and 95% confidence interval |
|----------------------------|--------------|-----------------------------------------|-------------------------------------------|---------------------------------------------------------------|
| Control Distilled water    | 1            | 0.80                                    | 0.82                                      |                                                               |
|                            | 2            | 0.97                                    | 0.90                                      |                                                               |
|                            | 3            | 1.04                                    | 0.92                                      |                                                               |
|                            | 4            | 0.96                                    | 0.92                                      | -5.7 (5- -16)                                                 |
| Ribena Toothfriendly       | 5            | 0.87                                    | 0.95                                      |                                                               |
|                            | 6            | 0.90                                    | 0.78                                      |                                                               |
|                            | 7            | 0.73                                    | 0.72                                      |                                                               |
|                            | 8            | 0.97                                    | 0.88                                      | -4.5 (6- -15)                                                 |
| Orange Juice               | 9            | 0.95                                    | 0.80                                      |                                                               |
|                            | 10           | 1.07                                    | 0.56                                      |                                                               |
|                            | 11           | 0.98                                    | 0.71                                      |                                                               |
|                            | 12           | 0.85                                    | 0.74                                      | -25.8 (-16- -36)                                              |
| Orange Cordial             | 13           | 0.81                                    | 0.65                                      |                                                               |
|                            | 14           | 0.85                                    | 0.65                                      |                                                               |
|                            | 15           | 0.96                                    | 0.83                                      |                                                               |
|                            | 16           | 0.96                                    | 0.90                                      | -15.1 (-5- -25)                                               |
| Fanta                      | 17           | 0.98                                    | 0.66                                      |                                                               |
|                            | 18           | 0.81                                    | 0.65                                      |                                                               |
|                            | 19           | 0.97                                    | 0.74                                      |                                                               |
|                            | 20           | 1.04                                    | 0.67                                      | -28.2 (-18- -38)                                              |

**Table 4.11:** Overall change in the hardness of dentine upon emersion in each test beverage.

NB: a positive number indicates an increase in hardness where as a negative number is a reduction in hardness.

From results Table 4.11 it is seen that overall Orange Juice, Orange Cordial and Fanta reduce the hardness of dentine much greater than either the control (distilled water) or Ribena.

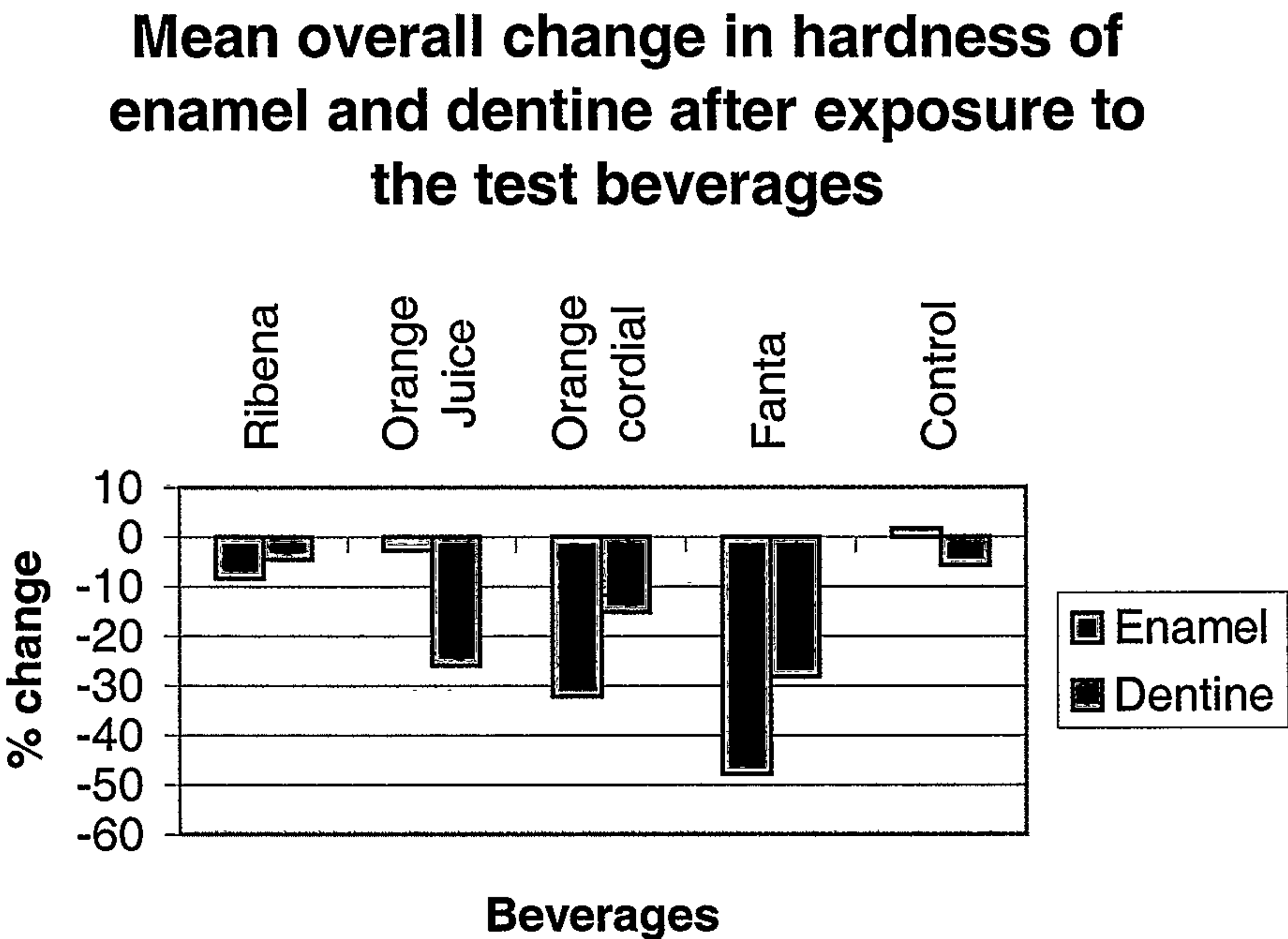


**Figure 4.15:** The change in hardness of enamel and dentine after emersion in each of the test beverages.

Figure 4.15 shows that overall Ribena causes the least change in hardness of enamel and dentine in comparison to the other beverages. The difference between the control and Ribena was not significantly different. Fanta caused the largest decrease in hardness overall followed by Orange Cordial in enamel. Interestingly Orange Juice did not cause a significant reduction in hardness to enamel but produced the second greatest reduction (25%) in hardness to dentine. Orange Cordial on the other hand had a much greater effect on enamel (32%) compared to dentine (15%). Except with Orange Juice enamel was affected more by each of the test beverages than the dentine.

Table 4.12 and 4.13 show the modulus of elasticity for each tooth before and after submersion of the teeth in each of the test beverages. It also shows the overall percent change in modulus of elasticity of enamel and dentine for each test beverage. Figures 4.16 shows the overall percent change of modulus of elasticity in enamel and dentine for each beverage. Note that the change in modulus of elasticity of teeth on immersion in water was close to zero.

| Beverage tooth exposed to: | Tooth number | Mean modulus of elasticity pre emersion in test beverage. Enamel GPa | Mean modulus of elasticity post emersion in test beverage. Enamel GPa | Mean percent % change in modulus of elasticity and 95% confidence interval for enamel |
|----------------------------|--------------|----------------------------------------------------------------------|-----------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Control distilled water    | 1            | 79.78                                                                | 89.03                                                                 |                                                                                       |
|                            | 2            | 74.47                                                                | 55.26                                                                 |                                                                                       |
|                            | 3            | 81.89                                                                | 79.28                                                                 |                                                                                       |
|                            | 4            | 80.77                                                                | 81.86                                                                 | -0.6 (17- -18)                                                                        |
| Ribena Toothfriendly       | 5            | 87.61                                                                | 68.40                                                                 |                                                                                       |
|                            | 6            | 82.33                                                                | 82.59                                                                 |                                                                                       |
|                            | 7            | 86.32                                                                | 81.68                                                                 |                                                                                       |
|                            | 8            | 90.65                                                                | 29.01                                                                 | -0.3 (18- -18)                                                                        |
| Orange Juice               | 9            | 79.78                                                                | 89.02                                                                 |                                                                                       |
|                            | 10           | 74.47                                                                | 55.26                                                                 |                                                                                       |
|                            | 11           | 81.89                                                                | 79.28                                                                 |                                                                                       |
|                            | 12           | 80.77                                                                | 81.85                                                                 | -4.0 (14- -22)                                                                        |
| Orange Cordial             | 13           | 86.60                                                                | 82.88                                                                 |                                                                                       |
|                            | 14           | 86.24                                                                | 80.37                                                                 |                                                                                       |
|                            | 15           | 73.95                                                                | 77.96                                                                 |                                                                                       |
|                            | 16           | 83.32                                                                | 78.49                                                                 | -3.4 (14- -21)                                                                        |
| Fanta                      | 17           | 87.61                                                                | 68.39                                                                 |                                                                                       |
|                            | 18           | 82.33                                                                | 82.59                                                                 |                                                                                       |
|                            | 19           | 86.32                                                                | 81.68                                                                 |                                                                                       |
|                            | 20           | 90.65                                                                | 74.01                                                                 | -23.7 (-6- -42)                                                                       |

**Table 4.12:** Overall change in modulus of elasticity for enamel upon emersion in each test beverage.

NB: a positive number indicates an increase in modulus of elasticity where as a negative number is a reduction in modulus of elasticity.

Table 4.12 shows that for enamel, the overall decrease in modulus of elasticity was very low except in the case of Fanta.

| Beverage tooth exposed to: | Tooth number | Mean modulus of elasticity of Dentine pre emersion in test beverage | Mean modulus of elasticity of Dentine post emersion in test beverage | Mean percent (%) change in Dentine modulus of elasticity and 95% confidence interval |
|----------------------------|--------------|---------------------------------------------------------------------|----------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Control Distilled water    | 1            | 16.60                                                               | 19.54                                                                |                                                                                      |
|                            | 2            | 19.49                                                               | 20.08                                                                |                                                                                      |
|                            | 3            | 23.07                                                               | 20.84                                                                |                                                                                      |
|                            | 4            | 20.33                                                               | 20.59                                                                |                                                                                      |
| Ribena Toothfriendly       | 5            | 23.55                                                               | 23.70                                                                |                                                                                      |
|                            | 6            | 19.93                                                               | 20.44                                                                |                                                                                      |
|                            | 7            | 14.99                                                               | 14.72                                                                |                                                                                      |
|                            | 8            | 18.94                                                               | 17.87                                                                |                                                                                      |
| Orange Juice               | 9            | 21.12                                                               | 19.55                                                                |                                                                                      |
|                            | 10           | 22.70                                                               | 15.74                                                                |                                                                                      |
|                            | 11           | 20.53                                                               | 16.66                                                                |                                                                                      |
|                            | 12           | 18.23                                                               | 16.94                                                                |                                                                                      |
| Orange Cordial             | 13           | 19.55                                                               | 18.16                                                                |                                                                                      |
|                            | 14           | 20.18                                                               | 17.43                                                                |                                                                                      |
|                            | 15           | 20.50                                                               | 20.35                                                                |                                                                                      |
|                            | 16           | 22.03                                                               | 20.32                                                                |                                                                                      |
| Fanta                      | 17           | 19.87                                                               | 17.33                                                                |                                                                                      |
|                            | 18           | 18.25                                                               | 18.40                                                                |                                                                                      |
|                            | 19           | 19.44                                                               | 17.84                                                                |                                                                                      |
|                            | 20           | 23.05                                                               | 18.11                                                                |                                                                                      |

**Table 4.13:** Overall change in modulus of elasticity of dentine of each tooth upon emersion in each test beverage.

NB: a positive number indicates an increase in modulus of elasticity where as a negative number is a reduction in modulus of elasticity.

Table 4.12 interestingly shows the greatest decrease in modulus of elasticity in dentine was by Orange Juice followed by Fanta. The decrease in dentine modulus of elasticity is consistent with the overall decrease in

hardness found for the beverages in dentine. It is interesting to note that overall dentine showed a greater decrease in modulus of elasticity than enamel (except in teeth exposed to Fanta), which is in direct contrast to the hardness data.

**Mean change in modulus of elasticity  
of enamel and dentine after exposure  
to test beverages**

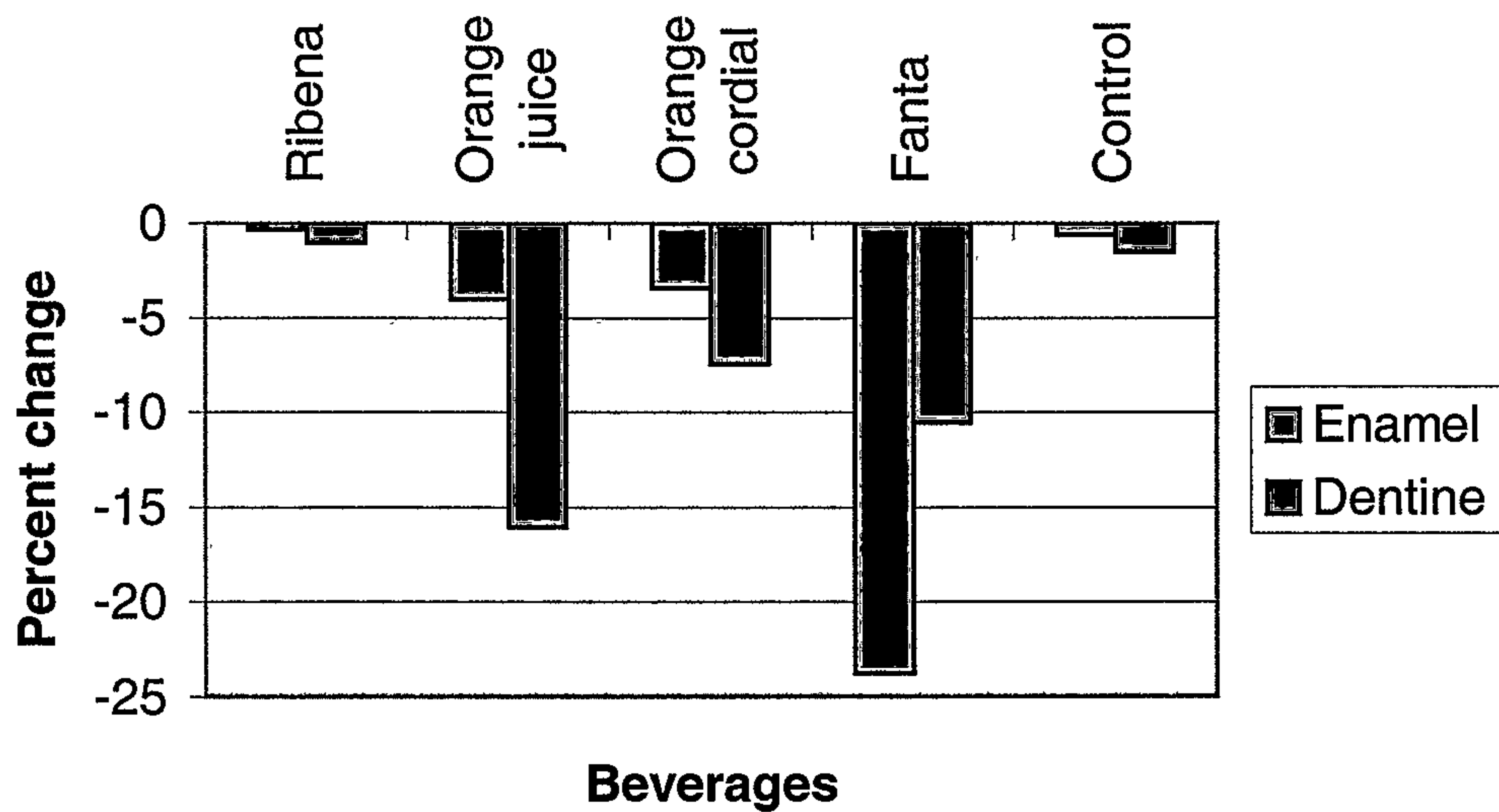


**Figure 4.16:** Showing the change in modulus of elasticity enamel and dentine after emersion in the test beverages.

**Statistical Analysis**

The results of the t-test conducted to determine if there was a statistically significant reduction in the hardness and/or modulus of elasticity of enamel or dentine are presented in Table 4.14.

| Beverages      | Enamel Hardness   | Enamel modulus of elasticity | Dentine hardness  | Dentine modulus of elasticity |
|----------------|-------------------|------------------------------|-------------------|-------------------------------|
| Ribena         | 1.3 <sup>NS</sup> | NS                           | NS                | NS                            |
| Orange Juice   | 0.6 <sup>NS</sup> | NS                           | 2.8 <sup>NS</sup> | NS                            |
| Orange Cordial | 4.3 <sup>*</sup>  | NS                           | 1.3 <sup>NS</sup> | NS                            |
| Fanta          | 6.4 <sup>**</sup> | NS                           | 3.2 <sup>*</sup>  | NS                            |

**Table 4.14:** Results of t-tests to determine if there is a statistically significant difference in the ability of each test beverage to reduce the hardness and modulus of elasticity of primary teeth.

All t-statistics have six degrees of freedom. P values have been Bonferroni-corrected for multiple correction.  $**0.0005 < P < 0.005$ ,  $*0.005 < P < 0.05$ ,  $NS = P > 0.05$ .

Table 4.14 shows that in comparison to distilled water only Fanta and Orange Cordial caused a significant reduction in the hardness of enamel. For dentine, Fanta was the only beverage to cause a statistically significant reduction in hardness. None of the test beverages caused a statistically significant change in the modulus of elasticity in enamel or dentine.

### Statistical Analysis of Test Beverages

Figures 4.17 to 4.21 are scatter plots of relating the percent reduction in hardness of enamel of teeth to the chemical properties of the solution in which they were immersed. Each data point is the % reduction in hardness of a tooth on immersion in a test beverage (not including distilled water). From the plots, the best predictor of the reduction in enamel hardness is the pH, followed by the phosphate. This is confirmed in the Table 4.15.

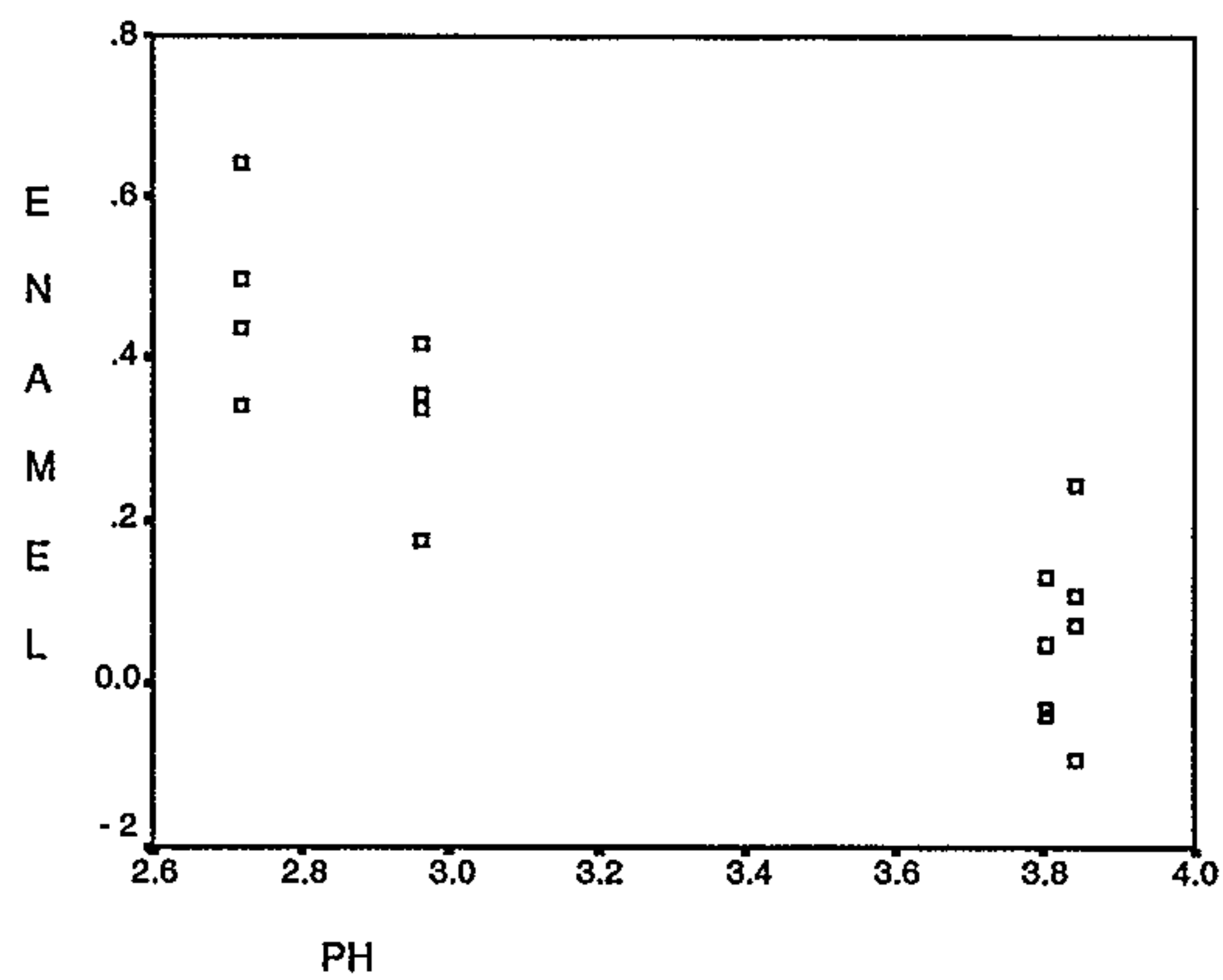


**Figure 4.17:** Showing the relationship between the pH of the test beverages and the percent reduction in enamel hardness.

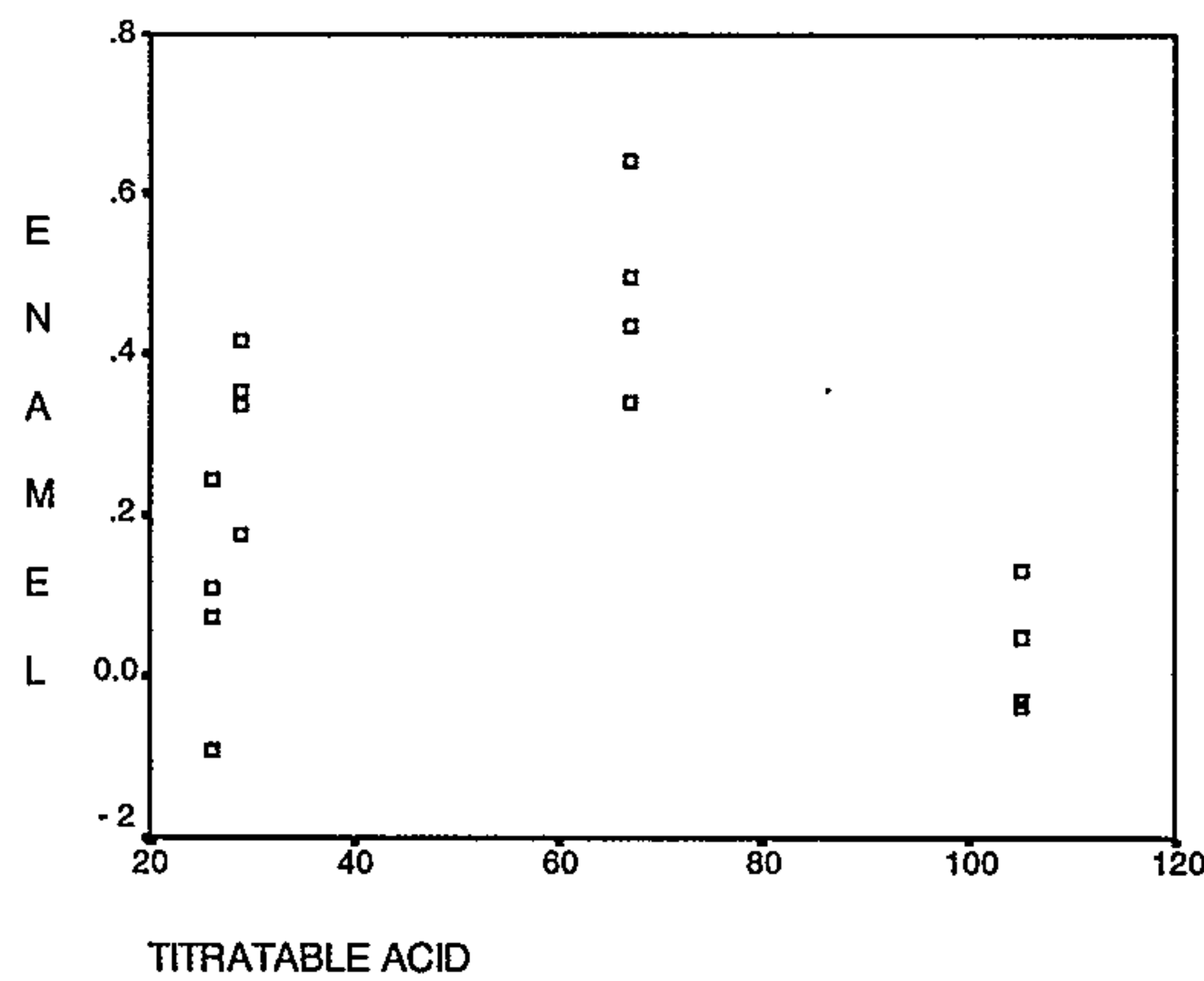


**Figure 4.18:** Showing the relationship between the titratable acidity of the test beverages and the percent reduction in enamel hardness.

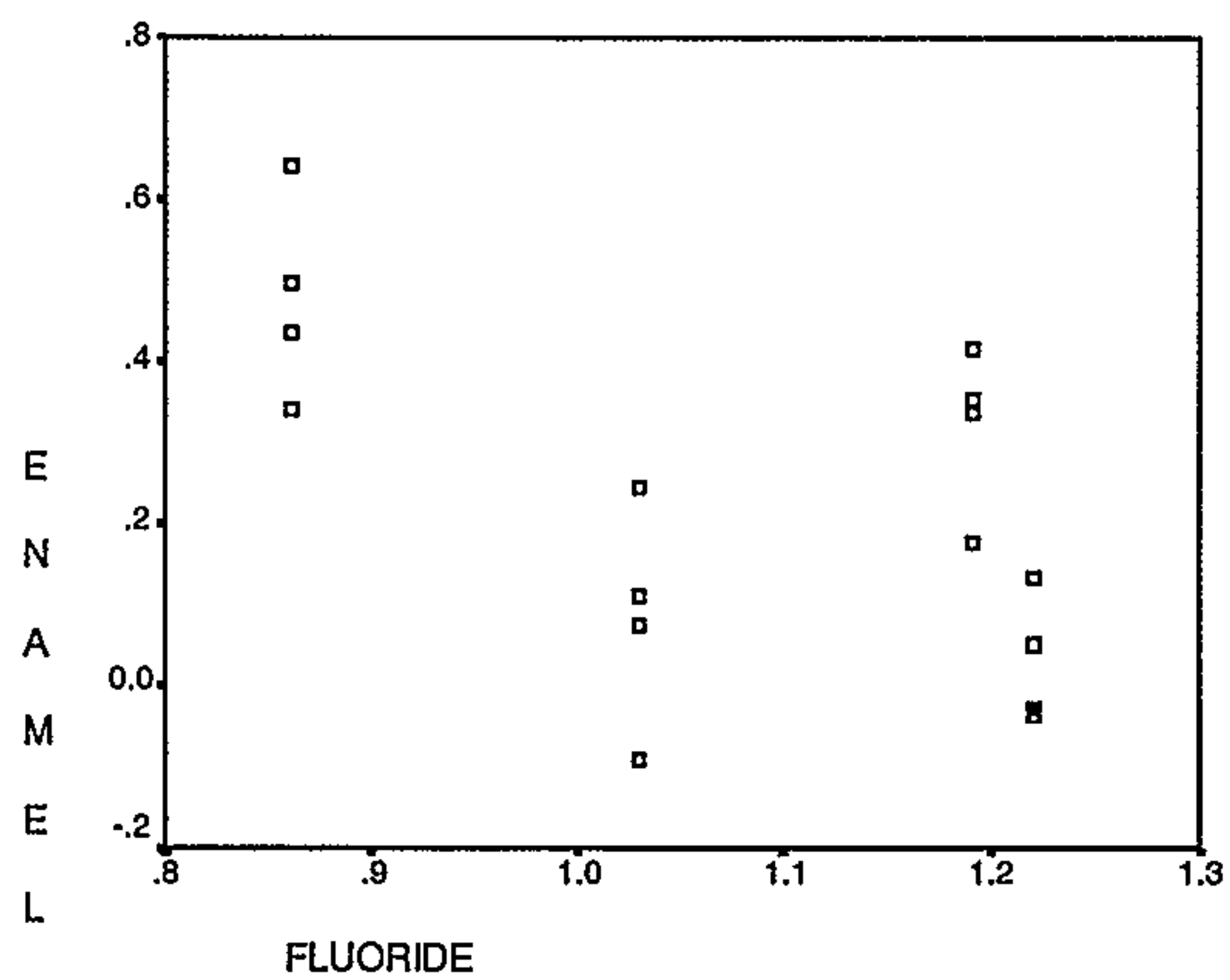


**Figure 4.19:** Showing the relationship between the fluoride concentration of the test beverages and the percent reduction in enamel hardness.

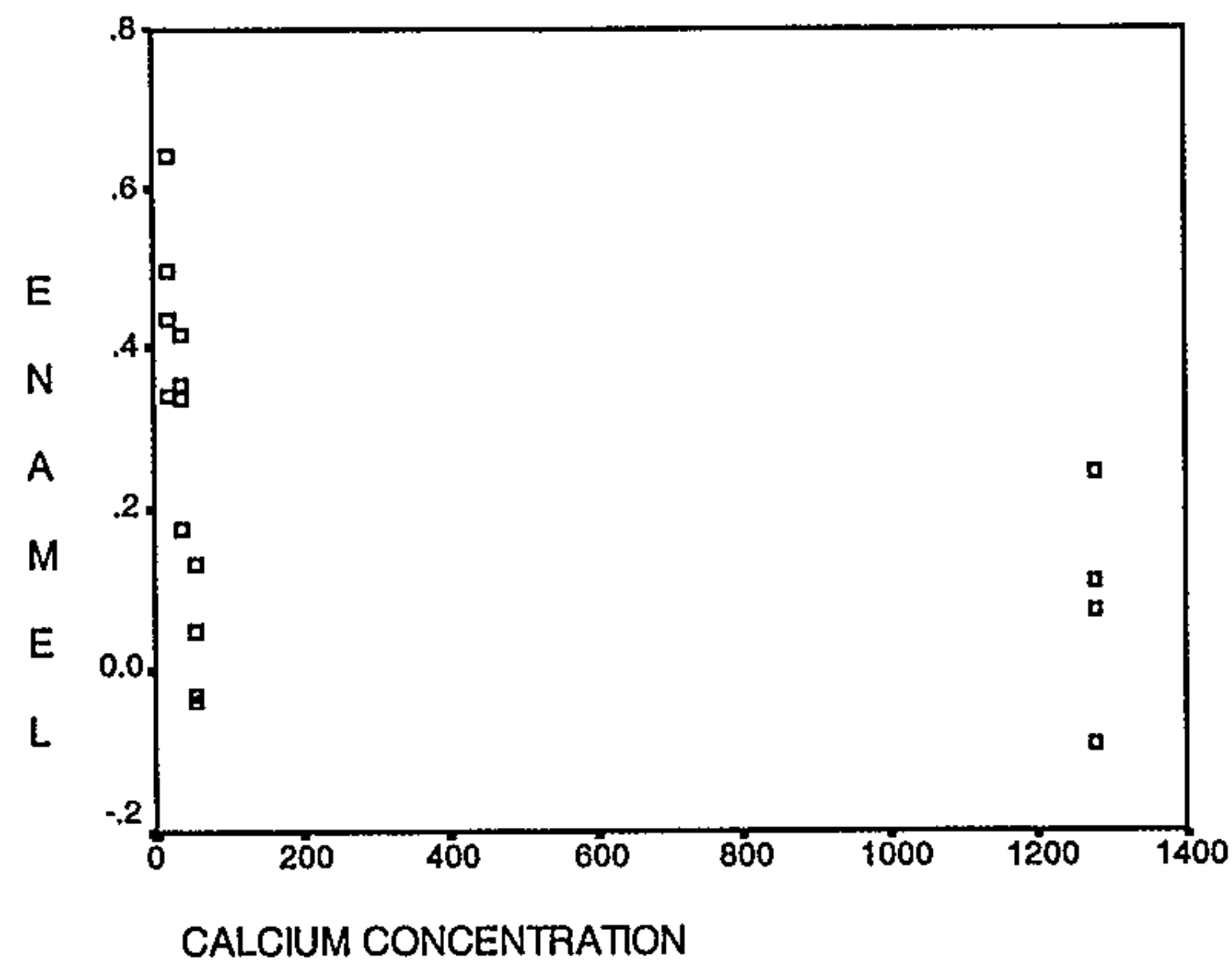


**Figure 4.20:** Showing the relationship between the calcium concentration of the test beverages and the percent reduction in enamel hardness.

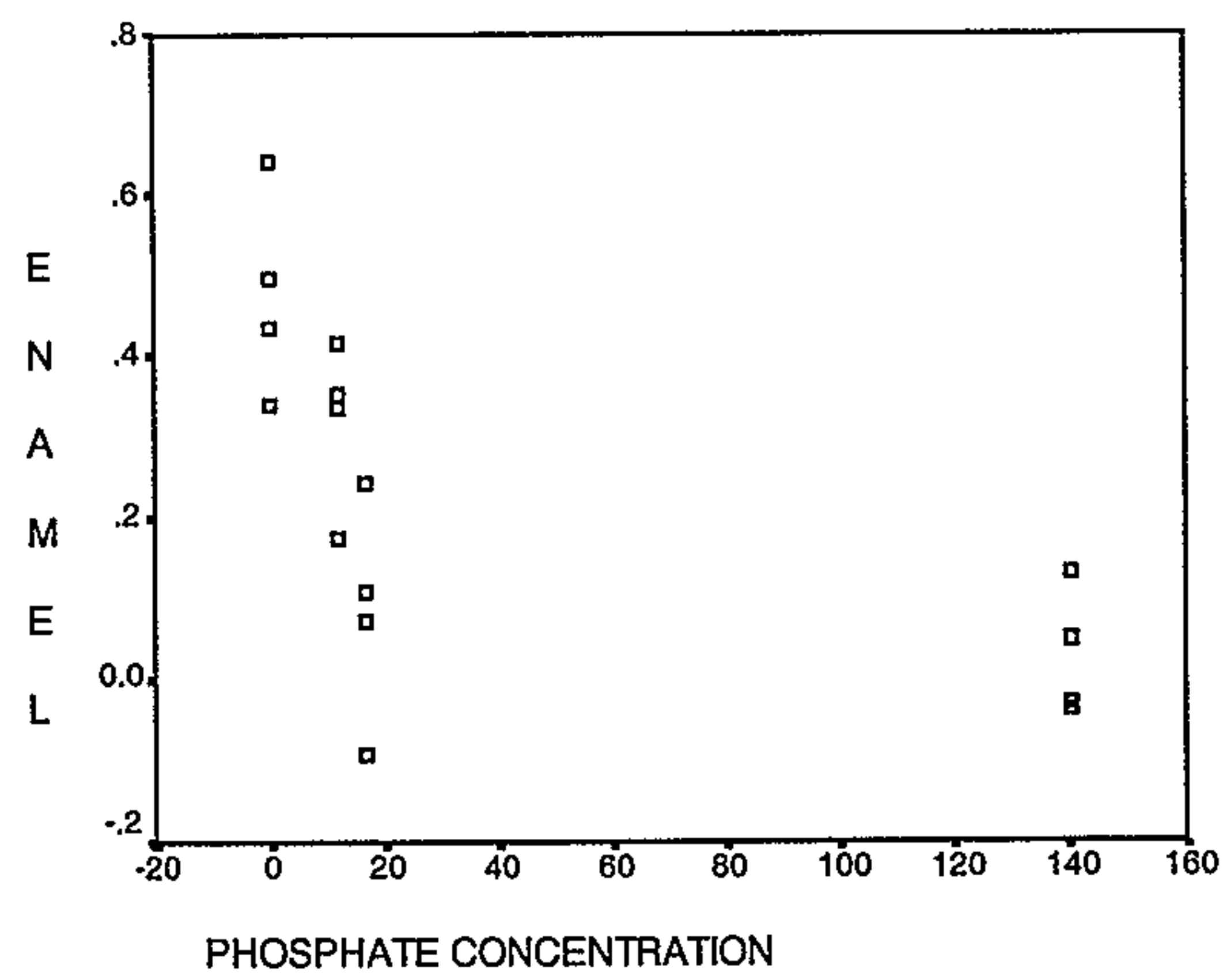


**Figure 4.21:** Showing the relationship between the phosphate concentration of the test beverages and the percent reduction in enamel hardness.

|                            | Enamel<br>hardness  | Enamel<br>elasticity | Dentine<br>hardness | Dentine<br>elasticity |
|----------------------------|---------------------|----------------------|---------------------|-----------------------|
| pH                         | -.862***            | -.373 <sup>NS</sup>  | -.324 <sup>NS</sup> | -.063 <sup>NS</sup>   |
| Titrateable<br>acidity     | -.196 <sup>NS</sup> | .142 <sup>NS</sup>   | .583*               | .572*                 |
| Calcium<br>concentration   | -.420 <sup>NS</sup> | -.246 <sup>NS</sup>  | -.620*              | -.505*                |
| Fluoride<br>concentration  | -.522*              | -.391 <sup>NS</sup>  | -.104 <sup>NS</sup> | .173 <sup>NS</sup>    |
| Phosphate<br>concentration | -.623*              | -.171 <sup>NS</sup>  | .259 <sup>NS</sup>  | .440 <sup>NS</sup>    |

\*\*\*  $P < 0.0005$ , \*\*  $0.0005 < P < 0.005$ , \*  $0.005 < P < 0.05$ , <sup>NS</sup>  $P > 0.05$ .

**Table 4.15:** The statistical significance of the change in hardness and modulus of elasticity with each test beverage.

From Figure 4.22 there appears to be a positive correlation between the increase in titrateable acidity increases and the percent reduction in enamel and dentine hardness. The relationship would be strong except for the Orange Juice which has a high titrateable acidity and a low erosive potential, especially in enamel. This relationship as stated before is not significant in enamel and therefore conclusions can not be drawn.

**Enamel and dentine hardness and change as a function of titrateable acidity**

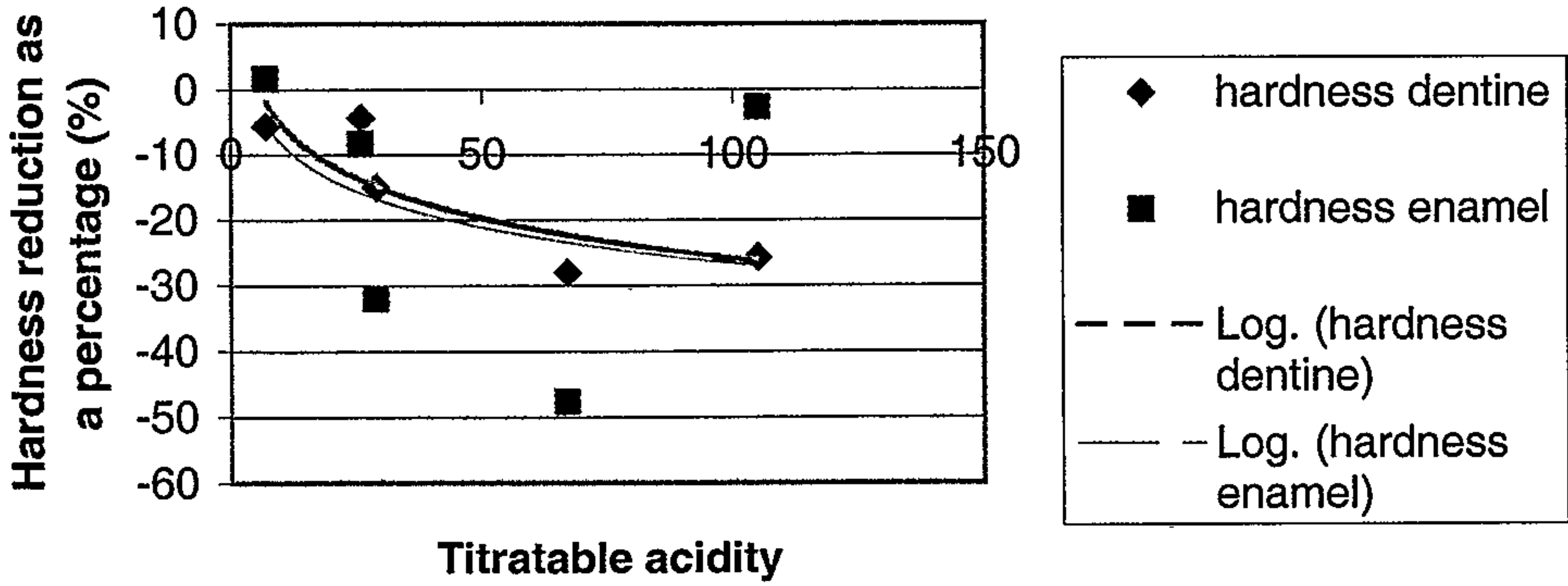


**Figure 4.22:** Enamel and dentine hardness change vs titratable acidity.

A stepwise multiple regression found that no variable explained significant variation in enamel hardness additional to that explained by pH (i.e. there is no statistical evidence for a combined effect on hardness of calcium and pH, or phosphate and pH). In the case of enamel hardness and elasticity, no variables explained significant variation additional to that explained by either calcium or titratable acidity.

## Chapter 5. DISCUSSION

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### Experiment one

*Experiment one has been published in Journal of Dentistry: See Appendix A.*

None of the traditional tests used to measure the physical properties of dental hard tissues and dental materials are ideal. The development of depth-sensing instruments, such as the UMIS offer advantages over conventional methods by providing high resolution and repeatability, as well as the ability to obtain information about the elastic and time-dependent plastic properties of the material (Doerner & Nix 1986).

Conventional hardness tests require direct imaging, usually visual, of the width or area of the indentations, to obtain a hardness value. When the indent is loaded, a certain amount of recovery in the elastic portion of a tooth occurs. As a result the indentation changes shape (Tabor D 1986). The UMIS has the advantage of testing the force versus penetration depth and therefore elastic recovery should have very little effect on the hardness as the load is removed. Large errors are also introduced with conventional hardness testing, particularly with measurement of the length of the diagonals at small scales as there are difficulties in focusing on both ends of the diagonals.

Other problems encountered include tester error in loading, rate of load application, duration of a contact period, and impact (Boyer HE 1987). If an indenter is placed on a surface of the specimen and the full load is applied instantly, the area of indentation will be three times larger than that observed

under conditions of incremental loading (as hardness = force/area). If the hardness is calculated from this area then it will be one third of the true value. The UMIS incrementally loads the specimen. In this study each of the indentations were incrementally loaded (25 increments) to a maximum 50 or 150 mN.

As the UMIS was originally developed for testing of thin films it requires only very small loads. Due to the small size of the indents, more tests can be conducted on an individual sample as well as creating a more standardised test area. This offers great advantage when testing the hardness of primary teeth.

For hardness tests, the type of indentation formed depends on the nature of the solid. In some cases the stress field will initiate cracks, particularly at the periphery of the indentations (Tabor D 1986). In this study approximately 15% of the enamel test indentations were discarded, which is similar to other studies (Craig RG & Peyton FA 1958). In the present study, the results from the indentations were discarded if they caused surface cracking or if the indentations were positioned out of the desired area being tested. Surface damage affecting micro-indentation results has been noted in other studies (Meredith N, Sherriff M, *et al.* 1996). Although no surface damage (by direct vision) was noted in the dentine, approximately 7% of the indentations in dentine were discarded as they fell upon surface imperfections. Such indentations gave force-displacement curves that varied from the mean by values greater than two standard deviations from the overall test indentation mean. The UMIS offers the advantage of allowing the visualisation of the

force-displacement curve to determine if cracking has occurred rather than the less accurate direct visualisation method of conventional hardness tests.

It is reported that for macro indentation techniques using a load ranging from 10-300 N that the indentation hardness is almost independent of the load unless the surface has been appreciably affected by work hardening or segregation of phases (Tabor D 1986). In general there is a reported increase in hardness at small loads (Stilwell NA & Tabor D 1961, Mott BW 1957, Boyer HE 1987, Brookes CA 1983). For conventional microhardness tests, the apparent increase in hardness with decrease in load is primarily caused by 1) errors in determination of the size of the indentation and 2) aberration in the elastic recovery of the indentation. As the size of the indentation decreases, reading will be less accurate because of these factors. The advantage of the UMIS is that it is a depth-sensing device which eliminates these two inaccuracies. The choice of load depends on the size and depth of the indentation considered to be most desirable (Boyer HE 1987). As long as a single load is used throughout a series of tests, the load dependence is of little significance (Boyer HE 1987).

In both dentine and enamel, the actual values for both the hardness and modulus of elasticity were not found to vary significantly in relation to the load applied (see Figures 4.3 and 4.4). Other researchers have reported that microhardness data are a function of the applied load (Salama SN & Kinawi NA 1989). Ryge and co-workers concluded that hardness is load dependant for all the materials that they tested, including dentine and enamel (Ryge G, Foley DE, *et al.* 1961). However, the loads applied in the present study are much

lower than previously reported. It was noted that the load of 150 mN proved to be slightly less satisfactory as there was more evidence of cracking especially in enamel.

Other variables are known to be important when determining the hardness of a material. Changes in temperature dramatically alter the properties of materials. The strength of most materials decreases as the temperature increases (Askeland DR 1989). The teeth were therefore stored at room temperature as well as allowed to cool to room temperature after being heated. The UMIS machine was kept at a constant temperature of 22 degrees Celsius throughout all experiments.

Other variables such as the shape of indenter, vibration, and surface preparation can also modify the microhardness (Boyer HE 1987) of conventional tests. The Berkovitch indenter, used in the UMIS has an advantage as it is brought to a much sharper point than the Vickers or Knoop indenter (Tabor D 1986). It is also the indenter of choice for picnoindentations, which are useful in determining the hardness of peritubular dentine and even individual crystals. Although the Berkovitch indenter has a very distinct point, over time it will become less so as the tip wears and blunts. At the beginning of the experiments a series of tests (evaluation of tip profile and compliance) were conducted to eliminate the effect of dulling of the point of the indenter. Vibration was controlled by an anti-vibration table and the surface of the specimens was highly polished as suggested for loads under 100g (Boyer HE 1987).

The hardness of enamel in primary teeth has been studied before, however it is difficult to make comparisons as the early studies used relative hardness or Knoop hardness numbers to describe their results (Caldwell RC, Muntz ML, *et al.* 1957, Saunbury P & Atkinson HF 1953). However values of  $3.62 \pm 0.2$  GPa and  $3.37 \pm 0.15$  GPa for both axial and occlusal enamel, respectively, in permanent teeth have been reported recently (Xu HHK, Smith DT, *et al.* 1998). These values are lower than those reported by Poolthong for permanent teeth using the same test method as used in this current study (Poolthong S 1998). The permanent teeth in Poolthong's study had slightly softer enamel ( $4.45 - 4.74$  GPa) when compared with the primary enamel in this study ( $4.88 \pm 0.35$  GPa).

No recent published data exist on the hardness of dentine in primary teeth however the value reported in this study,  $0.92 \pm 0.11$  GPa are comparable to those reported for permanent dentine,  $0.57 - 1.13$  GPa (Poolthong S 1998, Xu HHK, Smith DT, *et al.* 1998).

The modulus of elasticity for permanent dentine has been reported at between  $17.6 - 22.9$  GPa (Poolthong S 1998, Xu HHK, Smith DT, *et al.* 1998). However there are conflicting reports as to whether the reported elastic properties of dentine depend on the orientation of the cut surface tested and on what material is tested (Craig RG & Peyton FA 1958, Xu HHK, Smith DT, *et al.* 1998). A modified atomic force microscope has been used to measure the hardness and modulus of elasticity of peritubular and intertubular dentine

(Kinney JH, Balooch M, *et al.* 1996). The mean modulus of elasticity of peritubular dentine was  $29.8 \pm 8.9$  GPa and did not depend on the site of indentation. In contrast the mean modulus of elasticity of intertubular dentine did vary between test site and was lower than the modulus of peritubular dentine. This finding is not surprising as peritubular dentine has been reported to have a higher concentration of calcium, phosphate and magnesium ions (Hassan R, Caputo AA, *et al.* 1981). In the present study all teeth were tested under the same orientation conditions to control this variable. The overall modulus of elasticity for primary dentine in this study was  $19.89 \pm 1.92$  GPa which is comparable with Poolthongs (1998) study and Xu and colleagues (1998). The dentine in this study was tested half way between the DEJ and the pulp and the results for the modulus of elasticity in this study are similar with the reported modulus of elasticity for intertubular dentine found by Kinney and colleagues (1996) of between  $17.7 \pm 0.3$  GPa for intertubular dentine near the pulp and  $21.1 \pm 1.3$  GPa for dentine tested near the DEJ.

The modulus of elasticity of enamel is also influenced by the orientation of the indentations. Xu and co-workers (Xu HHK, Smith DT, *et al.* 1998) reported that the modulus of elasticity for occlusal permanent enamel was significantly higher at  $94 \pm 5$  GPa than axial enamel at  $80 \pm 4$  GPa. The eight primary teeth in the current study were sectioned horizontally and the modulus of elasticity of the axial enamel in the present study was  $80.35 \pm 7.71$  GPa which is very similar to previously reported values.

Throughout this study the mean values for the hardness of enamel and dentine have been quoted. It is obvious, however, that results of individual teeth vary in both enamel (4.66 to 5.15 GPa) and dentine (0.76 – 1.12 GPa). Such differences may be explained in several ways. Firstly in the case of the dentine it is very difficult to test the same location in each tooth as an arbitrary distance 'half way' between the CEJ and pulp chamber was selected. Secondly, although the test sites were crack and scratch free, minor surface and subsurface imperfections not evident under microscopic examination may have been encountered whilst testing, causing variation in results. Differences may also arise in the hardness of teeth due to exposure of individuals to different environmental factors during the time of tooth formation and mineralisation. It is widely known that fluoride exposure can influence the hardness of the enamel both during formation and post-eruptively. It would be very difficult to eliminate such variation from this study. Interestingly there was also some variation in the hardness within each tooth. Similarly fracture toughness is reported to also vary in enamel within the same tooth (Hassan R, Caputo AA, *et al.* 1981). This may arise as a result of variation in mineralisation of the tooth crown.

There is a weak but positive correlation between the modulus of elasticity and the hardness of both enamel and dentine. Previous studies on a wide range of calcified tissues have established that the modulus of elasticity correlates directly with the calcium mineral content per unit volume of a calcified material (Curry JD 1998). The positive correlation between hardness and modulus of elasticity of the enamel and dentine suggest that hardness as well as

modulus may correlate with calcium mineral content. This aspect warrants further work and may provide an insight in to the influence of demineralising and remineralising substrates on the properties of dental hard tissue.

## Experiment Two

### Changes in Hardness and Modulus of Elasticity of Enamel and Dentine

The second part of this study determined the change in hardness and modulus of elasticity of both enamel and dentine following exposure to four test beverages. The percent reduction was then taken as an indicator of erosive potential of a beverage. This is reasonable because a material's susceptibility to wear may be determined by observing the material's resistance to penetration. Indentation damage is known to simulate individual events in a range of cumulative surface removal processes, such as abrasive wear (Marshall DB & Lawn BR 1986)

The present study, using the mean change in hardness before and after immersion in four test beverages found that the only Fanta and Orange Cordial caused a statistically significant reduction in the enamel hardness. Fanta was the only beverage to cause a statistically significant reduction in dentine hardness. None of the test beverages were shown to cause a statistically significant reduction in enamel or dentine modulus of elasticity.

Using *in vitro* measurement of erosion obviously does not take into account any protective factors (such as saliva, salivary pellicle etc) for erosion in the oral environment (Hughes JA, West NX, *et al.* 2000). Therefore as with all *in vitro* method, this study reflects the worst potential scenario for acid erosion of enamel and dentine (Hughes JA, West NX, *et al.* 2000).

The literature reports multiple *in vitro* methods for determining the change in the hardness of dental hard tissue before and after placement of a tooth in a potentially erosive beverage, (Attin T, Koidl U, *et al.* 1997, Grobler SR, Du Toit IJ, *et al.* 1994, Lussi A, Jaggi T, *et al.* 1993, Lussi A, Jaeggi T, *et al.* 1995, Lussi A, Kohler N, *et al.* 2000). Change in surface microhardness with the UMIS, has many advantages over other *in vitro* methods, which were discussed in the previous section. Visualisation of the indent is not necessary using the UMIS. This is a particular advantage following exposure to erosive beverages which tend to etch the dental hard tissues making visualization of the diagonals difficult.

Whilst there is a relatively large amount of the literature dealing with erosive potential of various beverages, most address wear of enamel only and not both dental hard tissues together (Larsen MJ & Nyvad B 1999, Lussi A, Jaggi T, *et al.* 1993). Supported by the present study, other research that has tested dentine's susceptibility to erosion have reported that dentine is less susceptible to erosion than enamel (Mistry M & Grenby TH 1993, Hunter ML, West NX, *et al.* 2000a, Hunter ML, West NX, *et al.* 2000b, Hall AF, Buchanan DT, *et al.* 1999). In this experiment, all beverages caused a greater reduction in the

hardness of enamel than dentine except for Orange Juice. It has been suggested that the difference in susceptibility to erosion may be due to the higher organic content in dentine which may act as a buffer, thereby reducing erosion (Mistry M & Grenby TH 1993). Mistry and Grenby in an *in vivo* study conducted on rats teeth showed lower erosive wear on dentine than enamel and concluded that the organic matter helps to preserve the structure of the dentine (Mistry M & Grenby TH 1993). Hunter and colleagues (2000b) noted that dentine loss due to erosion was not linear with time, with significant early erosion plateauing after several days. The authors could not find an explanation for this observation. It is important to determine to what degree dentine is affected by erosion particularly in deciduous teeth, where the enamel is much thinner than in permanent teeth.

The relationship between reduction in hardness and elastic modulus is unclear although within dentine the two appear to mirror each other. It is known that with etching there is an increase in its porosity. It is possible that the greater the porosities, the greater will be the reduction in the modulus of elasticity however these mechanism have yet to be evaluated fully with respect to dental erosion. There is currently nothing in the dental literature on the influence of erosive beverages on the modulus of elasticity. A comparison to other studies is therefore impossible.

The UMIS gave reproducible results when observing the change in hardness and modulus of elasticity. The control (distilled water) gave a standard error for this UMIS to be up to 5.7% which is to be expected with any test method

on a material such as enamel or dentine. Even with the small numbers of teeth used in this study, statistically significant reductions in enamel and dentine hardness were shown for Fanta and Orange Cordial. We can conclude therefore that Fanta has a significant erosive potential in enamel and dentine whilst Orange Cordial has significant erosive potential in enamel. No other beverages had a statistically measurable erosive potential. Unlike a number of other studies orange juice did not appear to reduce the hardness of enamel or dentine significantly (Lussi A, Kohler N, *et al.* 2000, Grobler SR, Senekal PJC, *et al.* 1990, West NX, Maxwell A, *et al.* 1998 ).

## Chemical Analysis: Method and Relationship to Hardness and Modulus of Elasticity Reduction

In order to determine the chemical composition of the test beverages a number of techniques and analysing instruments was used. The recent literature on erosion and erosive potential of beverages has focused on the chemical composition of potentially erosive beverages to try and determine which property of a beverage will influencing erosive potential. From previous literature the pH, titratable acidity, calcium, phosphate and fluoride content were determined to be the most relevant properties of a beverage to test to determine if any one or combination of more than one factor most directly relates to the erosive potential of a beverage.

## pH

In this study the measurement of the pH of the test beverages was done using a pH meter. The same technique for measuring the pH of beverages has been used by other researchers reproducibly (Edwards M, Creanor SL, *et al.* 1999, Larsen MJ & Nyvad B 1999).

The pH of the beverages ranged from 2.7 (Fanta) to 6.38 (control, distilled water) which is consistent with the findings from other similar research (Edwards M, Creanor SL, *et al.* 1999, Larsen MJ & Nyvad B 1999, Mistry M & Grenby TH 1991, Mistry M & Grenby TH 1993). All test beverages besides distilled water had a pH lower than the critical pH for enamel dissolution. The pH of the test beverages was found in this study to have a highly significant relationship with the observed reduction in hardness of enamel, but not of dentine hardness or enamel or dentine modulus of elasticity.

pH is the most commonly cited factor to be related to erosive potential of a beverage. An *in vivo* study conducted on rats found the lower the pH of the beverages the rats consumed, the greater the observed erosion (Mistry M & Grenby TH 1993). Larsen and Nyvad (1999) found that as the pH of the beverages decreased the amount of enamel dissolved increased logarithmically. Lussi and colleagues (1993 and 1999) have suggested that they can predict (using pH along with other factors) the *in vitro* erosive potential of a beverage to within 7%. In agreement with these studies, using scatterplots and multiple regression analyses, this study found the best predictor of reduction in hardness

is the pH of a beverage. This is highly significant for enamel ( $P < 0.005$ ) but has no statistical relationship to dentine hardness or change in modulus of elasticity. The reason for the differences is unknown. It appears from this research that the pH of a test beverage is important in the reduction of hardness of enamel and dentine especially when it is lower than pH 3. Both beverages with a pH of less than 3 (Fanta and Orange Cordial) reduced the hardness of enamel and dentine to a greater percentage than the beverages above the pH 3. This may suggest that with a pH of less than 3 other potentially protective factors such as calcium or phosphate may not be effective irrespective of their concentration. Whilst pH does not affect calcium equilibrium it is known to influence phosphate. The first pKa of  $\text{H}_3\text{PO}_4$  is about 2.1, so at pH 3.1 90% of the phosphate is the monoanion  $\text{H}_2\text{PO}_4^-$ , but this decreases to 50% at pH 2.1. The effect of this on erosive potential is unknown.

Grenby and colleagues (1990) found that although there was a relationship between the pH and the amount of erosion seen *in vitro*, the titratable acidity was a better guide to the erosive potential of the infant fruit drinks they were testing. In the present study the pH of the test beverages was seen to be the best predictor of erosive potential in enamel but not necessarily in dentine.

In conclusion the pH is a useful factor when determining the erosive potential of a beverage on enamel but should not be used in isolation, as indicated most clearly in this study by Ribena, in which its pH is low but its erosive potential is minimal.

## Titrateable Acidity

In this experiment the titrateable acidity was determined with the use of the 0.1 M sodium hydroxide (NaOH) solution by recording the volume of NaOH required to reach neutrality. All research into titrateable acidity determination in the erosion literature is determined in a similar way. This test proved to be reproducible.

In the present study the titrateable acidity of the test beverages was found to have no statistical effect on the reduction in enamel hardness or modulus of elasticity but it was found to be a predictor of the reduction of hardness and of modulus of elasticity in dentine. This is in contrast to the findings for pH.

Titrateable acidity is the measure of the total acid present, bound and unbound in a beverage. Titrateable acidity has been used by Lussi and colleagues (1995) to predict the erosive potential of a beverage *in vitro*, along with other factors. Larsen and Nyvad (1999) made no conclusion as to the effect of the titrateable acidity of their test beverages on enamel erosion. They did note however that at a given pH beverages with the higher titrateable acidity had an increased erosive potential. These researchers also found that their Orange Juice, with a significantly higher titrateable acidity than the other beverages tested, had a very high erosive potential in enamel. This is in direct contrast to the current study in which our Orange Juice had a very high titrateable acidity but did not cause significant erosion. The reason for the differences in results is not known but may be due to different, unidentified constituents (for example calcium, phosphate and fluoride) of each beverage from the different countries.

It has been suggested that titratable acidity, which is the measure of the total acid content (free and bound) is a more important indicator than actual pH (which is a measure of free acid only) in determining erosive potential (Edwards M, Creanor SL, *et al.* 1999, Grenby TH, Mistry M, *et al.* 1990). It is possible that a beverage with a high titratable acidity has a better buffering capacity: as the free acid is consumed in erosion it is replaced by the bound acid, so the process keeps going at about the same pH. If there were no bound acid the pH would rise as the erosion consumes acid and therefore the erosion process would slow. The second possible mechanism is that the bound acid molecules may adsorb more effectively to the tooth surface and act more efficiently to erode than free acid molecules.

The present study however did not show any significant relationship between enamel hardness reduction and titratable acidity. However there was a statistically significant relationship between titratable acidity and change in dentine hardness and modulus of elasticity. The reason for the differences are not known. It is suggested that titratable acidity may be used to predict erosive potential with respect to dentine however it should not be used in isolation from other factors, particularly pH. Grenby and colleagues (1990) have also noted the difficulty in establishing clear relationships between acidity and erosive potential.

## Fluoride

This present study found, utilising stepwise multiple regression analysis, there was a statistically significant relationship between fluoride concentration and reduction in enamel hardness ( $0.005 < P < 0.05$ ). However there was no relationship between fluoride content and reduction in hardness of dentine nor elastic modulus of either enamel or dentine.

Fluoride has been used in the prevention of tooth decay for many decades and its presence in the drinking water is known to decrease dental caries by up to 40% (Newbrun E 1989, Brunelle JA & Carlos JP 1990). Fluoride can become incorporated into the hydroxyapatite crystals as fluoroapatite to decrease demineralisation. *In vitro*, fluoride in toothpaste has been shown to reduce the erosive effect of beverages on extracted teeth (Munoz CA, Feller R, *et al.* 1999). It is possible that the fluoride content of a frequently consumed beverage may also affect erosive potential by protecting the dental hard tissue from demineralisation (Amaechi BT, Higham SM, *et al.* 1998b, Lussi A, Kohler N, *et al.* 2000, Greenby TH 1996).

The use of the fluoride ion-specific electrode is well established in the dental literature as the method of choice for determining fluoride concentration (Larsen MJ & Nyvad B 1999, Lussi A, Jaggi T, *et al.* 1993, Lussi A, Jaeggi T, *et al.* 1995, Lussi A, Kohler N, *et al.* 2000). The level of fluoride in beverages determined in this research was overall higher than other studies. In this research the results for fluoride concentration varied between 0.86 to 1.22 ppm. Lussi *et al.* (1993 and 2000) report much lower fluoride concentrations in their

study beverages, the highest being 0.36 ppm. Fluoride concentrations may vary as a result of differences in fluoride concentrations in water used to mix drinks both pre, during and after processing (Silva M & Reynolds EC 1996, Johnson J Jr & Bawden JW 1987). This may be an explanation for the varying fluoride contents of the test beverages.

Using iodine permeability and surface microhardness as a measurement of erosion Lussi and colleagues (1993) observed that statistical correlation could be found between the potential erosiveness of a number of beverages and their fluoride content. These authors found that beverages with the highest fluoride content showed the smallest amount of surface softening (Lussi A, Jaggi T, *et al.* 1993). In contrast Larsen and Nyvad (1999) reported that fluoride content (of 18 soft drinks) had no effect on the depth of erosion of extracted teeth. The amount of fluoride reported to be present in most studies is small (i.e. less than 1 part per million). This makes statistical analysis of fluoride's effect on erosion difficult.

Results from Larsen and Nyvad's study (1999) on the erosion of various beverages in relation to their chemical composition report a fluoride concentration of between 0.09 to 0.8 ppm although one beverage (Maarum) had a fluoride concentration of 1.78. The Maarum water with a pH of 5.38 did not cause any noticeable erosion in contrast to two other beverages with a similar pH but much lower fluoride concentration.

Interestingly in the present study, Orange Juice had the highest fluoride content of the test beverages but the lowest overall reduction in enamel hardness. Conversely Orange Cordial with the very similar fluoride content as Orange Juice, caused a large reduction in enamel hardness. This makes conclusions for fluoride from the present study difficult.

The present study indicates that knowing the fluoride concentration of a beverage will aid in the prediction of the erosive potential of that beverage on enamel, although in agreement with Larsen and Nyvad (1999) it seems that fluoride alone does not have the ability to reduce erosion significantly, especially at a low pH. It may be however, that with the correct conditions, where the pH or other erosive factors are not as severe as in the present test beverages, then fluoride may have a role to play in reducing the erosive potential of a beverage. However this cannot be confirmed in the present study.

## Phosphate and Calcium Determination

It is postulated that the presence of suitable concentrations of calcium and phosphate may counteract the dissolution of dental mineral according to the Law of Mass action (Johnson J Jr & Bawden JW 1987, Greenby TH 1996, Grenby TH, Phillips A, *et al.* 1989). The Law of Mass Action states that the rate of a chemical reaction (i.e. the dissolution of enamel and dentine mineral in this case) is proportional to the concentration of the reacting substances (calcium and/or phosphate) present at any given time (Greenby TH 1996). This has resulted in measuring the concentration of both calcium and phosphate in beverages and attempting to relate this to the erosive capabilities of these

beverages (Grenby TH, Phillips A, *et al.* 1989, Grobler SR, Senekal PJC, *et al.* 1989, Larsen MJ 1975, Larsen MJ & Nyvad B 1999, Lussi A, Jaggi T, *et al.* 1993, Lussi A, Jaeggi T, *et al.* 1995, Lussi A, Kohler N, *et al.* 2000, Reussner GH, Coccodrilli G, *et al.* 1975).

### **Calcium Determination**

The calcium in this report was determined with atomic absorption (AA). This is the method used by multiple other researchers to determine the calcium for potentially erosive beverages (Grenby TH, Phillips A, *et al.* 1989, Larsen MJ 1975, Larsen MJ & Nyvad B 1999, Lussi A, Jaggi T, *et al.* 1999, Lussi A, Jaeggi T, *et al.* 1995, Lussi A, Kohler N, *et al.* 2000, Grobler SR, Senekal PJC, *et al.* 1989). Each of these authors reported no difficulties in their experimental technique.

In this experiment there was a number of compounding factors that made the determination of the calcium concentration difficult. Initially it was not known if the calcium in each of the test beverages were expected to be bound in solution to other minerals or compounds. To allow the calcium to become unbound it was decided to wet ash a portion of each of the test beverage samples. Wet ashing is used to decompose organic matter to bring metals into solution in a form which is suitable for analysis in a matrix comparable to the standards used in the determination of Ca. Each of the samples, especially Orange Juice required a substantial amount of nitric acid to allow decomposition of the organic matter. Unexpectedly the orange colour of each of the beverages was never completely removed.

Pure samples of each beverage were tested along with the wet ashed samples to allow comparison and determination as to the amount of calcium that was bound and unbound. By using both the newer of the atomic absorbance machine (AA220) and the older machine (AA800) reproducibility was assessed. To ensure that the pure samples did not clog the AA machines, each of the pure samples of the test beverages were filtered. The filtering removed most of the orange colouring for the Ribena and Orange Cordial although the orange colour was still present in the Fanta and Orange Juice. The Orange Juice was very difficult to filter due to the 'pulp' presence in the beverage and required a number of filterings through a vacuum filter. The results from the AA220 showed that there was very little difference in the amount of bound vs unbound calcium in the Orange Juice or Fanta. For Ribena the difference was significant with the concentration of the pure sample being 233 mg/L versus its total calcium of the wet ashed sample being 1277 mg/L. Cordial also showed a difference between the total calcium in the pure sample as being 26 mg/L versus the wet ashed, unbound calcium of 37 mg/L. Another difference noted between the pure samples and the wet ashed samples was that Orange Juice reported a higher level of calcium in the pure sample than the wet ashed sample. It is assumed that there is an interference with the colour or the complex organic component in the Orange Juice. As the beverage is not transparent there may be particles in the Orange Juice that cause scattering of the atomic absorbance flame which will cause an abnormally high reading. For final analysis of the calcium content the wet ashed results for each beverage was used because they were the most consistent between the two separate

samples run. There was between a 5-10% error in these results which is acceptable for the present purposes. These difficulties have never been reported in the dental literature before.

To test reproducibility the wet ashed test beverages were run through the older of the atomic absorption (AA800) machines with EDTA added. The EDTA is another releasing agent which is added to a sample to decrease chemical interferences. EDTA protects calcium from the interfering effects of sulfate and phosphate. The newer atomic absorbance machine (AA220) has never required the use of EDTA as a releasing agent in previous tests run on other test liquids, It is assumed that the design of the nebuliser/burner has improved in the newer AA machine to provide better volatilisation. Each of the samples were run multiple times and although each of the tests was consistent within each test, the tests were not consistent between the two machines. This inconsistency has never been reported within the dental literature. It is assumed that each of the previously cited researchers in other laboratories, working with test beverages have overcome the problem of whether EDTA should be added and which machine should be used for the final absolute calcium value, although how has never been mentioned in any of the erosive potential publications. For completeness the difficulties encountered with each test procedure should be mentioned in each publication.

Lussi and colleagues (1993) found a significant relationship between erosive potential and the phosphate content, titratable acidity, pH and fluoride concentration, but not the calcium content of the beverages. Overall the

literature has supported this finding and it appears that there is no clear relationship between the calcium concentration and the erosive potential of a beverage. This can be seen from the work of Lussi and colleagues (2000 and 1993) and Larsen and Nyvad (1999). This relationship may only be true for the beverages which have a naturally low level of calcium, as when calcium is added to raise the concentration significantly, it appears to provide a protective effect on erosion (Larsen MJ & Nyvad B 1999). An example of this is the Ribena Toothkind beverage, which was originally developed as a blackcurrant flavoured drink with minimal erosive potential. There have been five reports of *in vitro* and *in vivo* studies which have highlighted its low erosive potential which has been attributed to its high calcium concentration of between 195 to 400 ppm, in comparison to the control beverages used (9.9- 20 ppm) (Hughes JA, West NX, *et al.* 1999a, Hughes JA, West NX, *et al.* 1999b, Hughes JA, Hunter ML, West NX, *et al.* 2000, Hunter ML, West NX, *et al.*, 2000b, West NX, Hughes JA, *et al.* 1999). Larsen and Nyvad (1999) also report on the beneficial effect on hardness of adding calcium and phosphate to Orange Juice.

In this study, Ribena overall caused very little change in both enamel and dentine. As suggested by West and colleagues (1999), the very high concentration of calcium in these beverages may have a protective effect on the enamel hardness and modulus of elasticity, even though no statistical relationship exists. It is possible that a statistically significant relationship between calcium concentration and erosion does exist in both enamel and dentine, however a larger number of teeth and a greater range of calcium concentrations will need to be investigated. Unfortunately three out of the four

beverages tested in this study had a small concentration of calcium (17.59 to 53.89 mg/L) whilst Ribena had a 25 times higher calcium concentration. This wide range of samples made establishing a clear relationship between calcium and enamel hardness difficult but there was a significant relationship with dentine hardness and modulus of elasticity. This indicates that further work in this areas is required.

From this study it can be seen there is no statistical significant relationship between calcium concentration in the test beverages and enamel hardness or modulus of elasticity change. However this study has shown that the calcium concentration of a beverage is useful in the prediction of the erosive potential of a beverage on dentine. Interestingly although the reason for this difference is unknown, it is the opposite relationship to phosphate. The calcium concentration of a beverage cannot be used in isolation to determine its erosive potential.

### **Phosphate Determination**

The DIONEX ion chromatography unit was used in the determination of the phosphate concentration of the test beverages in this research. Ion chromatography has been reported elsewhere in the dental literature to be a standard phosphate determination method. Other methods used for phosphate determination include ammonium molybdate method and colour spectrometry (Larsen MJ & Nyvad B 1999, Lussi A, Jaggi T, *et al.* 1993).

This method of phosphate determination was reproducible over the two tests conducted. Pure samples of the beverages required filtering through a very

small filter to allow only the soluble fraction to be passed through the column. It is therefore unknown what effect the discarded material from the filter (for example the pulp from the Orange Juice) may have on the phosphate content of the drink.

This study found that the phosphate concentration of a beverage had a statistically significant inverse relationship with the reduction of hardness of enamel only. There was no relationship with the reduction of enamel or dentine modulus of elasticity or the reduction in dentine hardness. Using multiple regression analysis phosphate concentration was found to be the second most important factor in predicting erosive potential of a beverage, second only to the pH of the beverage.

In this research Orange Juice had a significantly higher phosphate concentration than all other test beverages. From the literature the phosphate levels reported here for Orange Juice was similar to the 160 mg/L (1.7 mmol/L) published by Greenby and colleagues (1989). Other reports by Lussi and colleagues (1995) and Larsen and Nyvad (1999) report the phosphate concentration of Orange Juice to be 2.88 and 3.55 mmol/L (264 and 330mg/L) respectively. A lower phosphate level of 1.47 mmol/L was found in this report and may be for two reasons. Firstly the Orange Juice here was not fresh (it came packaged, pre-mixed from a concentrate) and secondly there may be different packaging and processing (e.g. filtering) of Orange Juice in Australia, than Orange Juice made overseas. This stresses the importance of analysing

separately, the phosphate concentration of beverages made and packaged in different countries.

Very low levels of phosphate were reported for Fanta in the research carried out by Larsen and Nyvad (1999) and Lussi and colleagues (1995) which is consistent with this report. Although no specific international results have been published for Orange Cordial, Greenby and colleagues reported a phosphate concentration of an 'orange drink' to be approximately 5-10 mg/L which is similar to the 12 mg/L reported for Orange Cordial in this study. The Ribena Toothfriendly beverage has been reported to have a very high added calcium content making it low in erosive potential, however there are no reports on its phosphate content.

There is no doubt that a very high phosphate concentration is protective against dental erosion: however, there is debate as to whether the phosphate content in commonly consumed beverages is adequate to provide a protective effect. Greenby and colleagues (1989) report in their experiments on the dental properties of soft drinks that the cola drink with the highest phosphate content was consistent with the lowest level of demineralisation. Conversely the pure Orange Juice with very high levels of calcium and phosphate showed one of the highest demineralising activity. Larsen and Nyvad (1999) stated in their study that at or below a pH of 2.5 the phosphate in the beverages cannot be expected to have a noticeable effect. In their study, only calcium and phosphate experimentally added to the Orange Juice had a significantly preventive effect on erosion. Lussi and colleagues (1995 and 2000) report that phosphate has a

beneficial effect on the erosive potential of beverages. Furthermore they demonstrated that knowing the concentration of phosphate along with other parameters they could predict the percentage reduction in surface hardness of enamel to within 7%.

This study identified using multiple regression analysis, that besides pH, the phosphate concentration is the next best predictor of erosive potential of a beverage in enamel. Beverages with low phosphate showed the greatest reduction in enamel hardness. In this experiment, Orange Juice has a very high concentration of phosphate and in enamel, it caused minimal hardness reduction in contrast to test beverages with low phosphate concentration. In the case of Orange Juice therefore, a high phosphate concentration may be the protective factor minimising its erosive potential in enamel. Interestingly although the phosphate appears to have a protective effect on enamel hardness change, it does not have as great an effect on dentine hardness change. The reason for this is unknown. Change in modulus of elasticity did not have any significant relationship with phosphate concentration in either enamel or dentine.

In conclusion the phosphate concentration of the test beverages was found to have an statistically significant inverse relationship with its erosive potential in enamel. The phosphate concentration cannot however be used in isolation to assess erosive potential of an given beverage, especially in dentine.

## Chapter 6. CONCLUSION

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At the beginning of this study there were three objectives to be addressed.

1. To determine the mechanical properties of primary tooth enamel and dentine using the Ultra Micro-Indentation System.

This study found that the mean hardness of primary enamel and dentine was  $4.88 \pm 0.35$  GPa and  $0.92 \pm 0.11$  GPa respectively. The modulus of elasticity of primary enamel and dentine was found to be  $80.35 \pm 7.71$  GPa and  $19.89 \pm 1.92$  GPa respectively. The UMIS was found to be a reproducible method for the investigation of the mechanical properties of primary teeth.

2. To establish the erosive potential of a number of different orange flavoured beverages.

Using a reduction in surface hardness as the indicator of erosive potential, Fanta and Orange Cordial had a statistically significant effect on enamel whilst only Fanta had a statistically significant erosive potential in dentine. None of the test beverages caused a significant change in modulus of elasticity of enamel or dentine.

3. To define the relationship between chemical composition and erosive potential.

The pH of the four test beverages followed by the phosphate concentration were the most statistically significant factors in predicting erosive potential in enamel. For dentine the calcium content and titratable acidity were the most

statistically significant factors in predicting erosive potential. Fluoride content showed a statistically significant relationship with the reduction in enamel hardness although no relationship to the change in hardness of dentine. Overall there was no statistical relationship between the modulus of elasticity change and any of the chemical composition tested except for calcium concentration and titratable acidity in dentine. These results indicate that no single component of a beverage can be used to predict its erosive potential, but the pH, titratable acidity and phosphate, calcium and fluoride concentration combined will aid in the determination of a beverages erosive potential.

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## Appendix A.

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**E Mahoney**, A Holt, M Swain, Kilpatrick N. *The hardness and modulus of elasticity of primary molar teeth: an ultra-micro-indentation study*. The Journal of Dentistry, 28 (2000) 589-594.

## The hardness and modulus of elasticity of primary molar teeth: an ultra-micro-indentation study

E. Mahoney<sup>a</sup>, A. Holt<sup>b</sup>, M. Swain<sup>b</sup>, N. Kilpatrick<sup>c,\*</sup>

<sup>a</sup>Department of Paediatric Dentistry, Westmead Centre of Oral Health, Westmead 2145, NSW, Australia

<sup>b</sup>Biomaterials Research Unit, Faculty of Dentistry, The University of Sydney, Suite G11, National Innovation Centre, Australian Technology Park, Eveleigh 1430, NSW, Australia

<sup>c</sup>Department of Dentistry, Royal Children's Hospital, Flemington Road, Parkville, Vic. 3052, Australia

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### Abstract

**Objective:** Baseline information on the mechanical properties of and the effect of load upon dental hard tissue is important in the development of successful dental materials. Existing methods of measuring such properties of tissue are subject to significant experimental error. This study reports on the use of an Ultra-Micro-Indentation System (UMIS) to measure the hardness and elastic modulus of primary enamel and dentine.

**Methods:** Primary molar teeth were sectioned, set in resin and polished. Thirty indentations were made in enamel and dentine using a Berkovitch indenter, 15 of which were subject to a load of 50 mN and 15 to a load of 150 mN. An automated computerised system converted the force/penetration graph for each indentation into a hardness vs depth graph from which values for the mean hardness and elastic modulus were calculated.

**Results:** Primary enamel had a mean hardness of  $4.88 \pm 0.35$  GPa whilst the hardness of dentine was  $0.92 \pm 0.11$  GPa. The elastic modulus for enamel was  $80.35 \pm 7.71$  GPa and that of dentine  $19.89 \pm 1.92$  GPa. Using linear regression analysis a significant relationship could be shown between the hardness and the elastic modulus for both enamel and dentine when loaded to 150 mN but only for dentine at 50 mN ( $P < 0.05$ ). In general the elasticity of tooth structure increased as the hardness increased.

**Conclusion:** The UMIS offers a simple and reproducible method of measuring basic mechanical properties of small samples of enamel and dentine. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** Ultra-micro-indentation system; Primary molar teeth; Berkovitch indenter

### 1. Introduction

Matching properties of restorative materials to the properties of teeth is considered useful to enhance the longevity of the restoration. As a consequence, baseline mechanical property data of teeth are required. This knowledge is also important to help clinicians understand how these tissues react under clinical loading conditions [1] as well as to help predict the behaviour of the tooth/restoration interface [2]. To date the data available has been generated mainly from conventional compressive and tensile tests. These tests are associated with some general limitations related to the size and quality of test specimens obtainable from a tooth. For example tensile strength testing is associated with difficulties in specimen preparation and alignment which may

give rise to misleading data [3–6]. Such difficulties are further compounded by the very small dimensions required to obtain homogeneous specimens of tissue such as enamel [7]. Preparation for these tests becomes difficult and time consuming in order to achieve a few ideal specimens. As a consequence of these problems, the results reported demonstrate a wide variation.

Hardness is the ability of a material to resist a permanent indentation [5]. Hardness testing is widely used to determine mechanical properties of materials. Such hardness tests can be correlated with a number of mechanical properties. For example, indentation hardness has been used to study elastic modulus, creep and fracture of brittle materials [3,8]. The modulus of elasticity (stiffness) is the ratio of stress to corresponding strain below the proportional limit. It is an indication of the amount of deformation that will occur in the dental tissue when a load is applied to it [9]. A material's susceptibility to wear can be determined by

\* Corresponding author. Tel.: +61-3-9345-5462; fax: +61-3-9345-5488.  
E-mail address: nicky@bassdata.com.au (N. Kilpatrick).

observing the material's resistance to penetration. Indentation damage is known to usefully simulate individual events in a range of cumulative surface removal processes such as abrasive wear [10].

Most hardness studies have employed the micro-indentation techniques. These include the Brinell, Rockwell Knoop and Vickers indentors of which the Vickers and the Knoop indentor are the most common [1,5,11,12]. These tests all employ a small symmetrical indentor that penetrates into the surface of the enamel or dentine at a known, given load. The hardness is determined from the applied load divided by the projected area of the indentation [2]. The micro-indentation methods are useful when the specimen size is limited [6]. Micro-indentation tests are able to measure the hardness, the elastic properties, creep and fracture toughness of the dental hard tissues [1,3,8].

A nano-indentation technique has been described in which very small indentations ( $<1\ \mu\text{m}$ ) are produced using loads of a few milli-Newtons [13–15]. This technique involves recording the depth rather than the area of the indentation. The depth-sensing device continuously records changes in depth of an indentation during both loading and unloading cycles. From this information the hardness and Young's modulus can be automatically calculated from the load–displacement curve rather than from visual measurement of the indentation impression. The Ultra-Micro-Indentation System or UMIS developed by the Commonwealth Scientific and Research Organisation (CSIRO) is a further development on this principal.

The UMIS operates with a high degree of automation under computer control as has been described previously [16]. The indenter is driven down by a long-range piezo driver connected to an elastic element. A Linear Variable Differential Transformer (LVDT), secured to the frame of the instrument, records the position of the shaft. The indenter contacts the surface and the elastic element is deflected relative to the main carriage and this deflection is monitored by a second LVDT. The deflection of the elastic member on contact with the surface generates a contact force. The LVDT output is zeroed when this contact occurs — therefore establishing a datum for indentation depth. For each indentation the UMIS produces a force–displacement curve for both the loading and unloading process. Because the area of the indentation is proportional to the depth squared the load is applied in square root increments of force to give a more uniform measure of hardness at equal depth increments. The data is subsequently automatically analysed [14] and hardness as a function of plastic penetration depth plotted from which a mean hardness value for each indentation site is calculated. In addition the elastic modulus of the material can be determined at the maximum penetration depth. Also, immediately after testing the indentations are viewed by a microscope to inspect their geometry and position. This is done to evaluate the quality of the indentations and determine whether any values need to be discarded. For example, if an indentation falls directly on

the dentinoenamel junction, or if a large amount of cracking on loading has occurred in the dental tissue, then these values are discarded.

The properties of primary teeth have received little attention in the literature. The aim of this study was to describe the properties of primary molar teeth in terms of the micro-hardness and Young's modulus of enamel and dentine using the UMIS.

## 2. Methods

First primary maxillary molar teeth were collected for the study. They were placed in a 1% solution of hydrogen peroxide disinfectant (Milton's Solution) immediately after extraction and stored at 4°C for at least 24 h. After the disinfection procedure the teeth were stored in distilled water at 4°C until required for preparation. As reported previously caries free primary teeth are difficult to obtain [17]. When carious teeth were used, the testing was performed on non-carious regions. Eight teeth were selected where the donor's age ranged from 4 to 7 years.

The teeth were enclosed in rubber mould and set in a cold-curing epoxy resin [Epofix, Struers, Copenhagen, Denmark]. Once the resin had set, the embedded teeth were removed from their moulds and the site for testing was determined. To enable comparison between teeth the test site was standardised. The crown of each tooth was sectioned half way between the tip of the buccal cusp and the cemento-enamel junction. As a result despite the variation in size of tooth the same region was tested. Furthermore the prism orientation in the enamel was virtually horizontal to the plane chosen. The teeth were sectioned using a low speed saw (Isomet, Buehler Ltd., Lake Bluff, USA) under tap water irrigation to expose the testing surface. To ensure the surface of the exposed teeth were free from scratches and deformation the specimens were polished prior to the indenting. To achieve this result, surface material was removed by means of successively finer sizes of abrasive particles. The grinding and polishing procedure is summarised in Table 1 and was based upon previous work [15,18].

The finished tooth specimens were mounted on metal bases with wax using a paralleling machine (Leitz, Wetzlar, Germany). The mounting base contained a strong magnet to ensure adequate contact was obtained with the test base in the UMIS. Once the teeth had been prepared they were stored in distilled water at room temperature. The total time the specimens spent in storage was 24 h in the disinfectant and no more than 1 week in distilled water. Previous pilot work done in the unit demonstrated no change in surface hardness when stored in distilled water providing testing was carried out within 3 months of specimen preparation [18].

When ready for testing each specimen was placed on the 50 mm diameter work-table and positioned under the

Table 1  
Summary of the grinding and polishing steps

| Step                   | 1                  | 2                  | 3                  | 4                  |
|------------------------|--------------------|--------------------|--------------------|--------------------|
| Abrasive               | Silicon-carbide    | Silicon-carbide    | Diamond particle   | Diamond particle   |
| Grit/grain size        | #500               | #1000              | 9 µm               | 1 µm               |
| Lubricant              | Water              | Water              | DP-Green           | DP-Red             |
| Rotational speed [rpm] | 150                | 150                | 150                | 150                |
| Force [N]              | 5                  | 5                  | 10                 | 10                 |
| Time [min]             | 5 in ea. direction | 5 in ea. direction | 5 in ea. direction | 5 in ea. direction |
| Cleanser time [min]    | 10                 | 10                 | 10                 | 10                 |

microscope where the indentation area could be selected. A series of indentation positions were individually selected. In each tooth, 30 indentations were done on both enamel and dentine. The indentations in enamel for all eight teeth began 100 µm from the dento-enamel junction on the buccal aspect of the tooth. Half of the 30 indentations in enamel were conducted at a loading force up to 50 mN and the other half were up to a loading force of 150 mN. The distance between these indentations was 50 µm in both the *x*- and *y*-axis. To standardise the position of the testing site in dentine the indentations began approximately half way across the buccal aspect of the cut dentine surface. As dentine is softer than enamel the indentations in the dentine were physically much larger and consequently at 50 µm separation tended to overlap. To prevent this the indentations were made 100 µm apart in the *x*-axis and 75 µm apart in the *y*-axis. When ready to indent the indenter was lowered until the tip was about 1 mm from the specimen surface. The UMIS software lowered the indenter until a contact force of 0.2 mN was encountered. This was taken as the baseline datum point from which the load was gradually increased in 25 increments at a rate of one incremental increase per 0.1 s up to the maximum load (either 50 or 150 mN) where there was a delay of 30 s followed by the same incremental unload process. This protocol has been used in other studies [15]. Table 2 summarises the test parameters. Testing each tooth took approximately 6 h. There was some concern as to the possible drying effects during this period, however, earlier work showed that teeth can be dry for up to two days

without influencing the hardness and modulus of elasticity of either dentine or enamel [18].

Following completion of each test array the UMIS software calculated values for hardness as a function of penetration depth for each indentation. The UMIS program adjusts the data for penetration under the initial contact force (typically 0.2 mN) and makes corrections for indenter tip geometry. The indentations for each tooth at either 50 or 150 mN were averaged overall to give the hardness value for each tooth at 50 and 150 mN. The modulus of elasticity was recorded at the maximum penetration depth for each indentation and a mean value calculated for each tooth from the 15 indentations at each loading. Comparison of group means was carried out using Student's *t*-test and linear regression analysis was used to evaluate the relationship between hardness and elastic modulus of both enamel and dentine.

3. Results

Table 3 summarises the results for hardness and elastic modulus for both enamel and dentine. Whilst a total of 15 indentations were done under each load for both enamel and dentine for each tooth, between one and three indentations were subsequently discarded from each tooth. Data was discarded for two reasons; the indentation in enamel lay on dentino-enamel junction or outside the testing area or secondly cracking had occurred during the testing process and the data was unreliable.

The hardness of enamel between teeth under loads of 50 and 150 mN ranged from 4.66 ± 0.23 to 5.15 ± 0.53 (mean 4.88 ± 0.41 GPa) and 4.64 ± 0.22 to 5.12 ± 0.34 GPa (mean 4.87 ± 0.29 GPa), respectively. For dentine the hardness varied from 0.78 ± 0.07 to 1.07 ± 0.07 GPa (mean 0.95 ± 0.11 GPa) under a 50 mN load and 0.76 ± 0.03 to 1.07 ± 0.08 GPa (mean 0.90 ± 0.09 GPa) under 150 mN.

The modulus of elasticity for enamel ranged from 74.47 ± 2.85 to 99.94 ± 3.95 GPa (mean 80.94 ± 6.65 GPa) at 50 mN and 73.31 ± 3.53 to 95.15 ± 8.36 GPa (mean 79.77 ± 8.86 GPa) under the 150 mN load. For dentine under a load of 50 mN, the elastic modulus ranged from 18.23 ± 0.81 to 23.97 ± 0.72 GPa

Table 2  
The parameters assigned to the testing procedure

| Test parameters              |                                     |
|------------------------------|-------------------------------------|
| Indenter type                | Berkovich<br>(pyramidal triangular) |
| Contact force (mN)           | 0.2                                 |
| Max. indentation force (mN)  | 50 or 150                           |
| No. of increments            | 25                                  |
| Increment progression        | Square root                         |
| Dwell at load/max/unload (s) | 0.1/30.0/0.1                        |
| Array row size               | 3                                   |
| Array column size            | 17–35                               |
| Delay between locations (s)  | 30.0                                |
| Testing conditions           | 23 ± 1°C and (50 ± 10) %RH          |

Table 3

A summary of the mechanical properties of primary enamel and dentine

|                             | Enamel           | Dentine          |
|-----------------------------|------------------|------------------|
| Hardness (GPa)              | $4.88 \pm 0.35$  | $0.92 \pm 0.11$  |
| Modulus of elasticity (GPa) | $80.35 \pm 7.71$ | $19.89 \pm 1.92$ |

(mean  $20.55 \pm 2.00$  GPa) whilst under a load of 150 mN the elasticity was  $16.39 \pm 0.31$  to  $22.53 \pm 1.03$  GPa (mean  $19.22 \pm 1.84$  GPa). There was no significant difference between the mean values for either hardness or elasticity under loads of 50 mN compared with those tested under 150 mN ( $p < 0.01$ ).

Figs. 1 and 2 demonstrate the relationship between hardness and elastic modulus for enamel and dentine under a load of 50 mN. There was a statistically significant relationship ( $p < 0.01$ ) between the hardness and the elastic modulus for enamel ( $R^2 = 0.117$ ) and for dentine ( $R^2 = 0.477$ ). However, only under a load of 150 mN was there a statistically significant relationship ( $p < 0.05$ ) for dentine only ( $R^2 = 0.604$ ).

#### 4. Discussion

Conventional hardness testing methods requires direct imaging, usually visual measuring, of the width or area of the indentations, to obtain hardness. Large errors are introduced with such testing, particularly with measurement of the length of the diagonals at a small scale as there are difficulties in focusing on both ends of the diagonal. Other problems encountered include tester error in loading, rate of load application, duration of a contact period, and impact. Hence, the use of depth-sensing instruments offer advantages over the conventional method by providing high

resolution, repeatability, and the ability to obtain information about the elastic and time-dependent plastic properties of the material [13]. As only very small loads are required, the UMIS also offers the advantage of being able to space the indentations close together, enabling more tests to be conducted on an individual sample as well as creating a more standardised test area.

In this study approximately 15% of the enamel test indentations were discarded, which is similar to other studies [11]. In the present study, the results from the indentations were discarded if they caused surface cracking or if the indentations were positioned out of the area being tested. Surface damage affecting micro-indentation results have been noted in other studies [1]. Although no surface damage was noted in the dentine, approximately 7% of the indentations in dentine were discarded as they fell upon surface imperfections. Such indentations gave force–displacement curves that varied from the mean by values greater than two standard deviations from the overall test indentation mean.

In both dentine and enamel, the actual values for both the hardness and modulus of elasticity were not found to vary significantly in relation to the load applied. Other researchers have reported that microhardness data are a function of the applied load [19]. Ryge and co-workers concluded that hardness is load dependant for all the materials that they tested, including dentine and enamel [12]. However, the loads applied in the present study are much lower than previously reported. It was noted that the load of 150 mN proved to be slightly less satisfactory as there was more evidence of significant cracking especially in enamel.

The hardness of enamel in primary teeth has been studied before, however it is difficult to make comparisons as the early studies used relative hardness or Knoop hardness numbers to describe their results [17,20]. However values

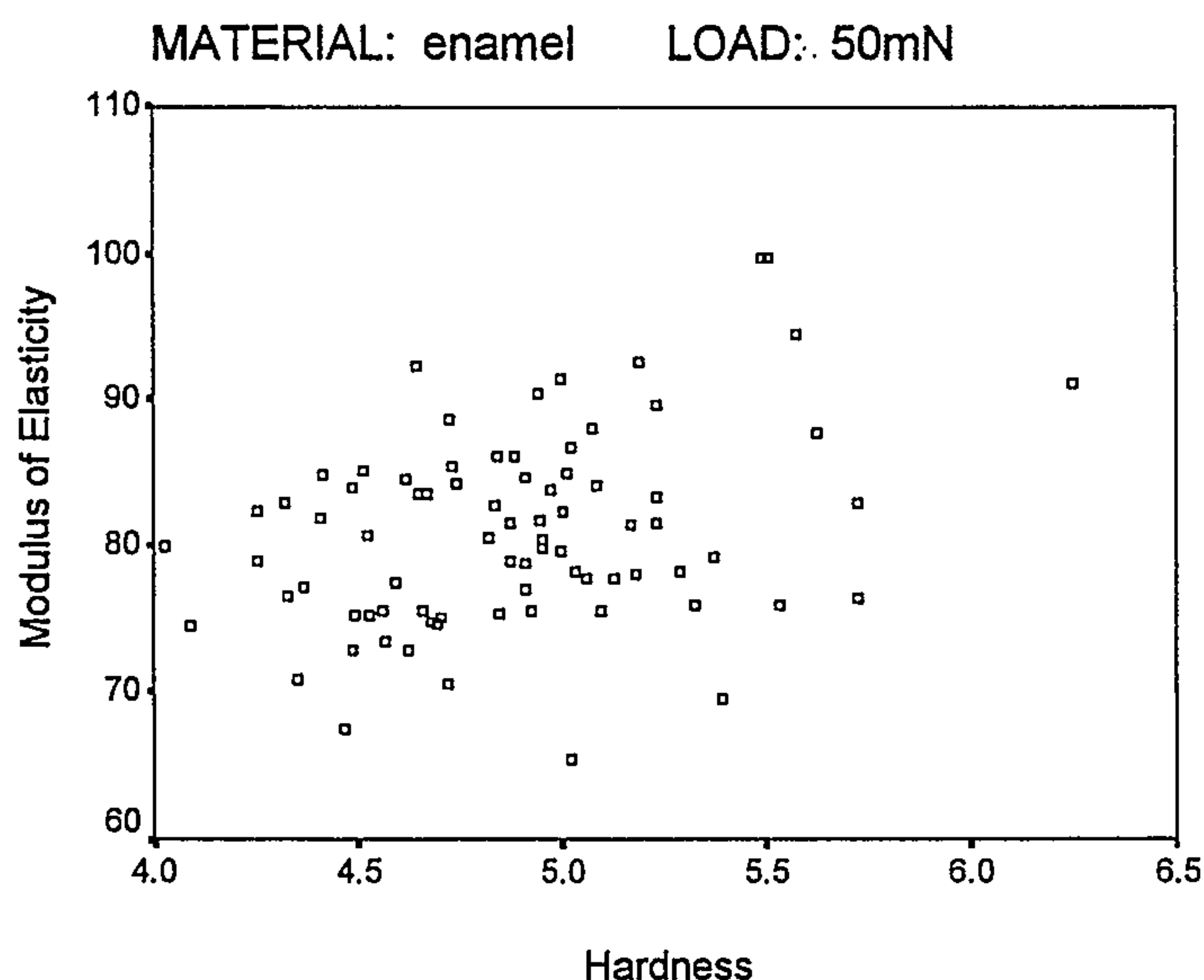


Fig. 1. Correlation between hardness and modulus of elasticity for primary enamel (Modulus of elasticity =  $53.77 \pm 5.56 \times \text{Hardness}$   $R^2 = 0.117$ ,  $p < 0.001$ ).

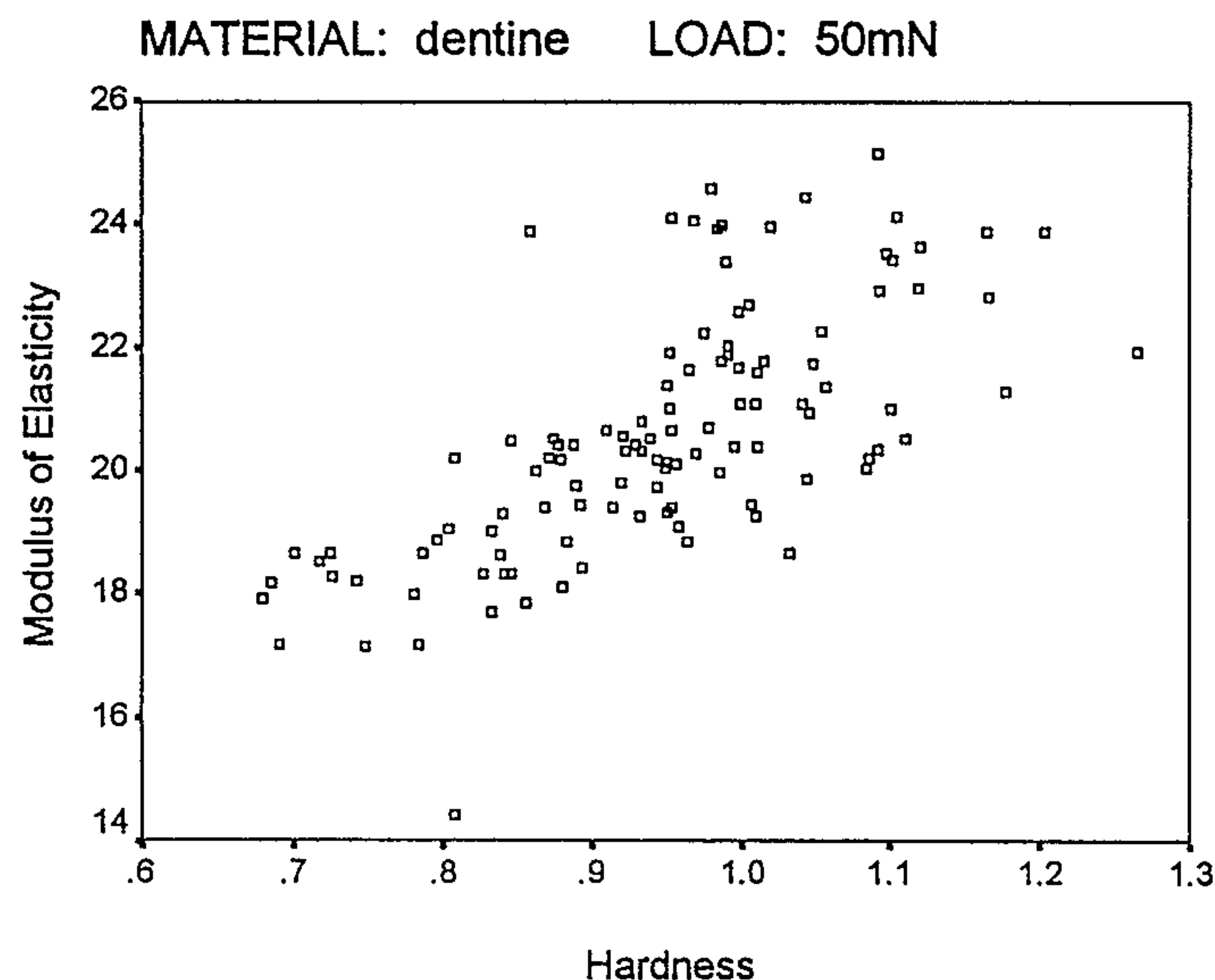


Fig. 2. Correlation between hardness and modulus of elasticity in primary dentine. (Modulus of elasticity =  $9.56 \pm 11.63 \times \text{Hardness}$   $R^2 = 0.477$ ,  $p < 0.001$ ).

of  $3.62 \pm 0.2$  GPa and  $3.37 \pm 0.15$  GPa for both axial and occlusal enamel, respectively, in permanent teeth have been reported recently [6]. These values are lower than those reported by Poolthong also for permanent teeth using the same test method as used in the current study [18]. The permanent teeth in that study had slightly softer enamel (4.45–4.74 GPa) when compared with the primary enamel in this study ( $4.88 \pm 0.35$  GPa).

No published data exists on the hardness of dentine in primary teeth however the results reported in this study,  $0.92 \pm 0.11$  GPa are comparable to those reported for permanent dentine, 0.57–1.13 GPa [6,18].

The modulus of elasticity for permanent dentine has been reported at between 17.6–22.9 GPa [6,18]. However there are conflicting reports as to whether the reported elastic properties of dentine depend on the orientation of the cut surface tested and on what exactly is tested [6,21]. A modified atomic force microscope was used to measure the hardness and modulus of elasticity of peritubular and intertubular dentine [2]. The mean modulus of elasticity of peritubular dentine was  $29.8 \pm 8.9$  GPa and did not depend on the site of indentation. In contrast the mean modulus of elasticity of intertubular dentine did vary between test site and was lower than the modulus of peritubular dentine. This finding is not surprising as peritubular dentine has been reported to have a higher concentration of calcium, phosphate and magnesium ions [22]. In the present study all teeth were tested under the same orientation conditions to control this variable.

The modulus of elasticity of enamel is also influenced by the orientation of the indentations. Xu and co-workers [6] reported that the modulus of elasticity for occlusal permanent enamel was significantly higher at  $94 \pm 5$  GPa than axial enamel at  $80 \pm 4$  GPa. The eight primary teeth in the current study were sectioned horizontally and the

modulus of elasticity of the axial enamel in the present study calculated to be  $80.35 \pm 7.71$  GPa, which is very similar to previously reported values.

Throughout this paper the mean values for the hardness of enamel and dentine have been quoted. It is obvious, however, that results of individual teeth vary in both enamel (4.66–5.15 GPa) and dentine (0.76–1.12 GPa). Such differences may be explained in several ways. Firstly in the case of the dentine it is very difficult to test the same location in each tooth as an arbitrary distance ‘half way’ between the CEJ and pulp chamber was selected. Secondly, although the test sites were crack and scratch free, minor surface and subsurface imperfections not evident under the microscope examination may have been encountered whilst testing causing variation in results. Differences may also arise in the hardness of teeth due to exposure of individuals to different environmental factors during the time of tooth formation and mineralisation. It is widely known that fluoride exposure can influence the hardness of the enamel both during formation and post eruptively. It would be very difficult to eliminate such variation from this study. Interestingly there was also some variation in the hardness within each tooth. There appears to be considerable variation in the fracture toughness of enamel within the same tooth [23]. This may arise as a result of variation in mineralisation of the tooth crown.

The present study shows a weak but positive correlation between the modulus of elasticity and the hardness of both enamel and dentine. This relationship is seen clearly for both enamel and dentine in Figs. 1 and 2. Previous studies on a wide range of calcified tissues have established that the modulus of elasticity correlates directly with the calcium mineral content per unit volume of a calcified material [24]. The positive correlation between hardness and modulus of elasticity of the enamel and dentine suggest

that hardness as well as modulus may correlate with calcium mineral content. This aspect warrants further work and may provide an insight in to the influence of demineralising substrates on the properties of dental hard tissue.

## 5. Conclusion

The present study uses an Ultra-Micro-Indentation System to provide baseline data on the mechanical properties of primary teeth about which little is known. Such data may assist in predicting the pattern of behaviour of the primary dentition under loads and at the tooth/restoration interface. Conventional methods to test the hardness and modulus of elasticity have many limitations. The Ultra-Micro-Indentation System is a reproducible and reliable system of measuring hardness and modulus of elasticity with relative ease. It also offers significant potential to measure the effect of erosive substances on the surface properties of enamel and dentine. This is the subject of ongoing research.

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## Appendix B.

Atomic absorption spectroscopy (AAS) Varian AA-800. Laboratory manual from Chemistry 2, University of Sydney.

# ATOMIC ABSORPTION SPECTROSCOPY (AAS)

## VARIAN AA-800

*Time required* < 3 hr

*Marks for this experiment* = 3

**Objectives:** To obtain a basic understanding of the operation of the instrument, produce calibration graphs and determine the concentrations of calcium in "unknowns".

The results also provide an insight into interference by combination, a type of interference commonly encountered in analysis by AAS; and show how this unwanted effect is reduced by the addition of edta (as a releasing agent).

You will use the Varian AA-800, located in Room 423, and carry out the tasks listed below.

### 1. *Operation of the instrument*

There is a folder near the instrument giving the steps involved in setting up the instrument, calibrating it, measuring "unknowns", printing the results and shutting down. *Use stored program number 7.*

### 2. *Calibration and measurements*

- i. Run the standard Ca solutions provided, without additives (SET 1). These contain 0.00, 1.00, 2.00, 3.00, 4.00, and 5.00 mg Ca L<sup>-1</sup> (ppm Ca). Produce a calibration graph using a suitable algorithm (linear, quadratic, cubic or new rational - find out what new rational means). Then run the "unknown" Ca solution A which contains Ca<sup>2+</sup> and orthophosphate ions. Print out the results and the calibration graph.
- ii. Now use the Ca standards containing Ca and 7500 ppm edta (SET 2). Measure "unknown" B which contains the Ca<sup>2+</sup> and orthophosphate ions plus 7500 ppm edta. Print out the results and calibration graph; and also a copy of the REVIEW METHOD page which gives the instrument operating conditions.
- iii. On the sheet provided, record the apparent concentrations of Ca<sup>2+</sup> ions in A and B and briefly comment on the results. Submit this sheet, stapled with the instrument print-outs.

### 3. *References*

1. Vogel's Quantitative Analytical Chemistry, 4th edn., pp. 821-824 (on interferences in flame spectroscopy).

*Copies of this reference (Folder 242) is available on 2-day loan from the Service Room.*

## Appendix C.

Fluoride determination using a ion-selective electrode. Laboratory manual from Chemistry 2, University of Sydney.

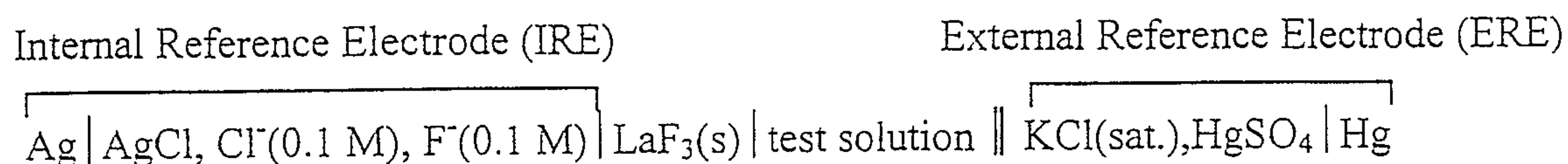
## EXPERIMENT 2-2

### FLUORIDE DETERMINATION USING AN ION-SELECTIVE ELECTRODE

In this experiment the fluoride ion concentration in an issued solution will be determined by potentiometry using a fluoride ion-selective electrode.

#### BACKGROUND

A typical cell arrangement for the potentiometric determination of fluoride ion is as follows:



This comprises two reference electrodes (silver-silver chloride and the saturated calomel electrode), the lanthanum fluoride sensor crystal and the test solution, all in series. The emf for this cell arrangement is given by:

$$E = E_{\text{IRE}} + E_{\text{LaF}_3} + E_{\text{JP}} + E_{\text{ERE}}$$

The first, third and fourth terms are respectively the emf's of the silver-silver chloride electrode, the junction potential, and the calomel electrode potential and are constant at a given temperature. The second term is the potential which develops across the lanthanum fluoride crystal. The overall cell potential is therefore controlled by the activity of fluoride in the test solution and is given by the Nernst equation:

$$E = K + \frac{RT}{nF} \ln(a\text{F}^-)$$

where K is a constant, R is the gas constant, F is the Faraday constant, T is the temperature,  $a\text{F}^-$  is the activity of the fluoride ion, and n is -1 for fluoride.

*Under what conditions can the activity of the fluoride ion be approximated by its concentration?*

Under these conditions,

$$E = K + \frac{RT}{nF} \ln[F^-]$$

where  $[F^-]$  is the concentration of the fluoride ion (in mol L<sup>-1</sup>).

Nernstian behaviour of the fluoride electrode cannot be assumed since deviations occur depending on the state of the electrode and the solution conditions. It is therefore necessary to calibrate the electrode. This is done by plotting  $E$  (in mV) for a series of standard fluoride solutions against  $\ln[F^-]$  and obtaining a least squares estimate of the slope ( $S = -RT/F$ ).

$E$  (in mV) is then measured for a series of solutions prepared by the *standard addition* of fluoride to the UNKNOWN. The concentration of *added* fluoride is plotted against  $e^{ES}$  and the concentration of fluoride in the UNKNOWN obtained as the least squares estimate of the intercept for  $e^{ES} = 0$ .

*Satisfy yourself that you understand the basis for this determination.*

## PREPARATION

1. Obtain from the Service Room your unknown solution and a fluoride kit, containing two plastic beakers and standard (10,000 ppm) fluoride solution.
2. Wash the beakers with distilled water and acetone and then dry them with a hair drier.
3. Pipette 100.0 mL of distilled water into the plastic beaker marked STANDARD.
4. Quantitatively transfer your unknown solution to a 500 mL volumetric flask and make up to volume with distilled water.
5. Pipette 100.0 mL of the diluted unknown solution into the plastic beaker marked UNKNOWN.  
*Once this solution has been diluted, it is important that it is immediately pipetted into the plastic beaker.*
6. Consult a demonstrator who will explain the use of the equipment required for the experiment, situated in the Second Year instrument room (Room 440/441).
7. After you have obtained the results for both the STANDARD and the UNKNOWN, a demonstrator will assist you to plot the results on the computer.

## PROCEDURE

### Calibration

Using a HANNA H1 8521 Digital pH-mV Meter, determine the slope for the electrode by the following procedure:

Carefully remove the rubber cap from the reference electrode before starting.

1. Rinse the electrodes well with distilled water and dry them **gently** with tissue. Turn the meter on by pressing the ON/OFF key once, then press the RANGE key twice; mV should now appear on the digital readout.
2. To the beaker marked STANDARD which contains 100.0 mL of distilled water, add 5.0 mL of buffer solution by pipette, immerse the electrodes in the solution, approximately 1-2 cm below the surface, then start the magnetic stirrer. Record the mV reading in the appropriate column of the result sheet. Using a precision micropipette, add 0.1 mL of the standard solution (10,000 ppm  $F^-$ ), taking care that there are no air bubbles in the pipette and that no drops adhere to the outer surface. Add the aliquot to the centre of the solution. Record mV reading.

Consult a Demonstrator before using the precision micropipette for the first time.

Do not allow the plunger to snap back as this will draw solution into the body of the pipette.

3. Add a further 0.10 mL of the standard solution; repeat six times, each time adding a 0.1 mL aliquot of standard solution. Note mV readings (the first two readings are usually unstable). Consult a demonstrator and use the computer in Room 440/441 to draw a graph, plotting mV against  $\ln(\text{fluoride ion concentration added})$ , *disregarding the first two readings and that of the standard solution*. The slope of this graph ( $RT/nF$ ) is S, which should be negative. Correct electrode operation is indicated by a value for S of  $-25.5 \pm 1$  mV, assuming the solution temperature is between 20° and 25°C. If the slope differs from this by more than  $\pm 1$  mV, repeat the experiment.
4. Switch the meter to OFF, raise the electrodes and rinse with distilled water.

### Standard addition

5. To the beaker marked UNKNOWN, containing 100.0 mL of the diluted unknown solution, add 5.0 mL of buffer solution

*The buffer solution contains 1 M NaCl and an acetate buffer pH~5. What is the role of each of these components?*

6. Place a clean, dry magnetic stirrer bar in the solution, then place the beaker onto the stirrer. Dry the electrodes by patting them gently with tissue paper, dip them approximately 1-2 cm below the solution surface, then start the stirrer. Select the mV mode of the meter. When the meter reading (in mV) has stabilised, record this as the zero-point reading.
7. Add **accurately**, using a precision micropipette (as in calibration step 2), 0.1 mL of the standard fluoride solution supplied (10,000 ppm). Record the meter reading after it has again stabilised. Now add 5 further 0.1 mL aliquots of standard solution, reading the meter after each addition as before. Record your mV readings on the result sheet.
8. Select the OFF position on the meter.
9. Raise electrodes, rinse several times with distilled water.

Carefully replace the rubber cap on the reference electrode when finished.

## CALCULATIONS

Use a computer to plot the concentration of added fluoride (in ppm) against  $e^{E/S}$ . The intercept for  $e^{E/S} = 0$  is the concentration of fluoride in the solution obtained by adding 5.0 mL of buffer to 100.0 mL of the diluted UNKNOWN.

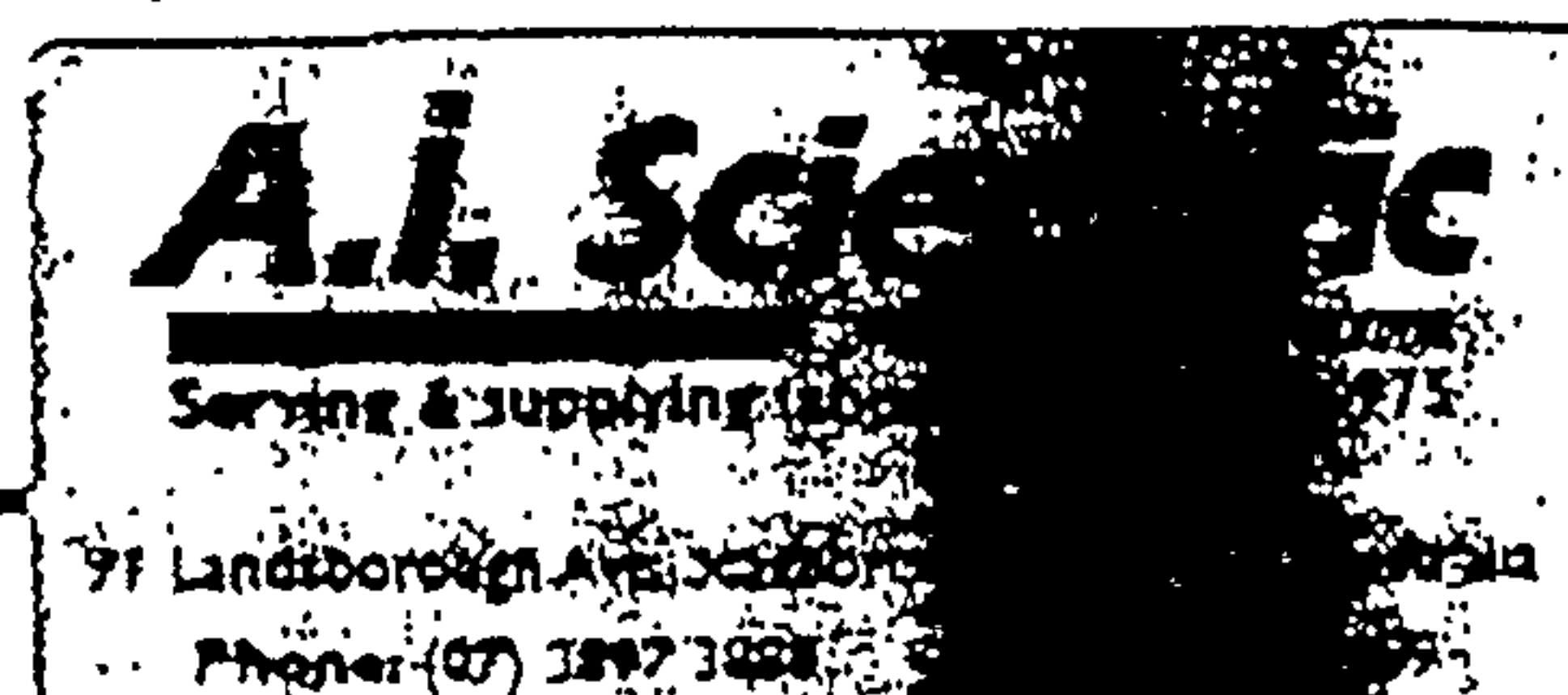
From this intercept, calculate the amount of fluoride issued (expressed as grams of NaF).

## REFERENCES

- J.S. Fritz and G.H. Schenk, *Quantitative Analytical Chemistry*, 5th ed., Ch. 16.  
G.D. Christian and J.E. O'Reilly, *Instrumental Analysis*, 2nd ed., Ch. 2.  
Vogel's *Textbook of Quantitative Inorganic analysis*, 4th ed., Ch. 14.  
APHA-AWWA&WPCE(USA) *Standard Methods for the Examination of Water and Wastewater*, 14th ed., p.387.

## Appendix D.

Determination of inorganic ions and organic acid in non alcoholic carbonated beverages. Instruction sheet. DIONEX Corporation.

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DIONEX

## Application Note 25

# Determination of Inorganic Ions and Organic Acids in Non-Alcoholic Carbonated Beverages

## INTRODUCTION

The determination of inorganic anions and cations and organic acids in non-alcoholic carbonated beverages is of importance from both health related and manufacturing perspectives. Organic acids such as citrate and malate, and inorganic anions such as phosphate are monitored due to their function as acidifiers or flavor enhancers. Chloride is monitored due to restrictions imposed by different countries and many Group I and II metals are monitored for purposes of mass balance. Thus, the content of these compounds needs to be monitored by the manufacturer to maintain product quality and to investigate possible patent infringements in competitive products.

Ion chromatography (IC) is a well established technique for the determination of ions in solution. This application note describes the use of ion exchange or ion exclusion chromatography (ICE) with suppressed conductivity detection for the determination of inorganic anions, cations, and organic acids in several popular carbonated beverages.

## EQUIPMENT

A Dionex chromatographic system consisting of:

- Gradient Pump
- Chromatography Module
- Conductivity Detector
- Eluent Organizer or Eluent Degas Module
- Autosampler

Dionex PeakNet Chromatography Workstation

## REAGENTS AND STANDARDS

Deionized water (DI H<sub>2</sub>O), 17.8 MΩ-cm or better

### Anion Analysis

Sodium hydroxide solution, 50% w/w (Fisher Scientific)

Methanol (EM Science)

### Cation Analysis

Methanesulfonic acid, >99% pure (Fluka Chemika-BioChemika)

### Organic Acid Analysis

Perchloric acid (Fluka Chemika-BioChemika)

Tetrabutylammonium hydroxide (Dionex, F/N 39602)

## PREPARATION OF SOLUTIONS AND REAGENTS

### 100 mM Sodium Hydroxide

Weigh 992 g (992 mL) of 17.8 MΩ deionized water into a 1-L eluent reservoir bottle. Vacuum degas the water for approximately 10 minutes. Tare the bottle on the balance and add 8.00 g (5.25 mL) of 50% sodium hydroxide directly to the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium.

### 1 mM Sodium Hydroxide

Place 990 g (990 mL) of 17.8 MΩ deionized water into a 1-L eluent reservoir bottle. Vacuum degas the water for approximately 10 minutes. Add 10 mL of 100 mM sodium hydroxide directly to the bottle. Quickly transfer the eluent bottle to the instrument and pressurize it with helium.

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#### 100 mM Methanesulfonic Acid

Weigh out 9.61 g of methanesulfonic acid (MSA). Carefully add this amount to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to the mark and mix thoroughly.

#### 0.8 mM Perfluorobutyric Acid

Perfluorobutyric acid (heptafluorobutyric acid) is supplied by Fluka in 10.0-mL bottles. Dilute the entire contents of one 10.0-mL bottle in 1 L to obtain a 0.0772 M stock solution. Dilute 10.4 g of the stock solution in 1 L to obtain the 0.8 mM working eluent.

#### 5 mM Tetrabutylammonium Hydroxide

Dilute 200 mL of the Dionex 0.1 M tetrabutyl ammonium hydroxide regenerant solution (P/N 39602) to 4 L with water. Alternatively, dilute 10 mL of 55% tetrabutylammonium hydroxide in 4 L of water.

### RESULTS AND DISCUSSION

#### Inorganic Anions

Inorganic anions such as chloride, nitrate, and sulfate present in carbonated beverages are usually derived from the water used in production. Some anions, however, such as phosphate may be added deliberately to impart a particular flavor or acidity. The water can be monitored by ion chromatography to ensure purity and consistency, while the final product is monitored to maintain product quality.

Inorganic anions are separated by anion-exchange chromatography, and monitored by suppressed conductivity detection; Table 1 lists the experimental conditions. When performing gradient elution on the AS11 column, a hydroxide eluent system is used instead of a carbonate eluent, because of its lower background conductivity. An Anion Trap Column (ATC) should be installed between the gradient pump and the injection valve to minimize baseline shifts resulting from the elution of anionic contaminants in the eluent.

Figures 1-4 show the separations of inorganic and organic anions in a variety of carbonated beverages using the AS11 column. The samples were degassed and diluted 1:10 prior to injection. The sodium hydroxide concentration in eluent 1 is weak enough that not only is fluoride eluted after the void, but several weakly retained monovalent organic acids are also resolved. Thus, using the conditions described in Table 1, it is

possible to separate not only the strong acid anions, but also a variety of weak organic acids. To obtain a flat baseline for these chromatograms, the baseline subtraction option in the PeakNet software was used.

Figures 1, 2, and 4 show that phosphate or citrate was used to acidify the beverages. Figure 3, which shows the separation of anions in a flavored carbonated water, naturally has no phosphate or citrate. All four beverages contain chloride and sulfate, with all but the water also containing some nitrate. A small amount of fluoride,

|              |                                                                               |    |    |    |
|--------------|-------------------------------------------------------------------------------|----|----|----|
| Column:      | IonPac AS11 Analytical (4 mm)<br>IonPac AG11 Guard (4 mm)<br>ATC-1 Anion trap |    |    |    |
| Eluent 1:    | Deionized water                                                               |    |    |    |
| Eluent 2:    | 1 mM Sodium hydroxide                                                         |    |    |    |
| Eluent 3:    | 100 mM Sodium hydroxide                                                       |    |    |    |
| Eluent 4:    | Methanol                                                                      |    |    |    |
| Gradient:    | Time                                                                          | E1 | E2 | E3 |
|              | Initial                                                                       | 80 | 20 | —  |
|              | 0.00                                                                          | 80 | 20 | —  |
|              | 5.00                                                                          | 66 | 20 | 14 |
|              | 18.00                                                                         | 42 | —  | 38 |
| Flow Rate:   | 2 ml/min                                                                      |    |    |    |
| inj. Volume: | 25 µL                                                                         |    |    |    |
| Detection:   | Suppressed conductivity, ASRS, recycle mode                                   |    |    |    |

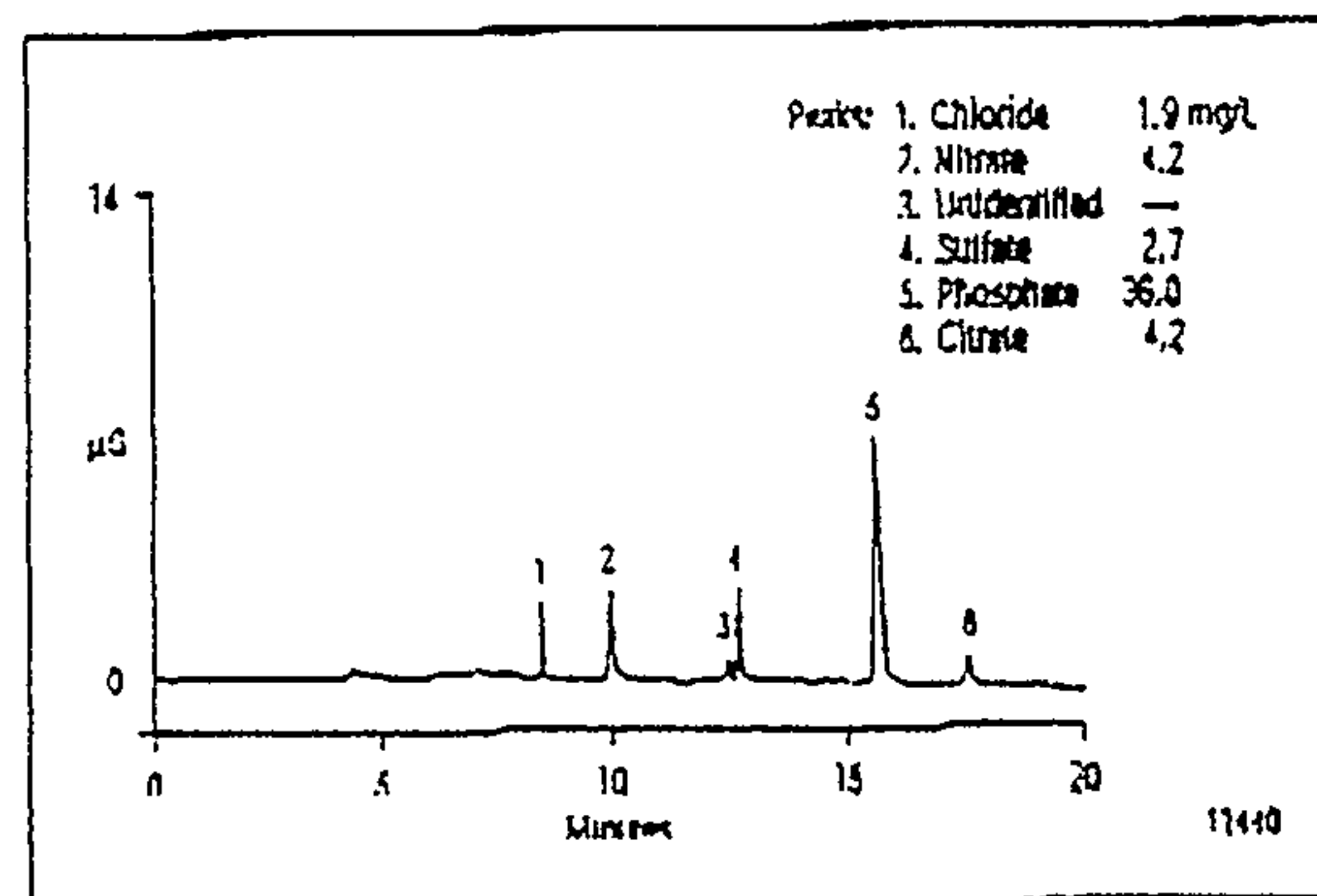


Figure 1 Separation of inorganic anions and organic acids in a cola by anion exchange chromatography. Conditions as listed in Table 1.

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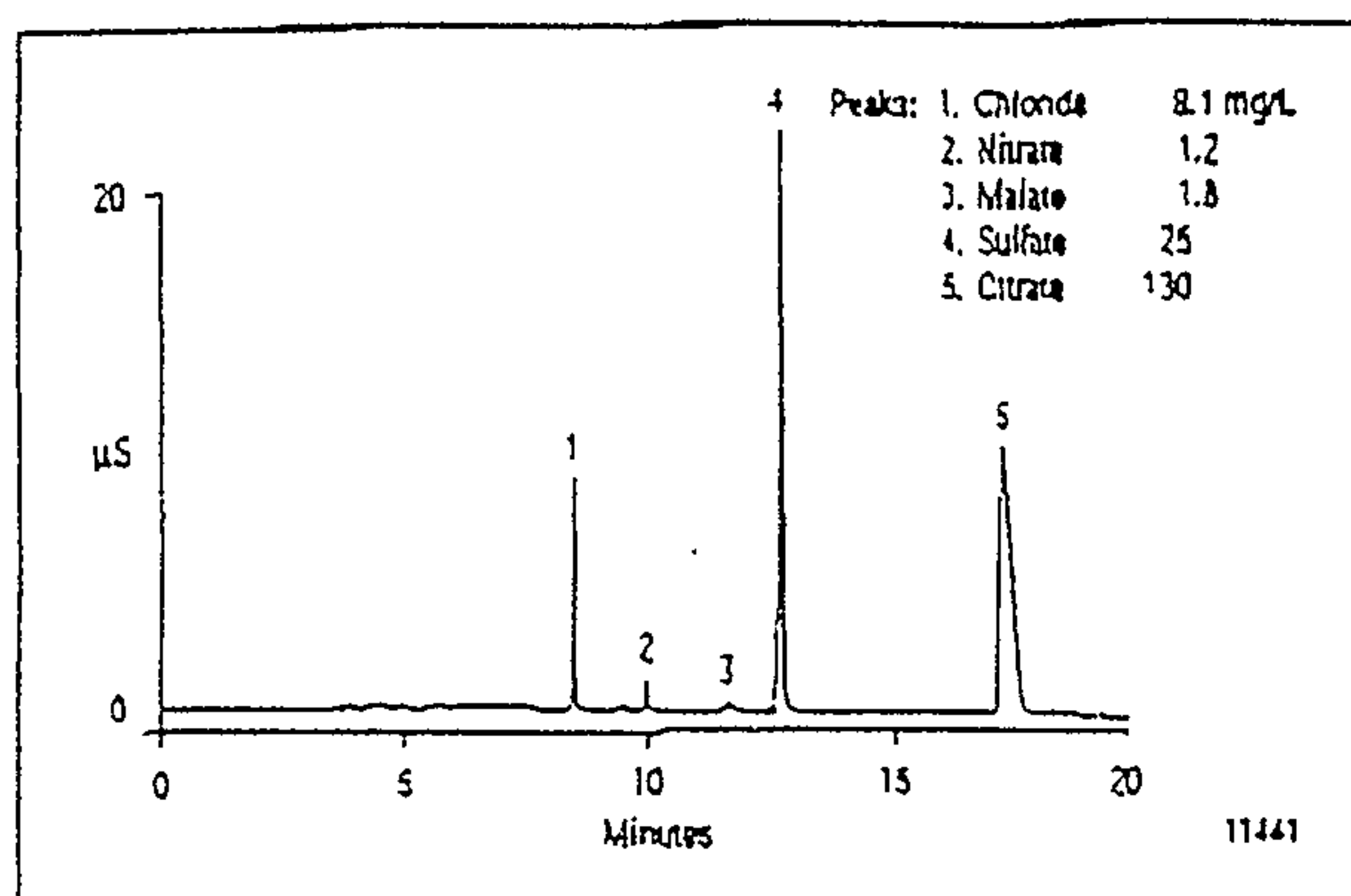


Figure 2 Separation of inorganic anions and organic acids in a carbonated lemon drink by anion exchange chromatography. Conditions as listed in Table 1.

which is sometimes added to municipal water supplies to prevent tooth decay, is also found in the synthetic grape flavored beverage.

Reproducibility for this method is on the order of 0.5% or better for retention times and 2% or better for peak areas. Linearity is good over the range tested (1.5 orders of magnitude), with a coefficient of determination,  $r^2 = 0.999$  for most of the analytes. These statistics were determined prior to baseline subtraction.

#### Inorganic Cations

As is the case with the inorganic anions, many inorganic cations are introduced into carbonated beverages from the water. Others are introduced as counterions to added ingredients. The four major cations in carbonated beverages are sodium, potassium, calcium, and magnesium.

Inorganic cations can be separated by ion exchange chromatography, and monitored by suppressed conductivity detection, as described in Table 2. The step gradient allows the separation of barium and strontium in addition to the standard 5 cations, sodium, ammonium, potassium, magnesium, and calcium. A step change at 5 minutes from the weak eluent to a stronger one allows for the elution of sharp peaks for the divalent cations. If it is not necessary to monitor for barium or strontium, the conditions can be changed to allow isocratic elution of the 5 cations shown in Figure 7, in less than 10 minutes. For isocratic elution, the eluent is 20 mM methanesulfonic acid.

Figures 5–8 show the separation of cations in a series of carbonated beverages by cation exchange chromatography. The samples were degassed and diluted

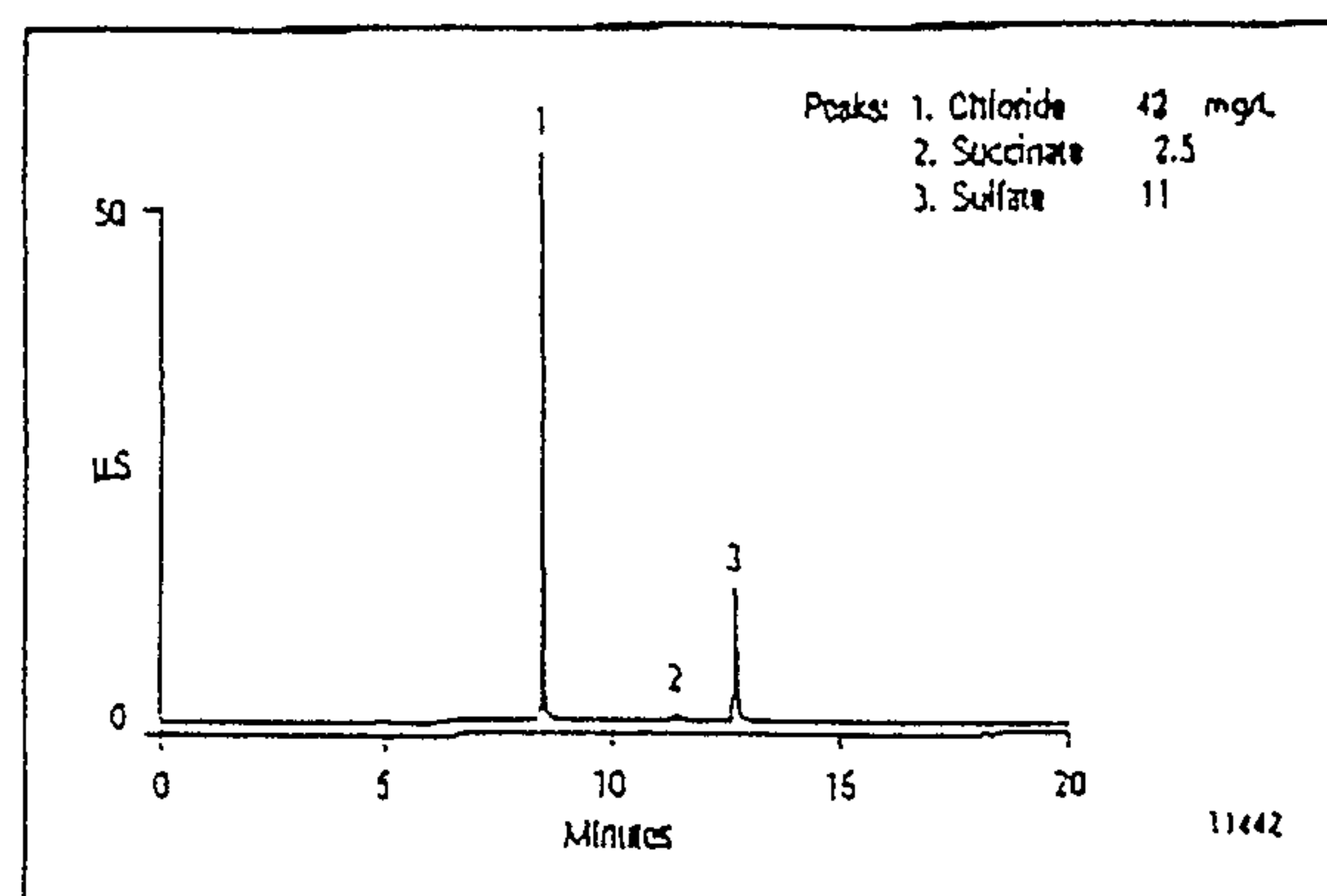


Figure 3 Separation of inorganic anions and organic acids in a flavored carbonated water by anion exchange chromatography. Conditions as listed in Table 1.

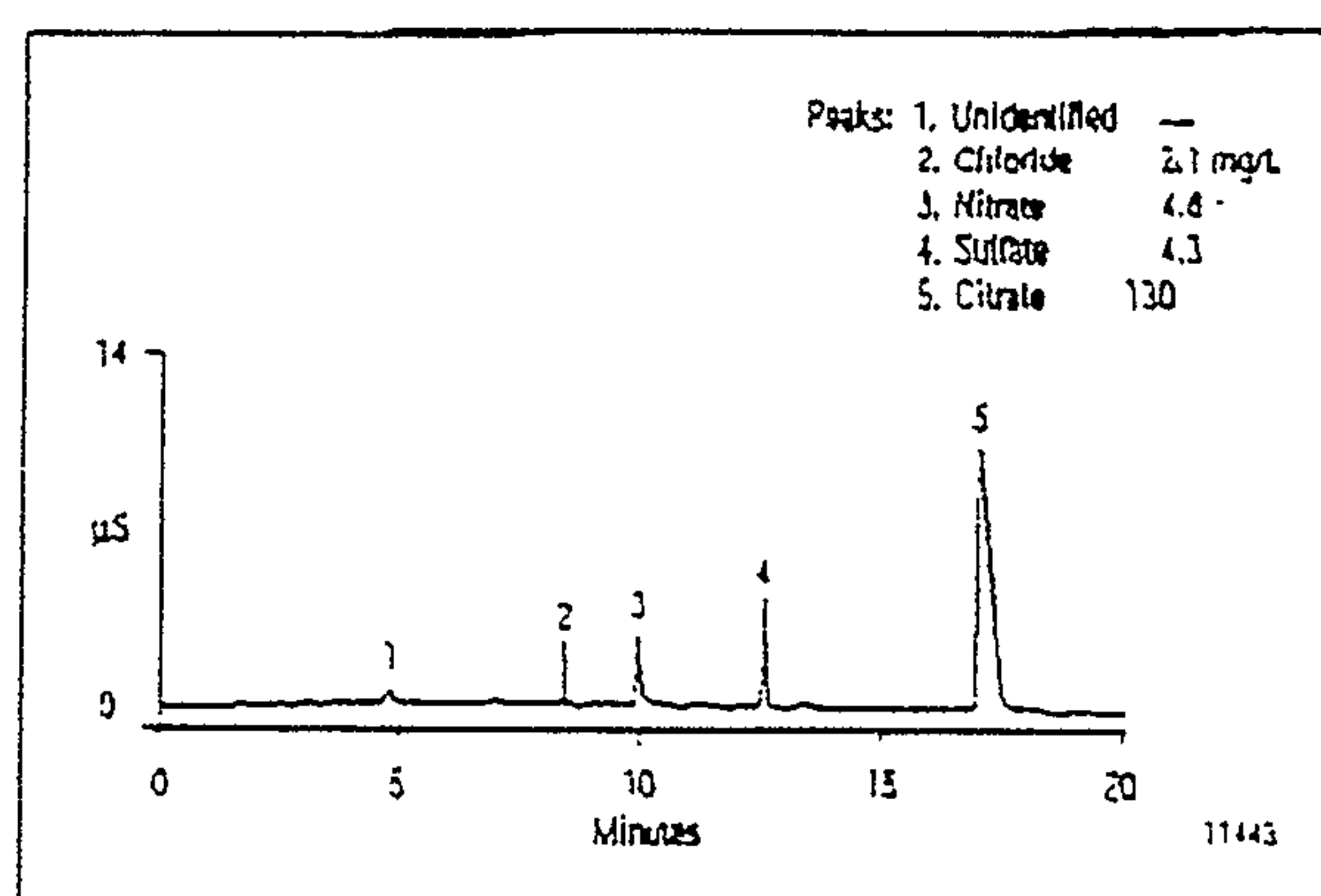


Figure 4 Separation of inorganic anions and organic acids in a carbonated synthetic grape drink by anion exchange chromatography. Conditions as listed in Table 1.

|              |                                                                                |    |    |
|--------------|--------------------------------------------------------------------------------|----|----|
| Column:      | IonPac CS12 Analytical (4 mm)<br>IonPac CG12 Guard (4 mm)<br>CTC-1 Cation trap |    |    |
| Eluent 1:    | Deionized water                                                                |    |    |
| Eluent 2:    | 100 mM Methanesulfonic acid                                                    |    |    |
| Gradient:    | Time                                                                           | E1 | E2 |
|              | Initial                                                                        | 84 | 16 |
|              | 5.00                                                                           | 84 | 16 |
|              | 5.01                                                                           | 60 | 40 |
|              | 10.00                                                                          | 60 | 40 |
| Flow Rate:   | 1.0 mL/min                                                                     |    |    |
| Inj. Volume: | 25 µL                                                                          |    |    |
| Detection:   | Suppressed conductivity, CSRS, recycle mode                                    |    |    |

Column: IonPac ICE-AS6 Analytical  
 Eluent: 0.8 mM Heptafluorobutyric acid  
 Flow Rate: 1.0 mL/min  
 Inj. Volume: 25  $\mu$ L  
 Detection: Suppressed conductivity, AMMS-ICE  
 Regenerant: 5 mM Tetrabutylammonium hydroxide at 5 mL/min

other monoprotic acids can be used as eluents, to do so will increase both the background conductivity and the noise. The experimental conditions are listed in Table 3.

Figures 9–12 show the separation of organic acids in a group of carbonated beverages by ion exclusion chromatography. The samples were degassed and diluted 1:10 (1:50 for the carbonated grape juice) prior to injection. Inorganic anions are eluted on either side of the water dip and do not interfere with the separation of most of the organic acids. The major exception is with oxalate, which is also eluted at the water dip. Figure 9 shows the organic acid profile in a flavored carbonated water, which contains no organic acids as expected. Figures 10 and 11 show the organic acid profiles in two synthetic carbonated beverages with only citrate being readily apparent. Citrate is often added to carbonated beverages to impart a certain acidity, but is also present in citric fruit; thus, explaining the higher concentration of citrate in the carbonated lemon drink compared to the cola. Figure 12 shows the organic acid profile in a carbonated apple juice. There are many different organic acids present naturally in apples, malate being particularly prevalent.

### CONCLUSION

IC has been applied successfully to the analysis of carbonated beverages for a variety of inorganic and organic components. Total analysis time is approximately 20 minutes for ICE analysis, 30 minutes for anion analysis, and 16 minutes for cation analysis due to the need for column reequilibration. Minimal sample preparation is required. The three methods described in this application note provide a rapid and convenient means to obtain complete profiles of the ionic components in carbonated beverages.

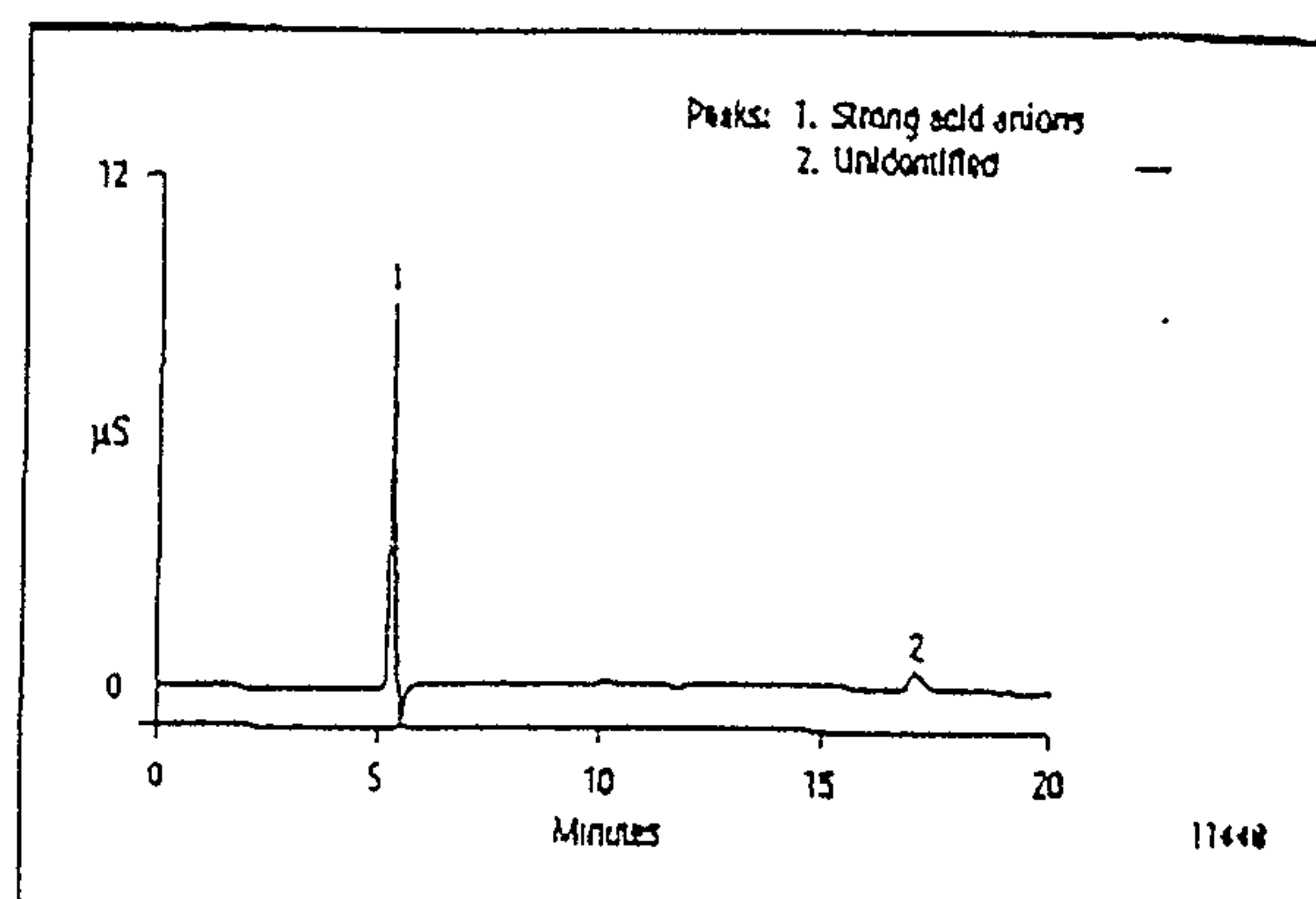


Figure 9 Anionic profile of a flavored carbonated water by ion exclusion chromatography. Conditions as listed in Table 3.

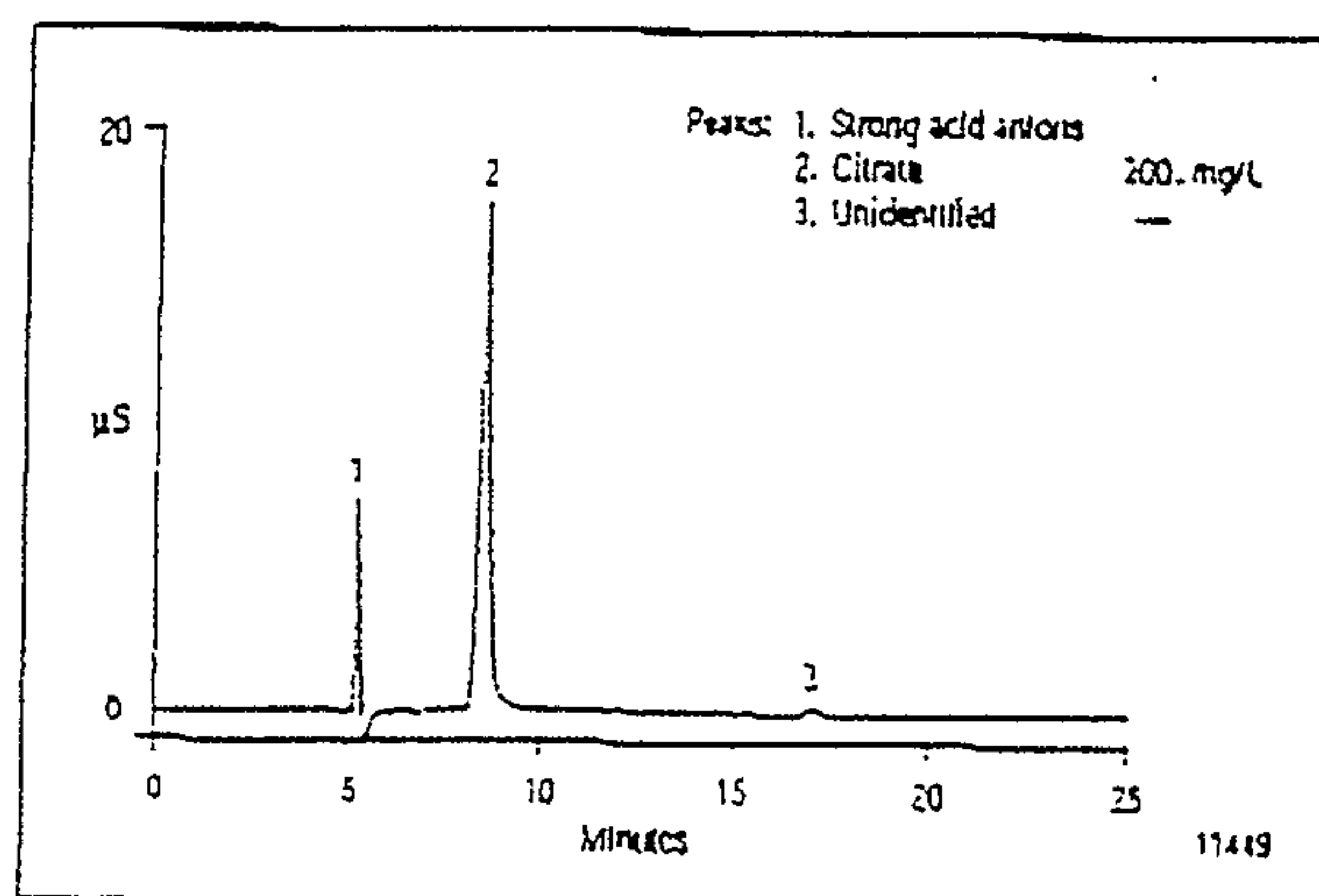


Figure 10 Separation of organic acids in a carbonated lemon drink by ion exclusion chromatography. Conditions as listed in Table 3.

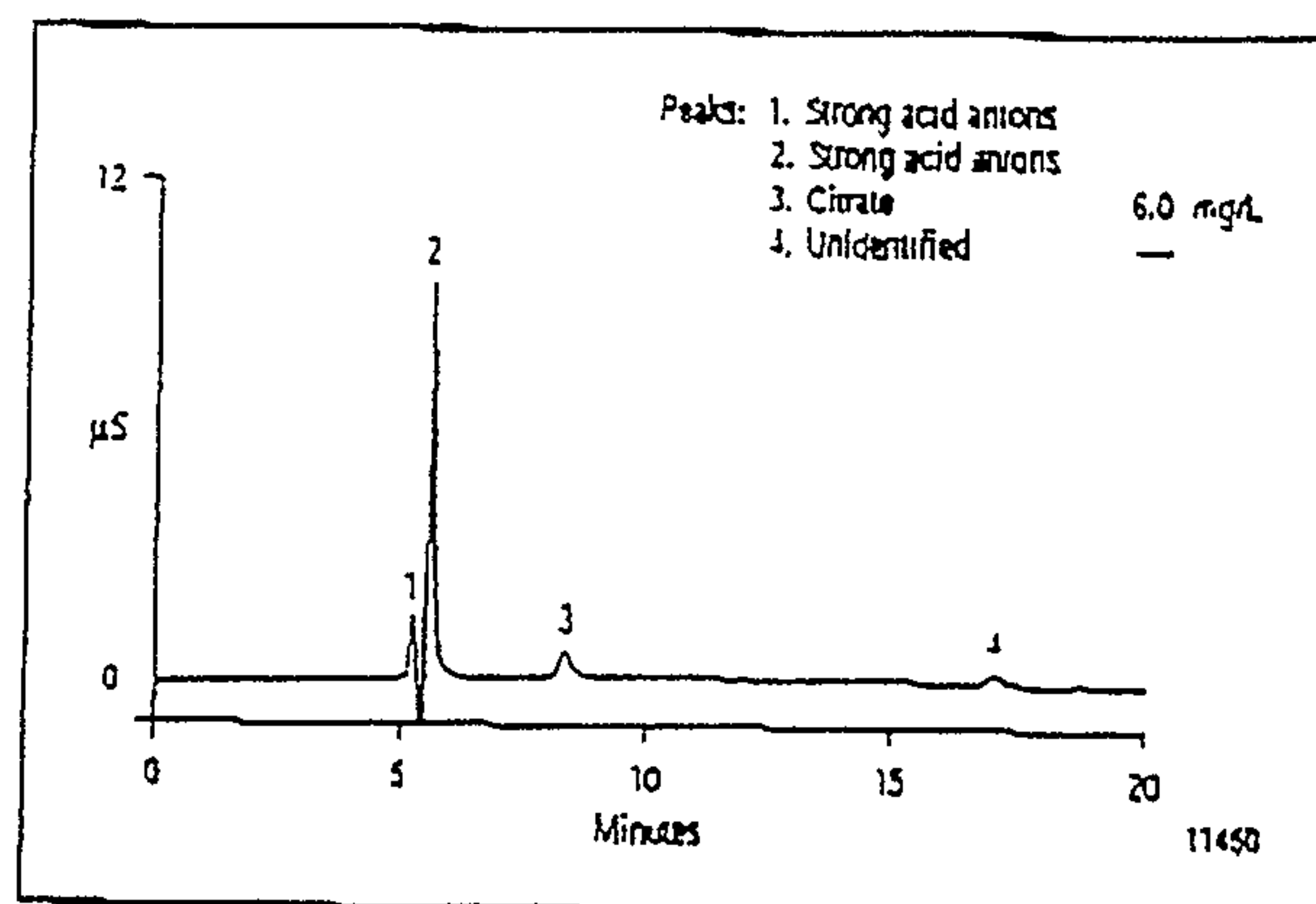
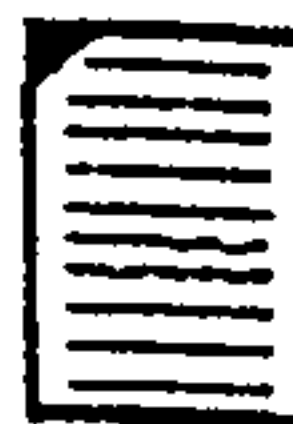


Figure 11 Separation of organic acids in a cola by ion exclusion chromatography. Conditions as listed in Table 3.

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### LIST OF SUPPLIERS

EM Science, P.O. Box 70, 480 Democrat Road,  
Gibbstown, New Jersey, 08027, USA,  
1-800-222-0342.

Fisher Scientific, 711 Forbes Ave., Pittsburgh,  
Pennsylvania, 15219-4785, USA.  
1-800-766-7000.

Fluka Chemika-BioChemika, Fluka Chemie AG,  
Industriestrasse 25, CH-9471 Buchs, Switzerland,  
+81 755 25 11.

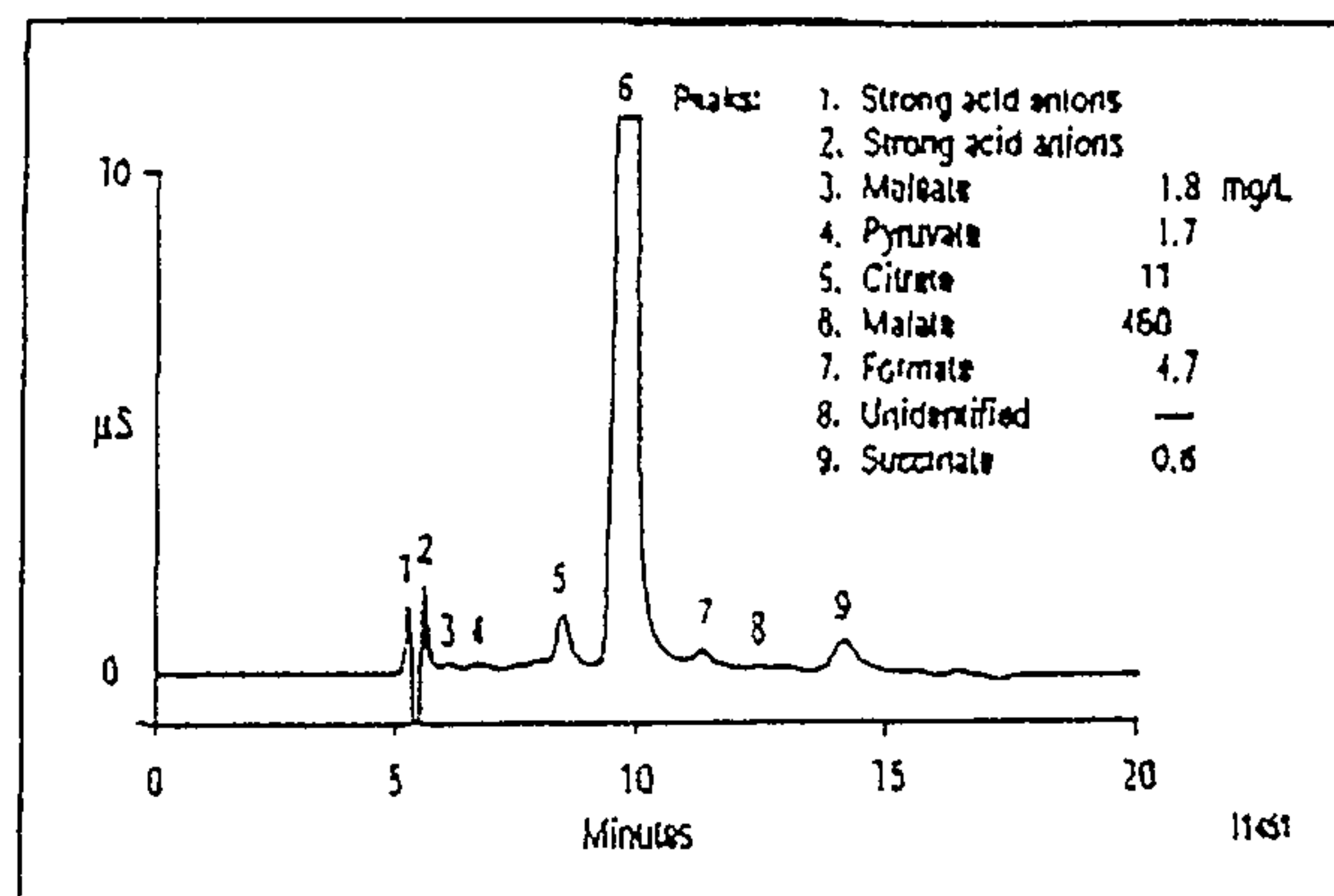
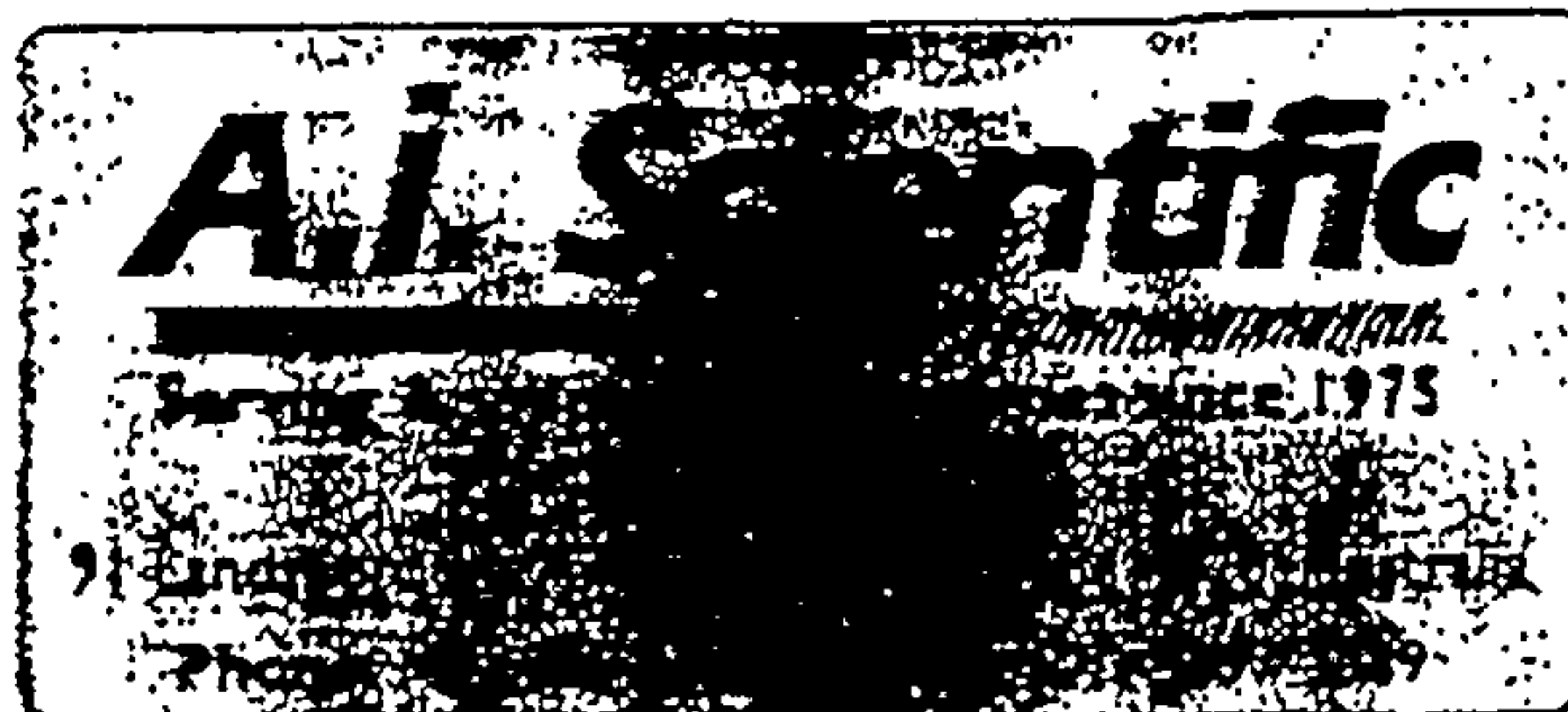


Figure 12 Separation of organic acids in a carbonated apple juice by ion exclusion chromatography. Conditions as listed in Table 3.



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Dionex Corporation  
1228 Tice Way  
P.O. Box 3005  
Sunnyvale, CA  
(408) 737-0700

Dionex Corporation  
Salt Lake City Technical Center  
1515 West 2200 South, Suite A  
Salt Lake City, UT  
(801) 972-8292

Dionex U.S. Regional Offices  
Sunnyvale, CA (408) 737-4522  
Westmont, IL (800) 729-3660  
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Dionex International Subsidiaries  
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Home page: [www.dionex.com](http://www.dionex.com)



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