

**THE EFFECT OF CASEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM
PHOSPHATE ON LOAD-DEFLECTION PROPERTIES OF BETA-TITANIUM WIRES
USED IN ORTHODONTICS**

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Objective: The objective of this in vitro study was to determine the effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on the load-deflection properties of beta-titanium wires, specifically loading and unloading flexural modulus and yield strength.

Materials and Methods: Ten 0.017 x 0.025 inch beta-titanium wires each from five companies were tested, using a three-point bend test apparatus, for a total of 50 control and 50 experimental samples. The experimental wires were exposed to MI Paste (CPP-ACP) for nine hours, to simulate three months of six-minute MI Paste application, before bend-testing in a water tank of 37 degrees Celsius, while the control wires were exposed to distilled water for nine hours before testing. A 2x5 multivariate analysis of variance was conducted to analyze the data.

Results: There were no statistically significant differences between the control group and experimental group for loading elastic modulus, 0.2% offset yield strength, or unloading 0.2% offset yield strength. There was a statistically significant difference for unloading flexural modulus, but it is most likely not a clinically significant difference, given there was a 1.92% difference between the control and MI paste group.

Conclusions: The results of this study suggest that CPP-ACP does not have any clinically significant effects on beta-titanium wires for the load deflection properties tested.

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1.0 INTRODUCTION

White spot lesions have always been one of the risks encountered in orthodontic treatment and continue to be an issue today. Aside from being the beginning stages of caries, they are also unsightly lesions, counterproductive to the esthetic benefits of orthodontics. Traditionally, the recommended prophylactic for white spot lesions has been topical fluoride treatments, along with homecare of daily tooth brushing with a fluoride toothpaste. Orthodontists may even prescribe high-concentration fluoride rinses or toothpastes to patients at a high risk for lesions.

Alternatively, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), a milk-casein derivative, has been demonstrated to remineralize enamel in cases of subsurface lesions, using delivery methods such as prepared topical solutions, sugar-free chewing gum, lozenges, and milk (Reynolds et al, 1995; Reynolds, 1997; Reynolds et al., 2003; Cai et al., 2003; Iijima et al., 2003; Walker et al., 2006). Furthermore, CPP-ACP has been shown to help prevent white spot lesions around orthodontic brackets *in vitro* (Sudjalim et al., 2007), and decrease enamel demineralization after *in vitro* incisor stripping (Giulio et al, 2009).

Beta-titanium wires are useful in orthodontics because they provide a combination of low stiffness, high springback or range, good formability, and can be welded to auxiliaries (Burstone and Goldberg, 1980). Among the clinical benefits of beta-titanium wires are their intermediate

modulus of elasticity and high springback. Also, they are very useful for the finishing stages of orthodontic treatment because of their good resilience and formability. However, several studies have shown that fluoride can have a negative effect on the surface topography (Kaneko et al., 2003; Watanabe and Watanabe, 2003; Kaneko et al., 2004; Kwon et al., 2005; Walker et al., 2007), tensile strength (Kaneko et al., 2003; Kaneko et al., 2004; Kwon et al., 2005), and unloading deflection properties of Beta-Titanium wires (Walker et al., 2007). Since these negative effects are due to the interactions of fluoride with titanium, beta-titanium wires are at the most risk of being deleteriously affected because, being composed of about 80% titanium, they contain the most titanium of all the orthodontic wire alloys. The purpose of this study, therefore, is to test the effects of CPP-ACP on load-deflection properties of beta-titanium wires *in vitro*, as a first-step in an effort to present a possible alternative to prescription-strength fluoride treatment during orthodontics.

2.0 LITERATURE REVIEW

2.1 BETA-TITANIUM WIRES

According to Proffit, properties of an ideal arch wire include: high strength, low stiffness, high range, high formability, and the ability to be welded. In the 1980s, beta-titanium, or titanium-molybdenum, wire was introduced for orthodontic use. Beta-titanium wires seem to fulfill the requirements for an ideal orthodontic wire, as they provide a combination of low stiffness, high springback or range, good formability, and can be welded to auxiliaries (Burstone and Goldberg, 1980). It is an intermediate type of wire because it has half the stiffness and twice the range of stainless steel, but double the stiffness and half the range of nitinol (Kusy, 1981; Proffit, 2000).

Among the clinical benefits of beta-titanium wires are their intermediate modulus of elasticity and high springback. Modulus of elasticity, or Young's Modulus, is equal to the ratio of stress to strain. It is proportionate to the delivered force magnitude and stiffness of the wire. The intermediate modulus of elasticity indicates that less counterproductive forces are needed to counteract the forces created by the beta-titanium wires, as opposed to stainless steel wires. This, in turn, suggests that less extraoral anchorage demands will be required for beta-titanium than stainless steel wires (Kapila and Sachdeva, 1989). Springback is proportionate to yield strength, which is the point at which 0.2% of permanent deformation is observed in the wire.

The combination of intermediate modulus of elasticity and high springback means that less loops and helices need to be bent in beta-titanium wires, as they usually are in stainless steel wires to decrease load deflection rate and increase range (Kapila and Sachdeva, 1989). The end result of all this is a simpler appliance design. Beta-titanium wires do, however, have good formability such that bends, loops, and helices could easily be bent into the wire if so desired.

Beta-titanium wires have better formability than stainless steel, but they have high friction (Kapila and Sachdeva, 1989). In fact, beta-titanium wires have the highest frictional resistance of any orthodontic wire. Beta-titanium is typically composed of 80% titanium. As the titanium content of an alloy increases, its surface resistance increases (Proffit, 2000). Therefore, beta-titanium wires are not very useful for sliding mechanics. They are, however, very useful for the finishing stages of orthodontic treatment because they have good resilience and formability.

2.2 WHITE SPOT LESIONS

2.2.1 Etiology

White spot lesions are essentially areas of demineralization on a tooth's surface. They are the beginning stages of carious cavitation, however, the enamel surface is still intact. They manifests as chalky-white, or opaque, tooth structure that has a roughened and slightly softer surface. The white appearance is caused by an optical phenomenon due to demineralization and becomes even whiter when dried (Ogaard et al., 1988). According to Ogaard et al. (1988) several experiments that used microradiography, polarized light microscopy, microhardness

tests, and electron microscopy have shown there to be two initial stages of enamel demineralization: 1) surface softening, which includes mineral loss most pronounced at the enamel surface, and 2) subsurface lesion, which has dissolution at a deeper part of the enamel. White spot lesions form because of a combination of host factors, diet, and hygiene. Host factors include cariogenic oral flora. *Streptococcus mutans* and *lactobacilli* are the oral bacteria most commonly associated with caries (Hardie, 1982). These bacteria, found in dental plaque, produce acids on the surface of teeth, which creates a lowered pH. If the oral environment is held at a lowered pH for an extended period of time, demineralization occurs, depleting the tooth of calcium and phosphates. If the pH is allowed to neutralize, remineralization will occur with ions from the saliva. If this neutralization does not occur, the tooth continues to demineralize, starting the process of white spot lesion formation.

Diet is another important factor in caries development because the bacteria in plaque metabolize carbohydrates and create acids as a byproduct (Ryan, 1983). For example, in one study, Schachtele and Jensen (1981) found that five grams of white bread caused a drop in plaque pH to 3.5. Therefore, a diet high in sugars will put a patient at an increased risk for caries or white spot lesion development. Also, increased time or frequency of exposure to these cariogenic foods will increase the risk of caries development because it does not allow for the pH of the mouth to neutralize and the teeth to remineralize.

Oral hygiene is also an important factor in the etiology of white spot lesions. Tooth brushing and flossing prevent plaque accumulation on teeth by mechanically disrupting bacterial colonization.

2.2.2 During orthodontics

White spot lesions are one of the major possible consequences of orthodontic treatment. Brackets and bands, used during orthodontic treatment, can be areas of plaque accumulation. In addition, appliances such as brackets and wires may make it more difficult or cumbersome for the patient to maintain adequate oral hygiene. White spot lesions may start to appear around ill-fitting bands within four weeks in the absence of fluoride (Ogaard et al., 1988 Part 1). In a study using SEM photographs and microradiography, marked and localized demineralization of the surface enamel was seen in premolar teeth that were poorly banded and not allowed fluoride for a month prior to extraction (Ogaard et al., 1988 Part 1). According to Geiger et al. (1988) the maxillary anterior segments, which is a high-esthetic zone, and the mandibular posterior segments are the most susceptible to white spot formation.

During orthodontic treatment, if the patient is non-compliant with regards to diet and oral hygiene practices and plaque is allowed to remain around the brackets, then the areas of the teeth surrounding the brackets may undergo demineralization, while the areas under the bracket will not (Ogaard et al., 1988 Part 2). Therefore, after debonding, if white spot lesions do occur during the course of orthodontic treatment, the teeth will display chalky-white outlines of the brackets. This is a risk that must be explained to the patient and his/her parents before the start of orthodontic treatment.

2.2.3 Prevention

Controlling the factors, such as diet and hygiene, that cause caries is important in the prevention of white spot lesions during orthodontics. However, one study found that even with daily use of a fluoride toothpaste, premolars that were bonded with brackets in anticipation of extraction exhibited up to 15% mineral loss in just one month (O'Reilly and Featherstone, 1987). These findings demonstrate that white spot lesions can develop in a period of time that equates the time between orthodontic visits, and suggests that some sort of prescription fluoride treatment may be needed to prevent white spot lesion formation. One study demonstrated that daily use of a 0.05% sodium fluoride rinse can significantly reduce decalcification seen on the labial surfaces of teeth during orthodontic treatment (Geiger et al., 1988). Another study, comparing the prevalence of white spot lesions in untreated versus orthodontically treated 19-year-olds, showed that even with a daily 0.05% sodium fluoride rinse in addition to daily brushing with a fluoride toothpaste orthodontic patients still had an increased prevalence of white spot lesions (Ogaard, 1989). In this study only 4% of orthodontically treated patients had no white spot lesions, as opposed to the untreated group in which 15% had no white spot lesions. These observations were made five years after completion of orthodontic treatment, which means that these visible lesions were very resistant to remineralization. The Cochrane Collaboration (Benson et al., 2004) concluded that there is some evidence that a daily sodium fluoride mouthrinse reduces the severity of enamel decay surrounding fixed orthodontic appliances. The authors of the Collaboration recommended rinsing with a .05% sodium fluoride mouthrinse, based on studies on non-orthodontic patients that showed rinses, gels, and toothpastes to be associated with a reduction in caries for children and adolescents.

2.3 THE EFFECT OF FLUORIDE ON BETA-TITANIUM WIRES

Fluoride, acidulated (APF) and neutral (NaF), has been shown to lead to the degradation of the mechanical properties of pure titanium (Yokoyama et al., 2003). Beta-titanium archwires are composed of 80% titanium. Studies that immersed beta-titanium wires in fluoride solutions have found that fluoride treatment of the wire leads to degradation of its properties, as well. *In vitro* studies have shown that after submersion in fluoride agents, changes occur in the beta-titanium wires such as color (Kaneko et al., 2003; Watanabe and Watanabe, 2003), decreased corrosion resistance (Kaneko et al., 2003; Watanabe and Watanabe, 2003; Kaneko et al., 2004; Kwon et al., 2005; Walker et al., 2007), decreased tensile strength (Kaneko et al., 2003; Kaneko et al., 2004; Kwon et al., 2005), fracture mode change from ductile to brittle on the surface (Kaneko et al., 2003), and decreased unloading modulus of elasticity and yield strength (Walker et al., 2007). Watanabe and Watanabe (2003) found that a single application of APF does not have an effect on the beta-titanium wire, however multiple applications within a month will change the surface topography of the wire.

Corrosion resistance of beta-titanium wires comes from a titanium oxide film that forms on the surface. When exposed to a fluoride agent, this titanium oxide layer reacts with the fluoride, creating different products (such as titanium fluoride, titanium oxide fluoride, or sodium titanium fluoride) on the surface of the wire, which lead to decreased corrosion resistance (Kaneko et al., 2003).

The decrease in tensile strength, alteration of fracture mode, and other mechanical changes seen in fluoride-exposed beta-titanium wires is thought to occur because of hydrogen embrittlement.

Hydrogen embrittlement is altered mechanical properties of a wire, due to the absorption of hydrogen. The loss of the titanium oxide layer due to fluoride exposure may lead to absorption of hydrogen ions because of titanium's affinity for hydrogen, leading to hydrogen embrittlement (Kaneko et al., 2003; Yokoyama et al., 2003). Another means of hydrogen embrittlement involves acetic acid. The oral environment produces acetic acid due to bacteria. Fluoride, then, reacts with the acid to form hydrofluoric acid, which dissolves the protective titanium oxide layer and allows hydrogen embrittlement due hydrogen absorption (Kwon et al., 2005). Another theory for the cause of increased fracture seen in fluoride-exposed titanium wires involves a combination of corrosion, surface layers of wire peeling off, and hydrogen absorption (hydrogen embrittlement) (Yokoyama et al., 2003).

Mechanical loading also seems to effect hydrogen absorption of beta-titanium wires in a fluoride solution (Kaneko et al., 2003; Kwon et al., 2005). Kwon et al. (2005) offer that mechanical tension increases interatomic space and density of dislocations, leading to accelerated hydrogen absorption.

The effects of fluoride agents on wires are thought to be associated with fluoride ion concentration and pH (Watanabe and Watanabe, 2003; Walker et al., 2007). That is, higher fluoride ion concentration and lower pH will lead to more and stronger negative effects on the beta-titanium wires.

Both APF and NaF have been shown to decrease the unloading flexural modulus and yield strength of beta-titanium wires (Walker et al., 2007). The load deflection characteristics of an orthodontic wire are very clinically relevant because these are the forces that are delivered to the tooth. The authors suggest that fluoride produces a negative effect on the unloading properties of beta-titanium wires due to trapped interstitial hydrogen after exposure to topical fluoride agents. First, hydrogen absorption occurs when the wire is exposed to fluoride agents. Then, when the wire has been loaded past the elastic range, lattice dislocations and slip can occur, releasing the trapped hydrogen, thereby increasing hydrogen ion concentration and leading to decreased unloading modulus and yield strength.

2.4 CASEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM PHOSPHATE

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a milk-casein derivative and has been shown to remineralize enamel subsurface lesions (Reynolds et al., 1995), (Reynolds, 1997; Reynolds et al., 2003; Shen et al., 2001; Cai et al., 2003; Iijima et al., 2004). CPP, containing cluster sequences of –Ser(P)-Ser(P)-Ser(P)-Glu-Glu-, seem to stabilize ACP, which is soluble calcium phosphate, in metastable solution (Iijima et al., 2003). In an *in situ* study, Reynolds et al. (2003) inspected dental plaque residues after exposure to CPP-ACP and found that there were major bonds with the CPP and the bacterial cell wall, suggesting that the localized CPP-ACP nanocomplexes get incorporated into dental plaque and onto the tooth surface. In this way CPP allows intimate contact of ACP with the surface of the tooth. In the plaque the CPP-ACP acts as a reservoir of calcium phosphate, which can diffuse into the enamel subsurface lesion and enhance remineralization (Reynolds et al., 1995). The bound ACP will also generate a high concentration of free calcium and phosphate ions by dynamic equilibrium (Reynolds et al., 1995). Although there is a state of supersaturation of the calcium and phosphate ions in the plaque, the multiple phosphoserine residues of the CPP bind to the nanoclusters of the ACP, preventing their growth to the critical size required for nucleation and phase transformation (Reynolds, 1998) and in this way there is no calculus formation. In 1997, Reynolds conducted an *in vitro* study in which he tested different concentrations of CPP solutions; he found that higher concentrations of CPP lead to increased concentrations of free calcium and phosphate ions, which lead to increased remineralization of subsurface enamel lesions. In 2001, Reynolds conducted an *in situ* study in which he demonstrated the ability of a sugar-free gum containing CPP-ACP (Recaldent) to remineralize enamel subsurface lesions. Sugar-free lozenges containing CPP-ACP have also been shown to remineralize enamel

subsurface lesions *in situ* (Cai et al., 2003). One *in vivo* study, using CPP-ACP chewing gum, has shown that the remineralized lesions were more resistant to subsequent acid challenge (Iijima et al., 2004). Recently Walker et al. (2006) demonstrated that milk enriched with CPP-ACP provided a dose-dependent increase in mineralization. Another *in vitro* study used extracted lower incisors that were then stripped, as you might clinically in interproximal reduction, and exposed to an acidic solution (Guilio et al, 2009); they found that the experimental group, which was treated with CPP-ACP tooth mousse, had less enamel demineralization. Furthermore, CPP-ACP has been proven in an *in vitro* study to prevent white spot lesions around orthodontic brackets that have been bonded with composite resin (Sudjalim et al., 2007).

The trade name for CPP-ACP is Recaldent. Recaldent can be found in products such as sugar-free chewing gum (Recaldent; GC Corp., Japan and Trident White; Cadbury Adams USA, Parsippany New Jersey, USA), mints (Recaldent Mints; Cadbury Japan Ltd., Japan), and topical gels (Tooth mousse; GC Corp., Japan and PROSPEC MI Paste; GC America Inc.).

3.0 STATEMENT OF THE PROBLEM

Topical fluoride has been routinely advocated and prescribed for the prevention of white spot lesions during orthodontics. However, studies have shown that all three types of commonly used prescription-strength fluorides (APF, NaF, and SnF₂) have destructive effects on beta-titanium orthodontic wires. Furthermore, APF and NaF have been shown to negatively affect the unloading forces of beta-titanium wires. Changing the mechanical properties of these archwires can have a deleterious effect on the progression of orthodontic treatment. CPP-ACP has been shown to remineralize subsurface enamel lesions and aid in the prevention of white spot lesions in orthodontics, but its effect on beta-titanium wires has not been tested.

4.0 OBJECTIVE

The objective of this study is to compare load-deflection curves of several beta-titanium wires between controls and those treated with CPP-ACP to determine what effects, if any, CPP-ACP has on beta-titanium load-deflection properties, specifically loading and unloading elastic modulus and yield strength.

5.0 RESEARCH QUESTION

What kind of effects, if any, does CPP-ACP have on loading and unloading modulus of elasticity and 0.2% offset yield strength of beta-titanium wires?

6.0 METHODS AND MATERIALS

6.1 Materials

The materials consisted of MI paste (GC America) and beta-titanium wires from each of five orthodontic manufacturers (Table 1):

- 1.Ormco TMA (Ormco)
2. GAC Resolve (GAC)
3. 3M Unitek Beta III Titanium (3M)
4. American Orthodontics B-Ti (AO)
5. Opal Orthodontics Beta Titanium (Opal)

The dimension of all of the wires that were tested is .017x.025 inch rectangular.

Specimens were prepared by cutting 25 mm segments from the straight ends of the archwire. GC America recommends one or two three-minute applications of MI paste, per day, for orthodontic patients. Accordingly, to simulate the clinical situation of three months of six-minute topical MI Paste application, the experimental wires were treated with MI paste for nine hours. The controls were treated with distilled water for nine hours. After the treatment period, the control and experimental wires were rinsed with distilled water before testing.

A three-point bend test apparatus was utilized to test for flexural loading and unloading properties of the wires. The testing machine is a MTI-1K (Measurements Technology, Inc., Roswell, GA) with a 2.5 pound load cell (Figure 1). The support span of the three-point bend test fixture was 12 mm, with radii of 0.05–0.13 mm for each support and the striker, according to ADA specification 32 (Figure 2).

6.2 Method of archwire testing

Three-point bending tests are most commonly used in orthodontic wire testing since it represents a wire engaged in a bracket and is easily reproducible (Kusy and Dilley, 1984), the results of this testing can be collected through a machine, and it is a convenient way to demonstrate how the archwire behaves before and after it is loaded past its elastic range. Therefore, a three-point bending test was used, per Walker et al.'s study (2007), with a few modifications. There were two testing conditions:

1. Control: treated with distilled water for nine hours
2. Experimental: treated with MI paste (GC America) for nine hours

Ten beta-titanium wires from each of the five manufacturers underwent the experimental treatment, and the other 50 underwent the control treatment, making it a total of 100 wires that were tested. The wires were deflected 3 mm, and then unloaded to zero deflection at a cross-head speed of 3 mm/min. To simulate an aqueous oral environment, the wires were tested in a distilled water bath at 37 degrees Celsius (Figures 3, 4). The load was measured in Newtons and deflection in millimeters for both loading and unloading with the MTI software program, which created read outs and load deflection curves for each wire tested (Appendix A). From these

curves, loading and unloading flexural modulus and 0.2% offset yield strength were calculated and evaluated (Appendix B).

Table 1. Beta-titanium wires used in this study.

Archwire	Company/Supplier	Address
Beta III Titanium	3M Unitek	2724 South Peck Road Monrovia, CA 91016, USA
B-Ti	American Orthodontics	1714 Cambridge Avenue P.O. Box 1048, WI 53082, USA
Resolve	GAC	185 Oval Drive Central Islip, NY 11722, USA
Beta Titanium	Opal Orthodontics	505 West 10200 Street South Jordan, UT 84095, USA
TMA	Ormco	1717 West Collins Avenue Orange, CA 92867, USA

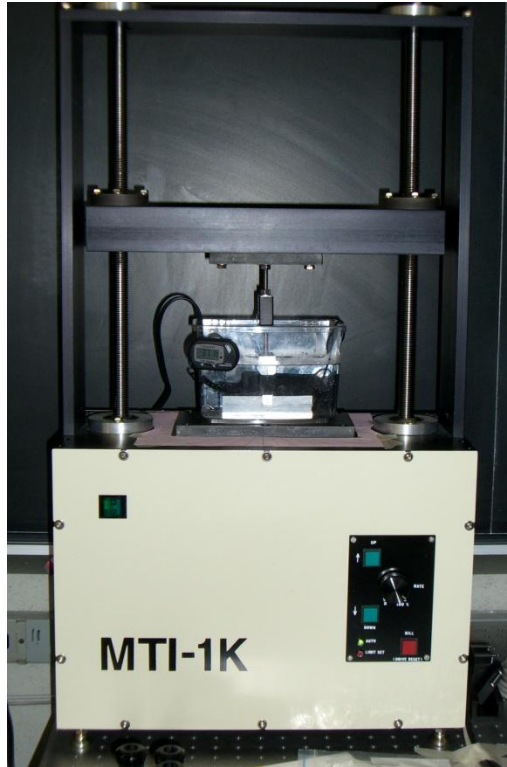


Figure 1. Load frame from MTI and testing set-up.

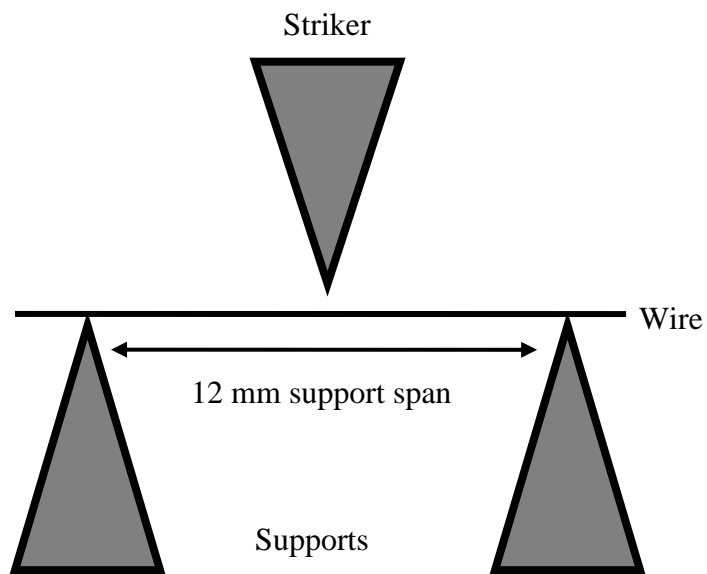


Figure 2. Schematic of bend test apparatus



Figure 3. Bend test apparatus in water tank of 37 degrees Celsius.

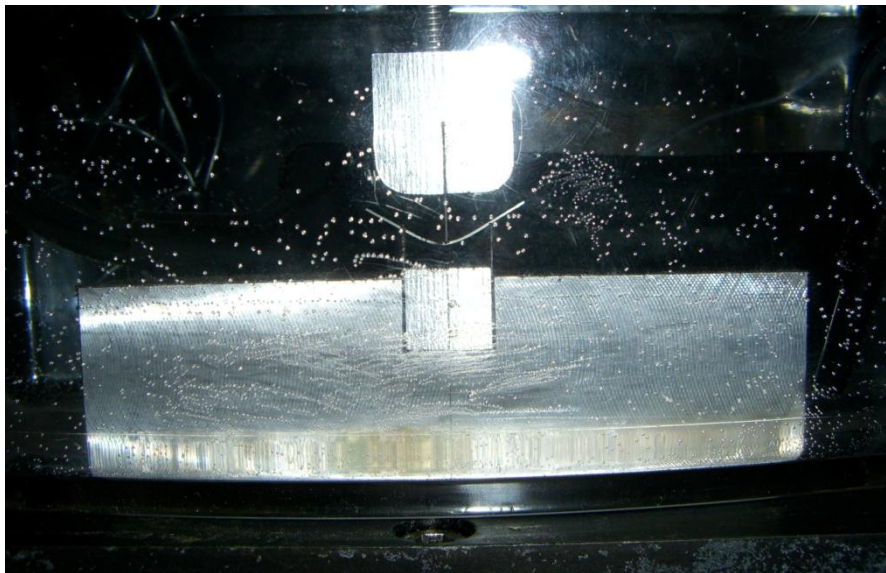


Figure 4. Bend test apparatus with wire deflected 3 mm.

6.3 Data Analysis

The means and standard deviations were calculated for loading elastic modulus (Loading E), loading 0.2% offset yield strength (Loading YS), unloading flexural modulus (Unloading E), and unloading 0.2% offset yield strength (Unloading YS) for the archwires from the five companies. The data were analyzed with a 2x5 multivariate analysis of variance (MANOVA), using Wilks' Lambda values and alpha equal to 0.05, to test for mean differences between the five companies and between the control and experimental groups. If the multivariate test revealed statistically significant differences between at least two of the measures for companies and/or groups, then a post hoc univariate test was done to assess which measures were statistically different from the others. Further post hoc pairwise comparisons were done to see which companies differed from one another, using a Student Newman-Keuls test with an alpha of 0.05.

7.0 RESULTS

The multivariate tests showed that two or more companies ($p \leq 0.0004$) and groups, i.e. control versus MI Paste, ($p \leq 0.0064$) significantly differ from one another on at least one of the measures, i.e. loading E, loading YS, unloading E, and unloading YS. The univariate tests revealed that, for the companies, there was a statistically significant difference across all four of the measures ($p \leq 0.0004$), unlike for the control versus experimental groups, where the statistical difference was only for unloading E ($p \leq 0.0024$). There was no significant interaction between the companies and the groups (Tables 2,3,4,5).

Differences between the control and MI Paste groups

Table 6 and Figure 5 show the means and standard deviations for the control and MI Paste groups, for all the measures. Using Student-Newman-Keuls multiple comparison tests at a 0.05 level for all pairwise differences, no statistically significant difference was found between the control and MI Paste group for Loading E, Loading YS, and Unloading YS. However, there was a statistically significant difference between the control and MI Paste group for Unloading E ($p \leq 0.0024$).

Table 2. Mean values of Loading E for group and company interaction.

Group x Company	Mean (MPa)	Standard Deviation
3M Unitek Beta III Titanium – Control	59616.60	1069.61
American Orthodontics B-Ti – Control	62443.27	1179.03
GAC Resolve – Control	61442.80	1017.02
Opal Beta Titanium – Control	59561.21	1515.59
Ormco TMA – Control	63898.92	1130.16
3M Unitek Beta III Titanium – MI Paste	59648.18	1318.01
American Orthodontics B-Ti – MI Paste	64150.34	1341.25
GAC Resolve – MI Paste	61294.68	1624.05
Opal Beta Titanium – MI Paste	59049.62	1328.51
Ormco TMA – MI Paste	64333.25	1659.28

Table 3. Mean values of Loading YS for group and company interaction.

Group x Company	Mean (MPa)	Standard Deviation
3M Unitek Beta III Titanium – Control	1275.45	43.06
American Orthodontics B-Ti – Control	1412.49	45.69
GAC Resolve – Control	1331.90	41.76
Opal Beta Titanium – Control	1308.51	29.85
Ormco TMA – Control	1340.00	35.88
3M Unitek Beta III Titanium – MI Paste	1274.88	62.35
American Orthodontics B-Ti – MI Paste	1402.20	39.22
GAC Resolve – MI Paste	1344.77	43.28
Opal Beta Titanium – MI Paste	1296.25	36.71
Ormco TMA – MI Paste	1355.65	29.98

Table 4. Mean values of Unloading E for group and company interaction.

Group x Company	Mean (MPa)	Standard Deviation
3M Unitek Beta III Titanium – Control	20506.69	593.48
American Orthodontics B-Ti – Control	21089.34	324.07
GAC Resolve – Control	19230.81	691.40
Opal Beta Titanium – Control	19448.17	610.77
Ormco TMA – Control	12587.16	391.50
3M Unitek Beta III Titanium – MI Paste	20002.73	816.07
American Orthodontics B-Ti – MI Paste	21119.36	614.35
GAC Resolve – MI Paste	18730.24	576.87
Opal Beta Titanium – MI Paste	19032.17	522.42
Ormco TMA – MI Paste	12194.55	289.02

Table 5. Mean values of Unloading YS for group and company interaction.

Group x Company	Mean (MPa)	Standard Deviation
3M Unitek Beta III Titanium – Control	715.79	30.37
American Orthodontics B-Ti – Control	716.59	48.22
GAC Resolve – Control	705.66	59.05
Opal Beta Titanium – Control	637.02	28.93
Ormco TMA – Control	649.94	39.53
3M Unitek Beta III Titanium – MI Paste	696.69	47.79
American Orthodontics B-Ti – MI Paste	700.03	52.18
GAC Resolve – MI Paste	690.05	48.54
Opal Beta Titanium – MI Paste	658.99	33.11
Ormco TMA – MI Paste	666.60	32.74

Table 6. Mean values for loading and unloading modulus of elasticity and yield strength of control and MI Paste groups ($n = 50$).

Mechanical Property	Mean (MPa)	Standard Deviation
Loading E Control	61392.56	2036.63
Loading E MI Paste	61695.21	2659.85
Loading YS Control	1333.67	59.51
Loading YS MI Paste	1334.75	61.22
Unloading E Control	18572.43	3143.36
Unloading E MI Paste	18215.81	3206.17
Unloading YS Control	684.99	53.69
Unloading YS MI Paste	682.47	43.97

Differences between the companies

The differences between the companies across all the measures had a p-value ≤ 0.0004 .

Loading E: Ormco had the highest mean (64116.08 MPa), followed by AO, then GAC, 3M, and finally Opal (59305.42). Opal was statistically significantly different from GAC, AO, and Ormco; 3M was statistically significantly different from GAC, AO, and Ormco; GAC was statistically significantly different from Opal, 3M, AO, and Ormco; AO was statistically significantly different from Opal, 3M, and GAC; and Ormco was statistically significantly different from Opal, 3M, and GAC (Table 7, Figures 5,9)

Loading YS: AO had the highest mean (1407.34 MPa), followed by Ormco, then GAC, Opal, and finally 3M (1275.17 MPa). Opal was statistically significantly different from 3M, GAC, Ormco, and AO; 3M was statistically significantly different from Opal, GAC, Ormco, and AO; GAC was statistically significantly different from 3M, Opal, and AO; AO was statistically significantly different from 3M, Opal, GAC, and Ormco; and Ormco was statistically significantly different from 3M, Opal, and AO (Table 8, Figures 6,10).

Unloading E: AO had the highest mean (21104.35 MPa), followed by 3M, then Opal, GAC, and finally Ormco (12390.85 MPa). Opal was statistically significantly different from Orco, 3M, and AO; 3M was statistically significantly different from Ormco, GAC, Opal, and AO; GAC was statistically significantly different from Ormco, 3M, and AO; AO was statistically significantly

different fromOrmco, GAC, Opal, and 3M; andOrmco was statistically significantly different from GAC, Opal, 3M, and AO (Table 9, Figures 7,11).

Unloading YS: AO had the highest mean (708.31 MPa), followed by 3M, then GAC, Ormco, and finally Opal (648 MPa). Opal was statistically significantly different from GAC, 3M, and AO; 3M was statistically significantly different from Opal and Ormco; GAC was statistically significantly different from Opal and Ormco; AO was statistically significantly different from Opal and Ormco; and Ormco was statistically significantly different from GAC, 3M, and AO (Table 10, Figures 8,12).

Table 7. Mean values and post hoc comparisons for Loading E for the five companies.

Company (<i>n</i> = 10)	Mean (MPa)	Standard Deviation	Different From
3M Unitek Beta III Titanium	59632.39	1194	AO, GAC,Ormco
American Orthodontics B-Ti	63296.80	1260	3M, GAC, Opal
GAC Resolve	61368.74	1321	3M, GAC, Opal,Ormco
Opal Beta Titanium	59305.42	1423	AO, GAC,Ormco
Ormco TMA	64116.08	1395	3M, GAC, Opal

Table 8. Mean values and post hoc comparisons for Loading YS for the five companies.

Company (<i>n</i> = 10)	Mean (MPa)	Standard Deviation	Different From
3M Unitek Beta III Titanium	1275.165	53	AO, GAC,Opal,Ormco
American Orthodontics B-Ti	1407.344	43	3M, GAC,Opal,Ormco
GAC Resolve	1338.331	43	3M, AO, Opal
Opal Beta Titanium	1302.381	34	3M, AO, GAC,Ormco
Ormco TMA	1347.824	33	3M, AO, Opal

Table 9. Mean values and post hoc comparisons for Unloading E for the five companies.

Company (<i>n</i> = 10)	Mean (MPa)	Standard Deviation	Different From
3M Unitek Beta III Titanium	20254.71	705	AO, GAC, Opal, Ormco
American Orthodontics B-Ti	21104.35	469	3M, GAC, Opal, Ormco
GAC Resolve	18980.52	634	3M, AO, Ormco
Opal Beta Titanium	19240.17	567	3M, AO, Ormco
Ormco TMA	12390.85	341	3M, AO, GAC, Opal

Table 10. Mean values and post hoc comparisons for Unloading YS for the five companies.

Company (<i>n</i> = 10)	Mean (MPa)	Standard Deviation	Different From
3M Unitek Beta III Titanium	706.2365	39	Opal, Ormco
American Orthodontics B-Ti	708.3075	50	Opal, Ormco
GAC Resolve	697.8544	54	Opal, Ormco
Opal Beta Titanium	648.0046	31	3M, AO, GAC
Ormco TMA	658.2694	37	3M, AO, GAC

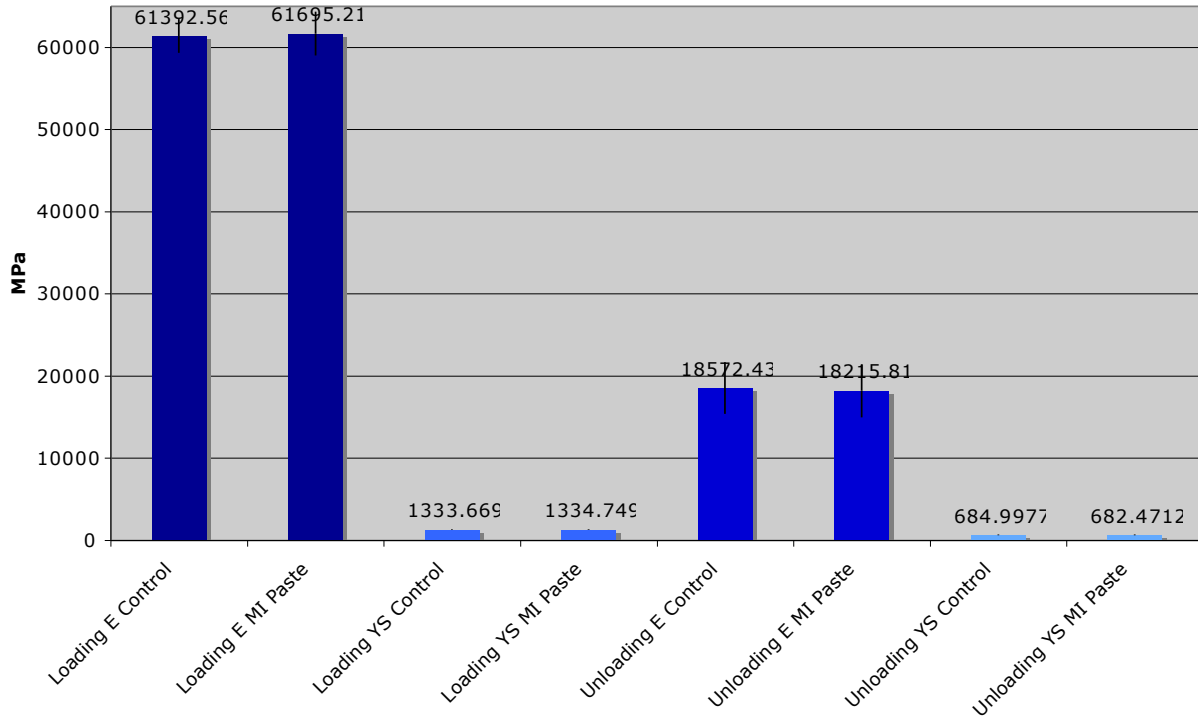


Figure 5. Mean values for loading and unloading E and YS for control and MI Paste groups.

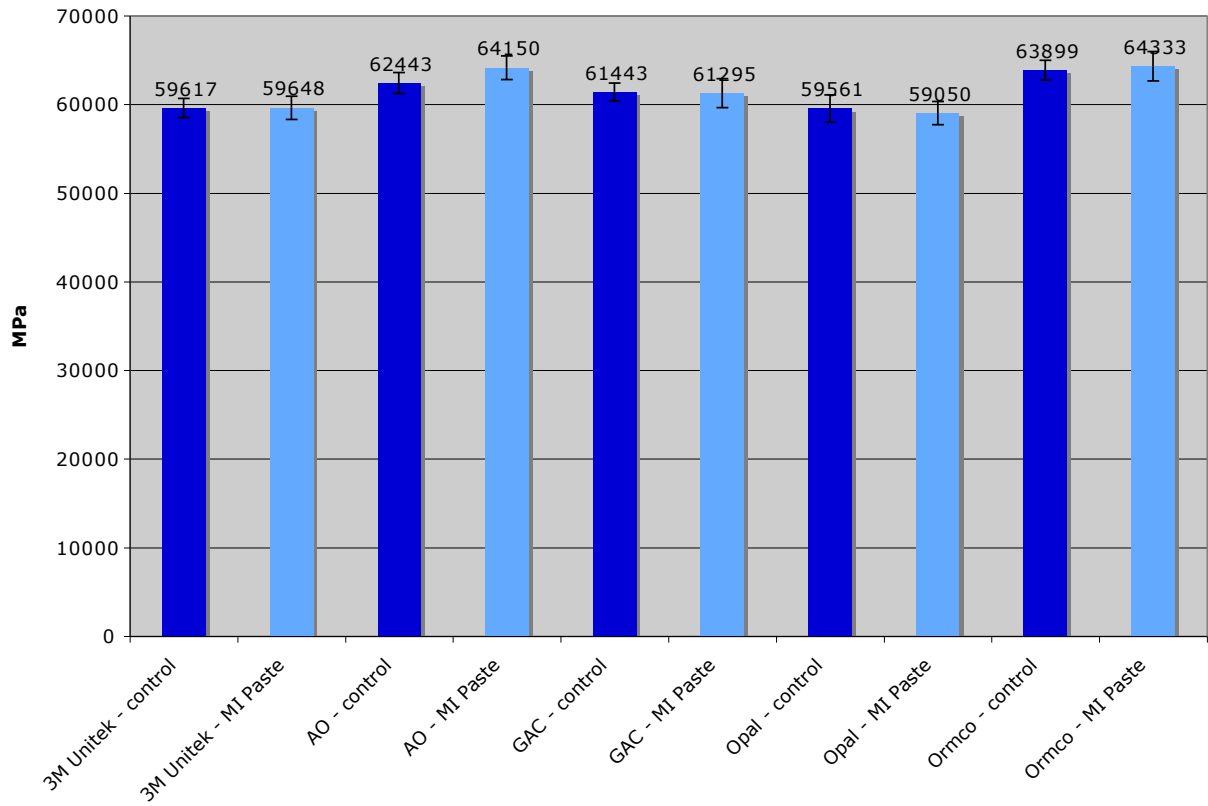


Figure 6. Mean values for Loading E for the five companies.

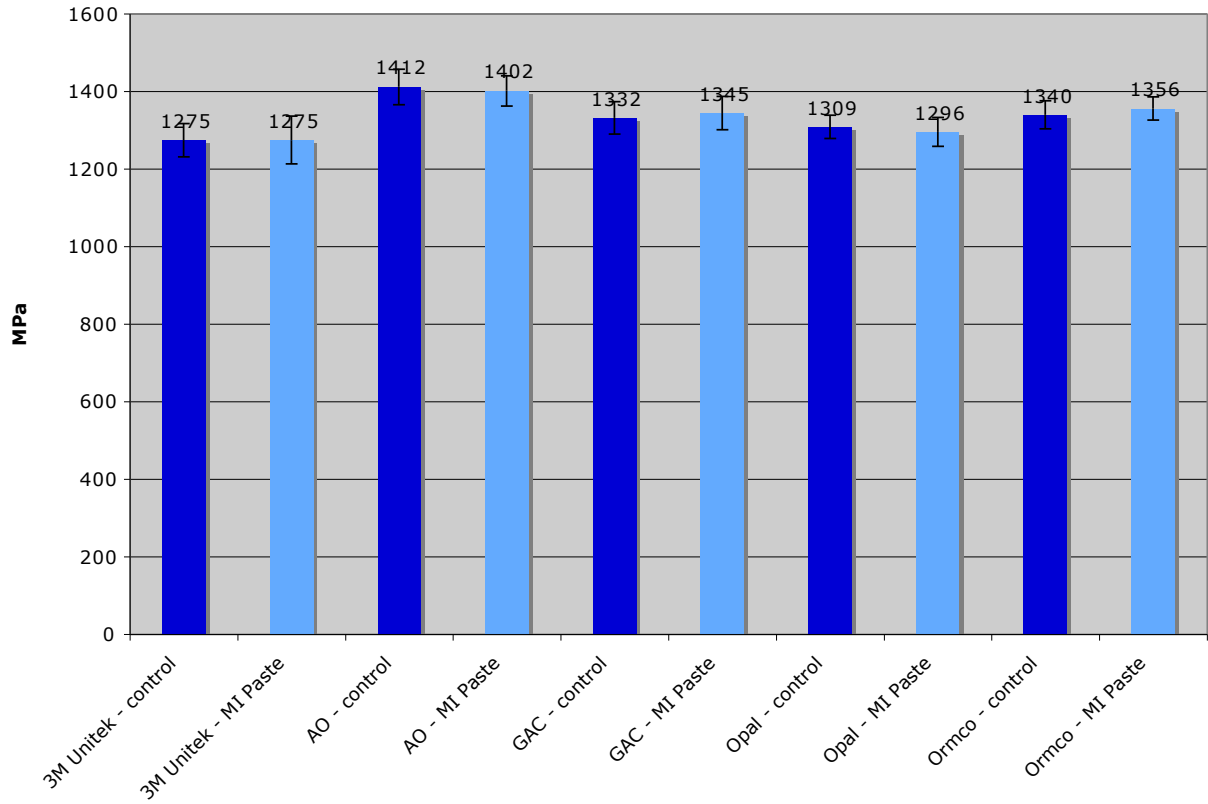


Figure 7. Mean Values for Loading YS for the five companies.

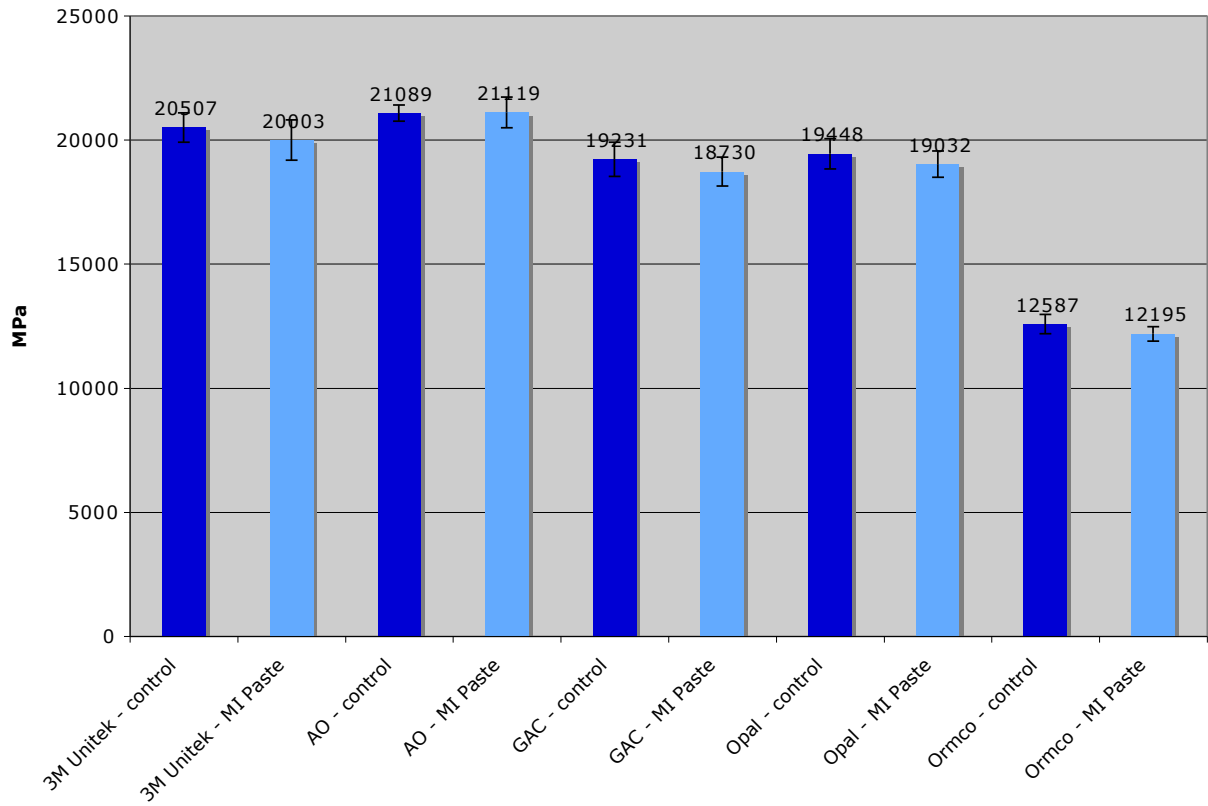


Figure 8. Mean values for Unloading E for the five companies.

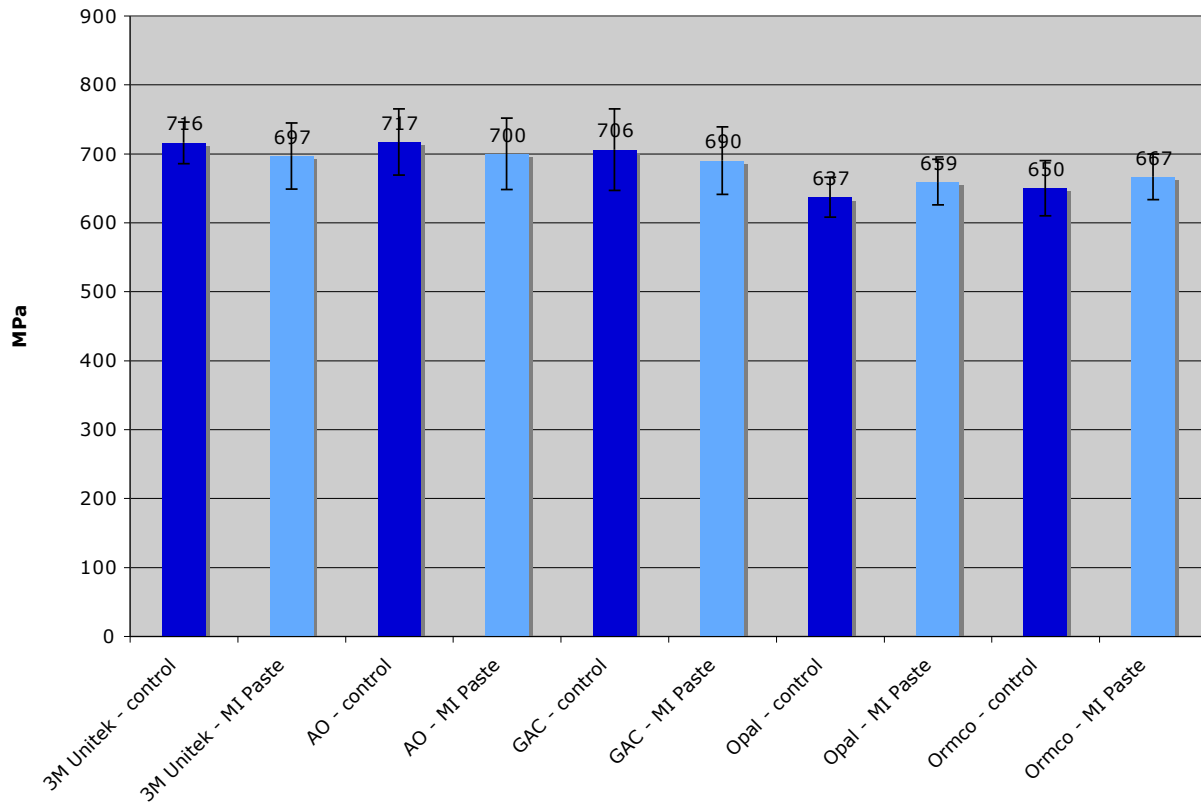


Figure 9. Mean values for Unloading YS for the five companies.

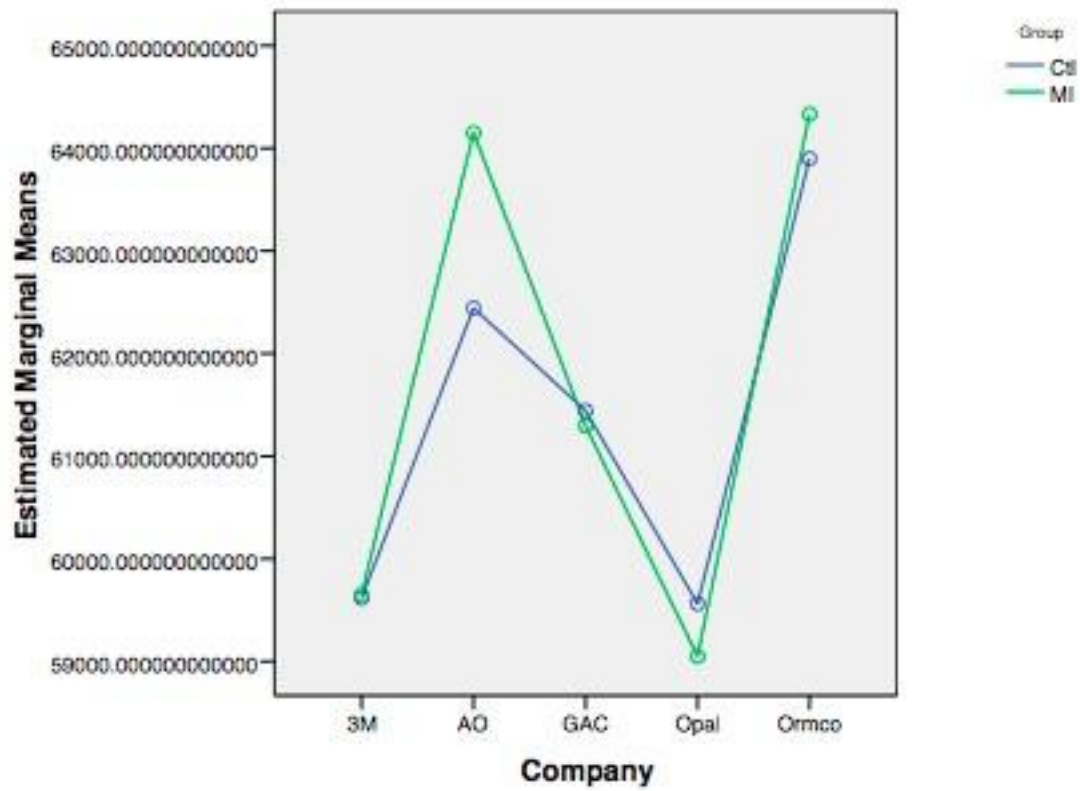


Figure 10. Estimated marginal means in MPa for Loading E.

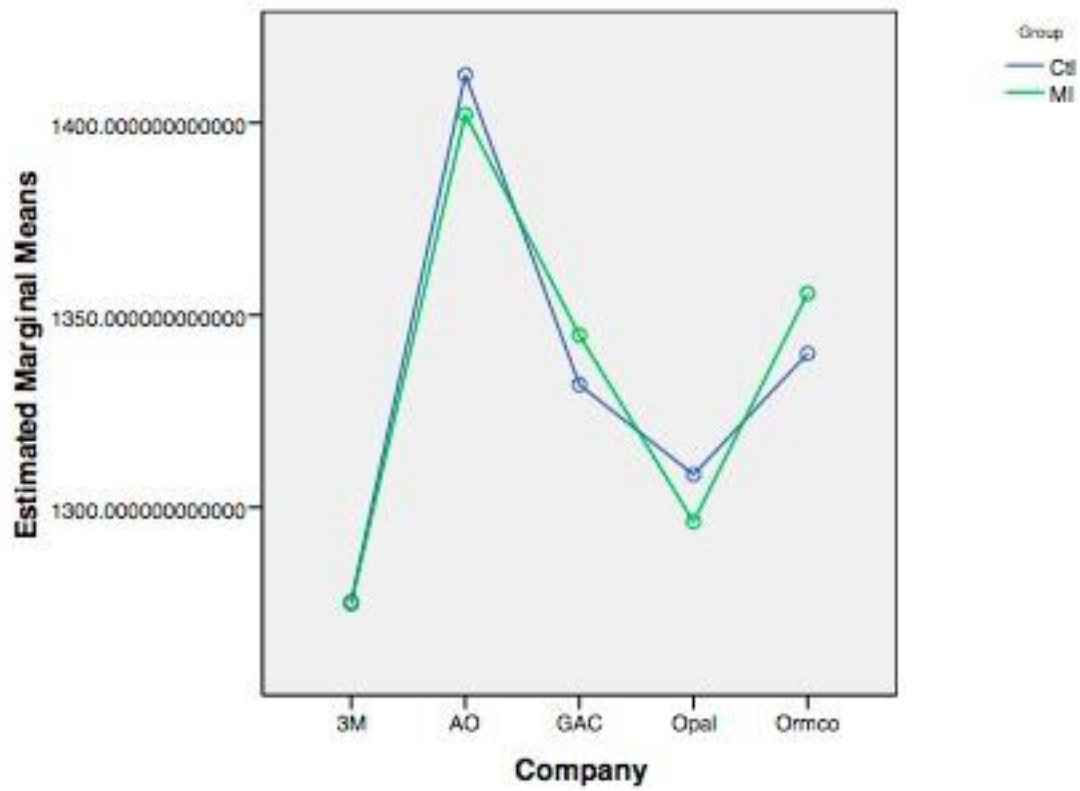


Figure 11. Estimated marginal means in MPa for Loading YS.

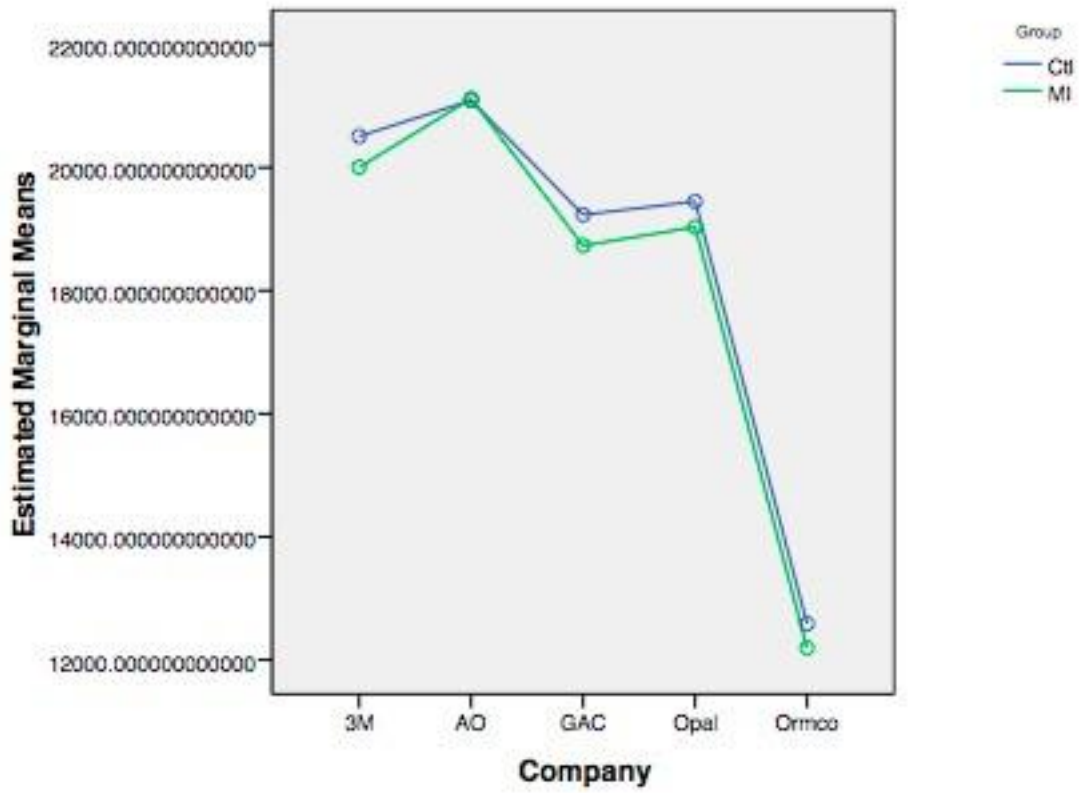


Figure 12. Estimated marginal means in MPa for Unloading E.

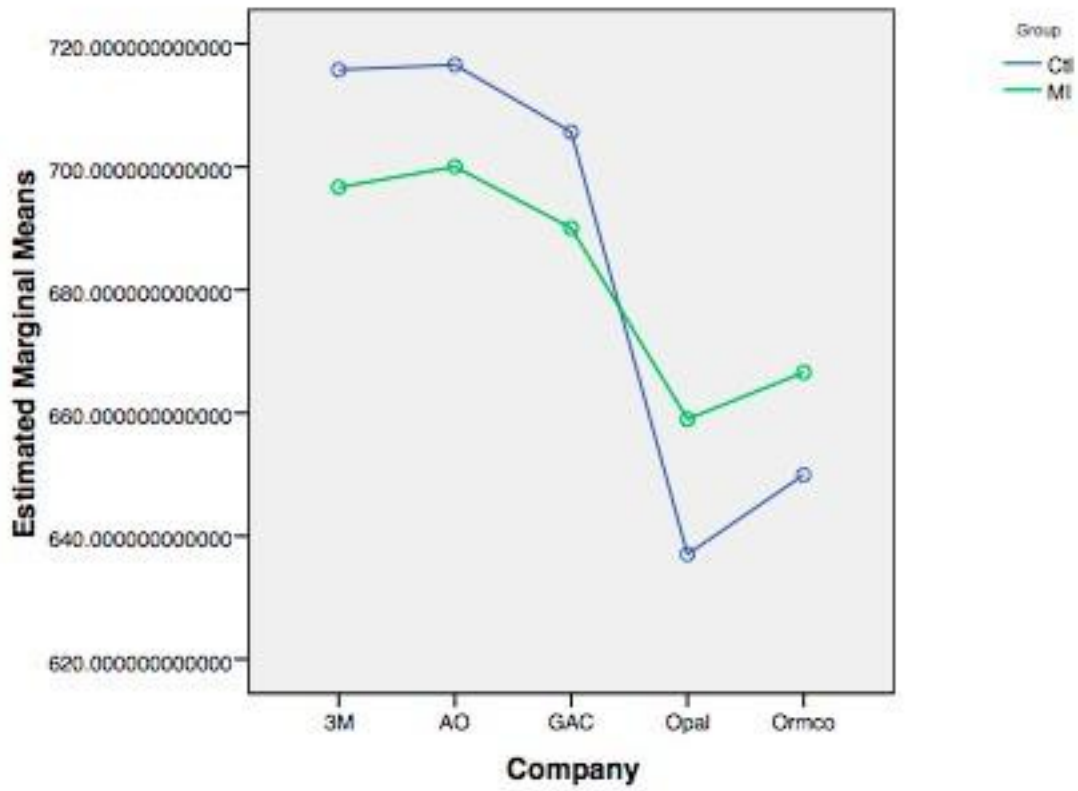


Figure 13. Estimated marginal means in MPa for Unloading YS.

8.0 DISCUSSION

The negative effects of fluoride on beta-titanium wires seem to be inherent to fluoride's reaction with titanium. Walker (2007) proposes that an increase in hydrogen ion concentration, and subsequent change in archwire behavior, is due to hydrogen release in a fluoride-treated wire that has been loaded past its elastic range. In other words, the increase in hydrogen ion concentration is causing the archwire to behave differently, as witnessed by the change in the unloading flexural modulus and yield strength, found in their study. It has also been cited above that fluoride negatively affects corrosion resistance, tensile strength, and fracture mode for beta-titanium archwires. Therefore, increasing hydrogen ion concentration by loading a fluoride-treated archwire past its elastic range, say with a loop or finishing bend, can lead to expression of these other negative effects.

The results of the current study showed that there was no statistically significant difference between the control and MI Paste groups for Loading E, Loading YS, and Unloading YS. However, there was a statistically significant difference for Unloading E. The control group had a mean Unloading E value of 18572.43 MPa, while the MI Paste group had a mean value of 18215.81 MPa. Although statistically significant, this difference is probably not clinically significant because the MI Paste value is only 1.92% different from the control value. The results obtained in the current study cannot be compared to values found in other studies,

because no other studies have been done to test beta-titanium wires treated with CPP-ACP. However, the results can be contrasted with Walker's 2007 study, which three-point-bend tested beta-titanium wires treated with fluoride, and reported a statistically significant difference between control and experimental groups for both unloading modulus and yield strength.

The current investigation had a very high power value, due to low variance and a high specimen number. However, being an *in vitro* experiment, it had limitations because it was impossible to exactly simulate clinical conditions. In the experimental set up there was no exposure to saliva or fluoride, which may have altered the results. Also, the experimental wires were treated with MI Paste for 9 hours to simulate two three-minute applications of MI Paste everyday for 3 months, but in the clinical situation there would be shorter repeated exposures to the MI Paste. Due to the limitations in this study, it is difficult to draw any realistic conclusions about the benefit of using MI Paste versus prescription strength fluoride on orthodontic patients. However, that was not the purpose of this study. The purpose of this study was to test whether CPP-ACP had any effect on the unloading properties of beta-titanium wires. There have not been any studies conducted to test the properties of beta-titanium wires treated with a fluoride alternative. Therefore, this study merely serves as a springboard for further studies that need to be done before any clinically relevant conclusions can be derived on the topic. Future studies could include shorter repeated exposures of MI Paste diluted with some amount of artificial saliva, with artificial saliva and/or fluoride exposures in between the MI Paste exposures. Eventually, *in vivo* studies would be the most clinically relevant. If future studies concur with the results found in this investigation, then CPP-ACP might be a viable alternative to prescription strength fluoride.

9.0 SUMMARY

Topical fluoride has been routinely advocated and prescribed for the prevention of white spot lesions during orthodontics. However, studies have shown that prescription strength fluoride can have destructive effects on beta-titanium orthodontic wires, including negatively affecting the unloading forces of beta-titanium wires. Changing the mechanical properties of these archwires can have a deleterious effect on the progression of orthodontic treatment. CPP-ACP has been shown to remineralize subsurface enamel lesions and aid in the prevention of white spot lesions in orthodontics, but its effect on beta-titanium wires has not been tested. Therefore, the objective of this in vitro study was to compare load-deflection curves of several beta-titanium wires between controls and those treated with CPP-ACP to determine what effects, if any, CPP-ACP has on beta-titanium load-deflection properties, specifically loading and unloading flexural modulus and yield strength.

Ten .017x.025 inch beta-titanium wires from each of the five manufacturers underwent the experimental treatment, and the other 50 underwent the control treatment, making it a total of 100 wires that were tested, using a three-point-bend test apparatus. The experimental wires were exposed to MI Paste (CPP-ACP) for nine hours, to simulate three months of two three-minute MI Paste applications, before bend-testing in a water tank of 37 degrees Celsius and the other half were exposed to distilled water and tested as controls, making it a total of 100 wires that were

tested. A 2x5 multivariate analysis of variance was conducted to analyze the data. If the multivariate test revealed statistically significant differences between at least two of the measures for companies and/or groups, then a post hoc univariate test was done to assess which measures were statistically different from the others. Further post hoc testing was done, using a Student Newman-Keuls pairwise comparison at a 0.05 level, to determine which companies were different from one another.

The results showed that there were no statistically significant differences between the control group and experimental group for loading elastic modulus, 0.2% offset yield strength, or unloading 0.2% offset yield strength. There was a statistically significant difference for unloading flexural modulus, but it is most likely not a clinically significant difference, given there was only a 1.9% difference between the control and experimental mean values. The results suggest that CPP-ACP does not have any clinically significant effects on beta-titanium wires, but further studies need to be done before any clinically relevant conclusions can be drawn.

10.0 CONCLUSIONS

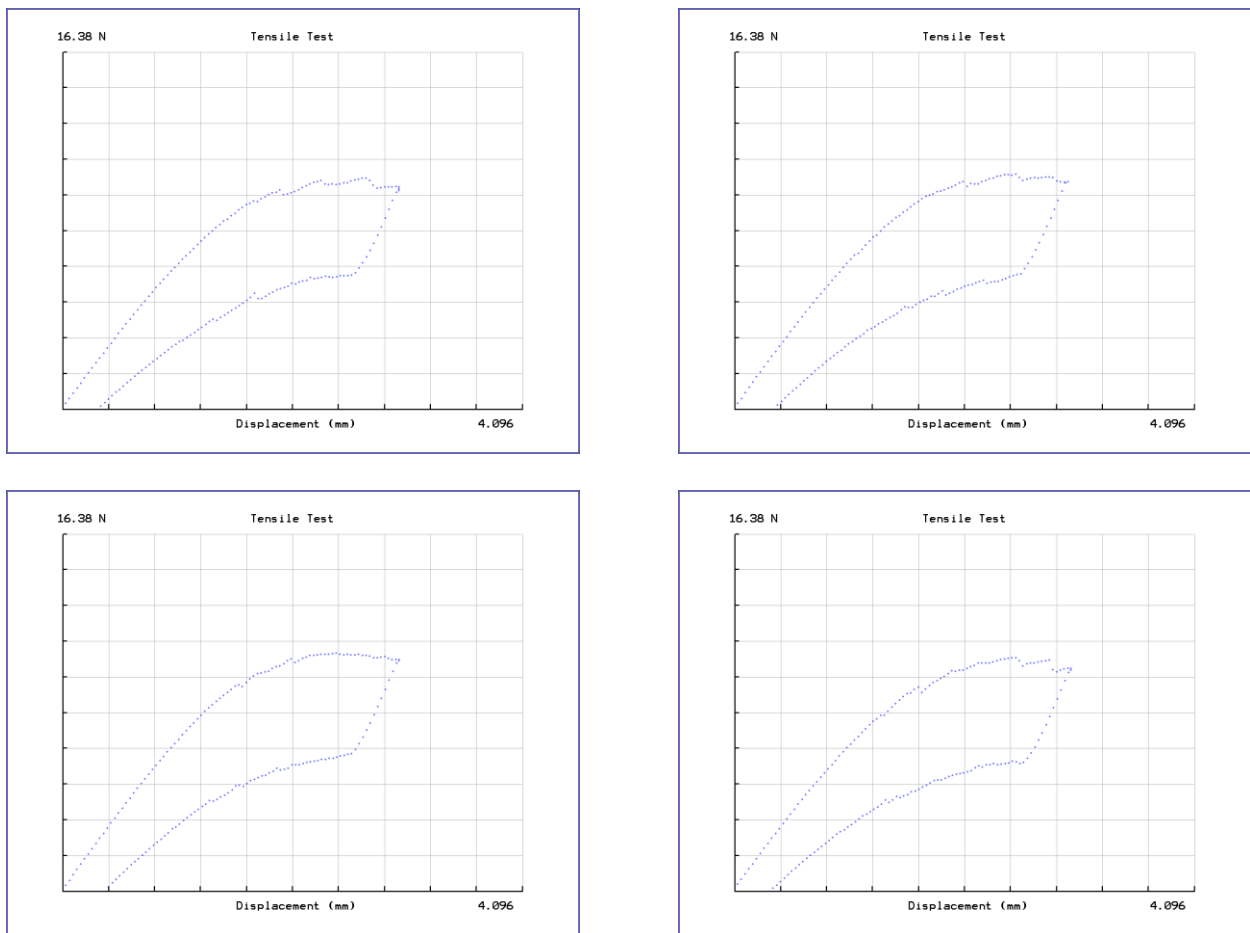
The following conclusions can be drawn from this study:

1. CPP-ACP (found in MI Paste) did not have a statistically significant effect on the loading modulus of elasticity or 0.2% offset yield strength of a .017x.025 inch beta-titanium orthodontic archwire.
2. CPP-ACP did not have a statistically significant effect on the unloading 0.2% offset yield strength of a .017x.025 inch beta-titanium orthodontic archwire, deflected past its elastic limit.
3. CPP-ACP did have a statistically significant effect on the unloading flexural modulus of a .017x.025 inch beta-titanium orthodontic archwire, deflected past its elastic limit, however this is most likely not a clinically significant difference.

APPENDIX A

LOAD DEFLECTION CURVES

Figure 14. Load deflection curves for ten control 3M Unitek wires:



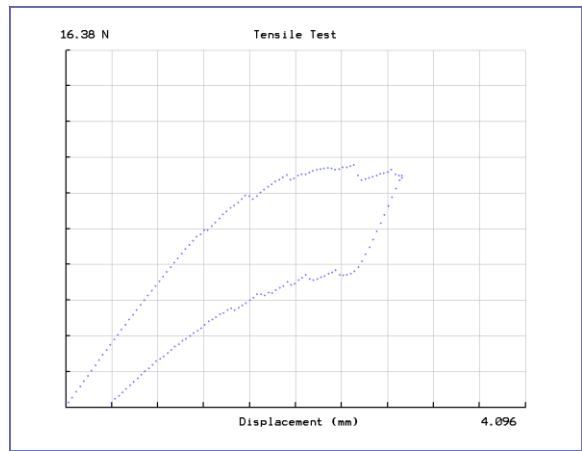
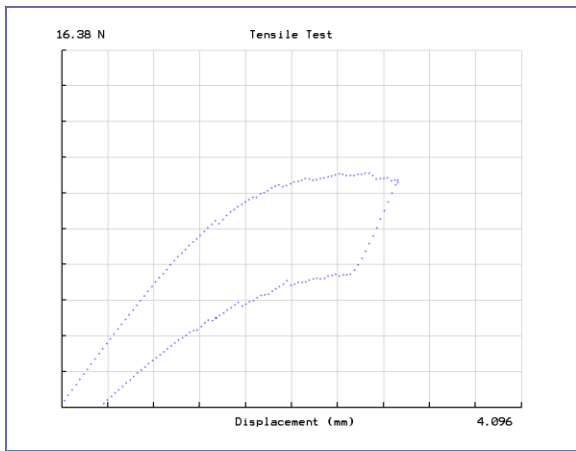
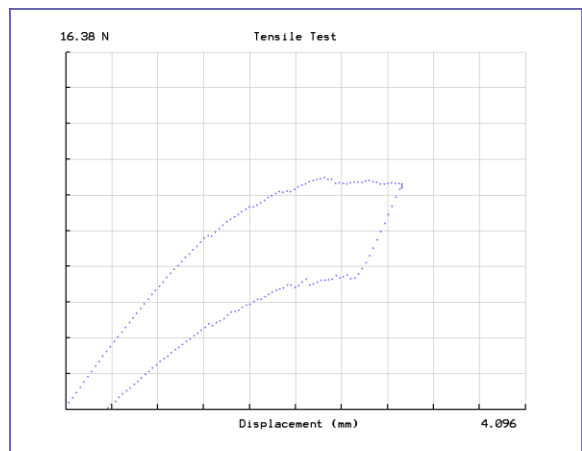
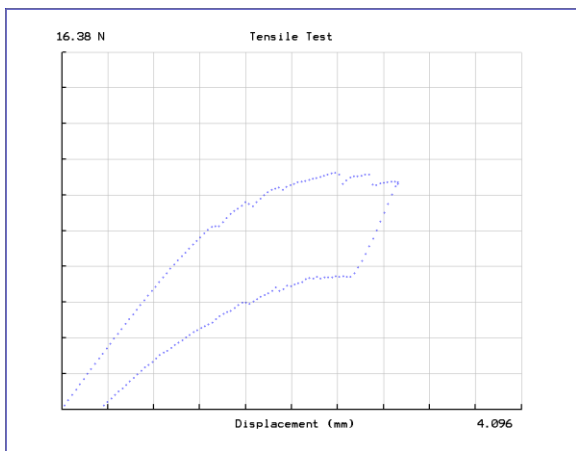
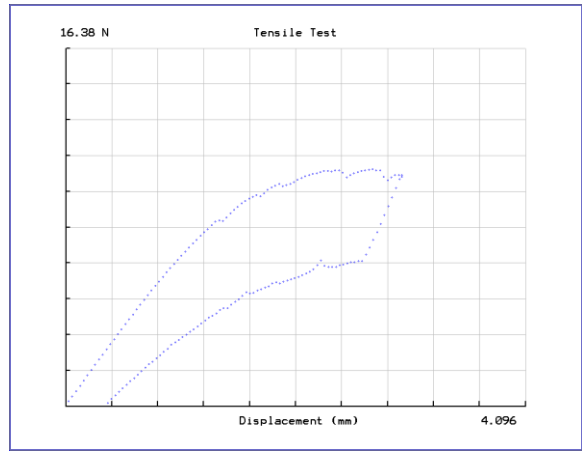
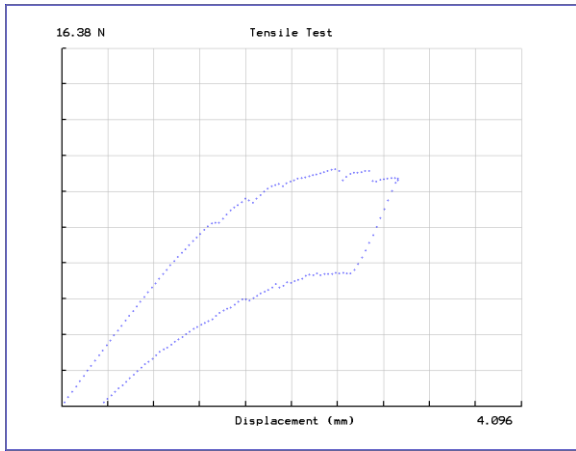
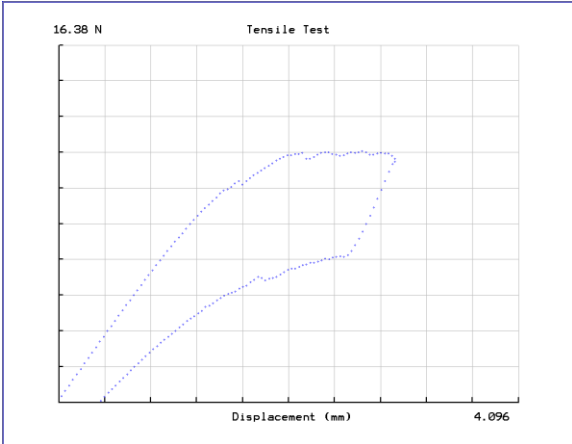
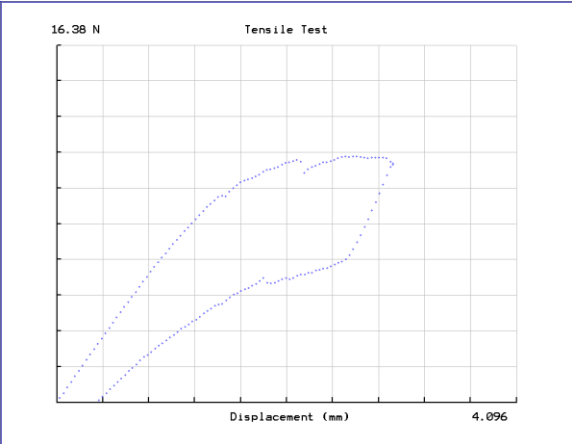
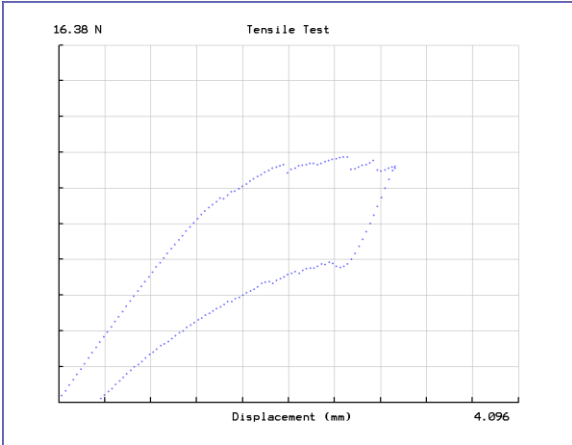
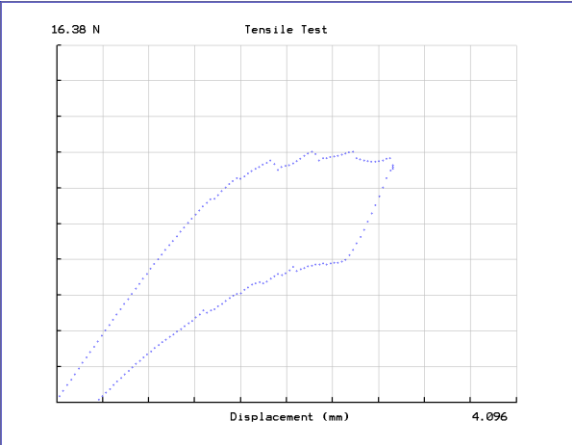
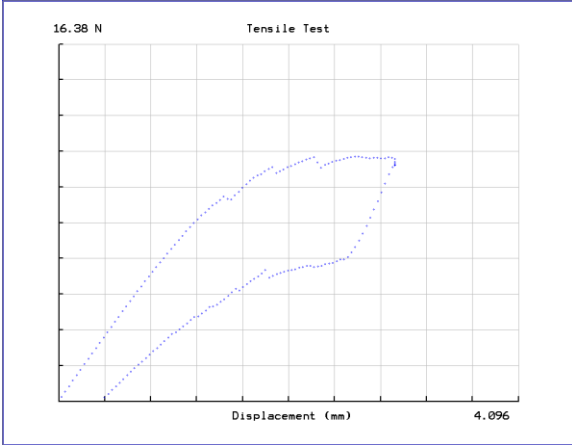
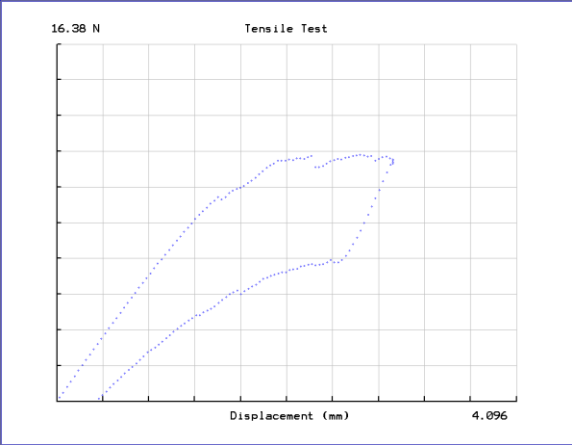


Figure 15. Load deflection curves for ten control American Orthodontics wires:



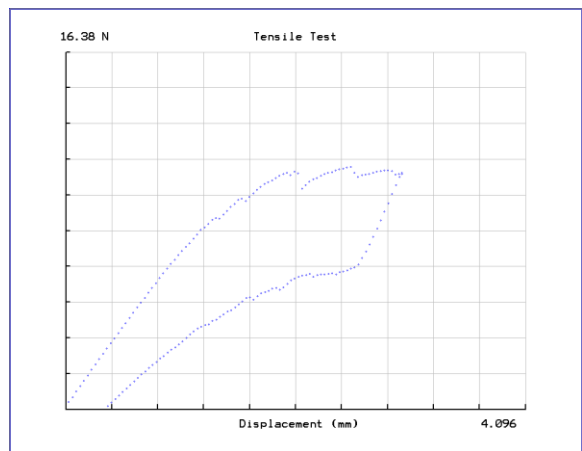
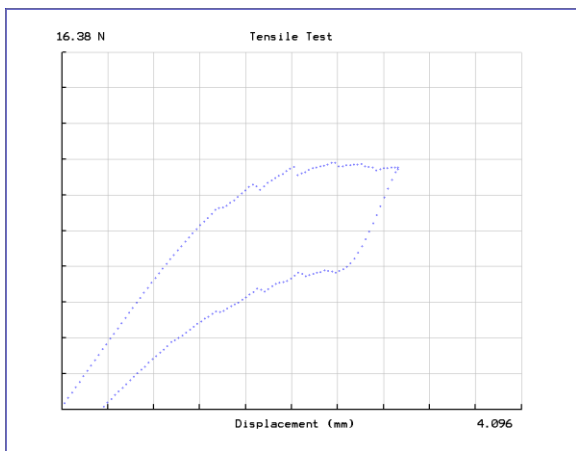
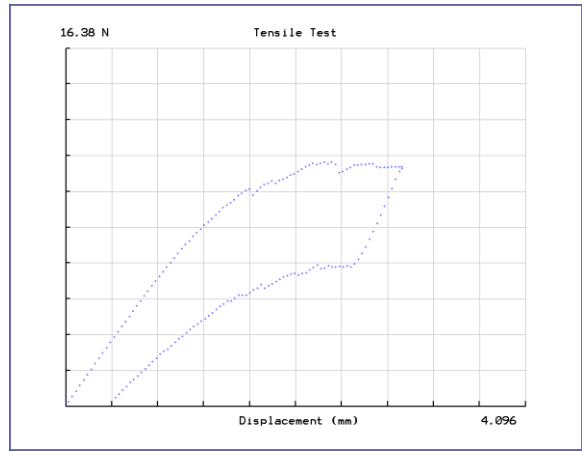
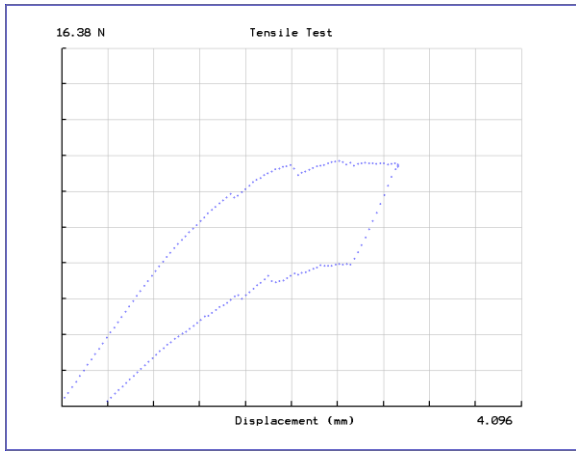
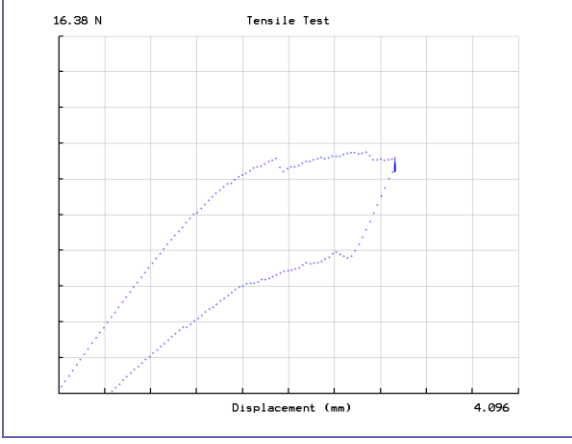
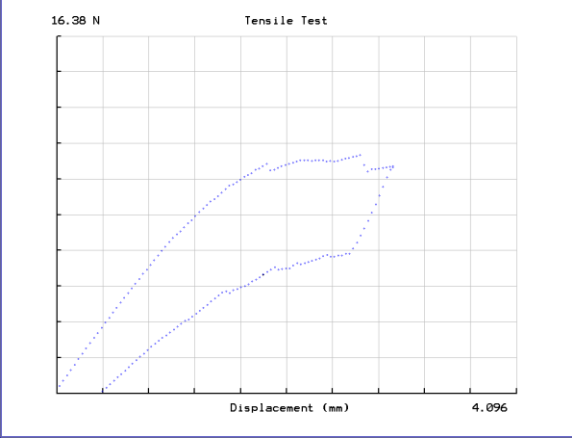
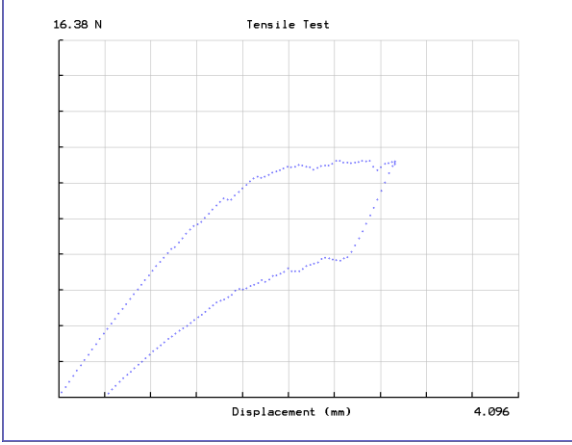
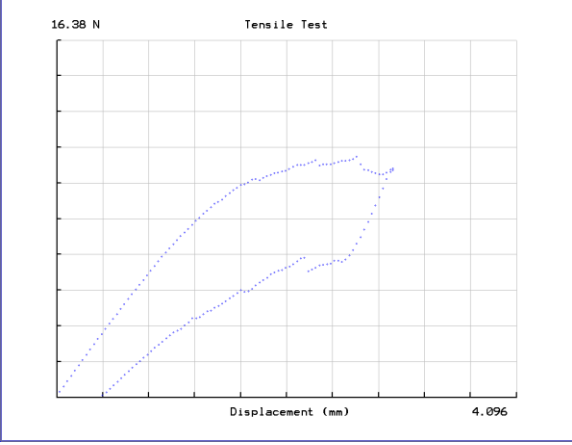
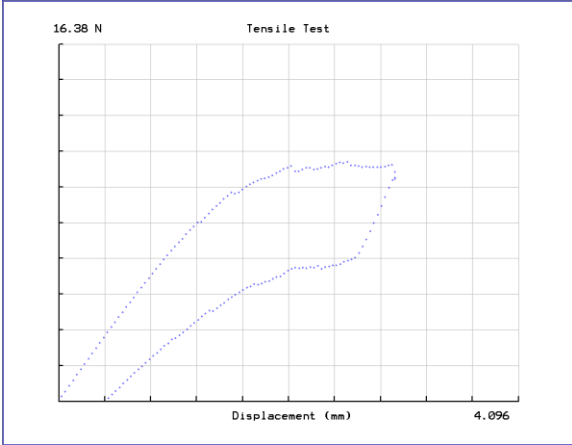
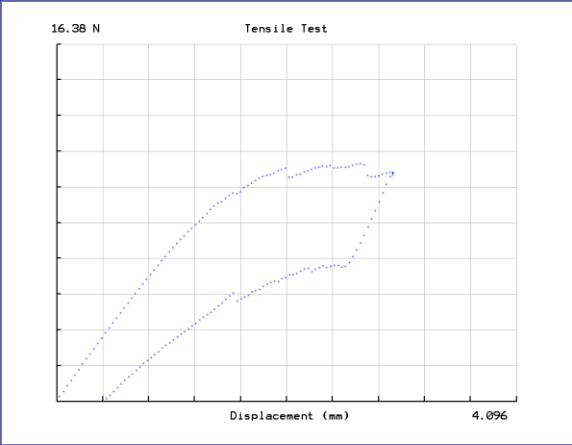


Figure 16. Load deflection curves for ten control GAC wires:



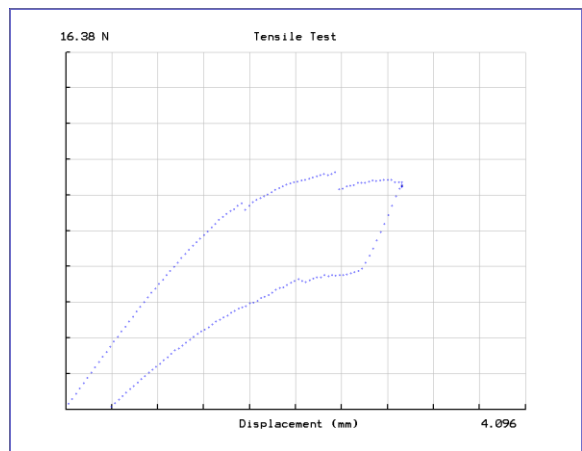
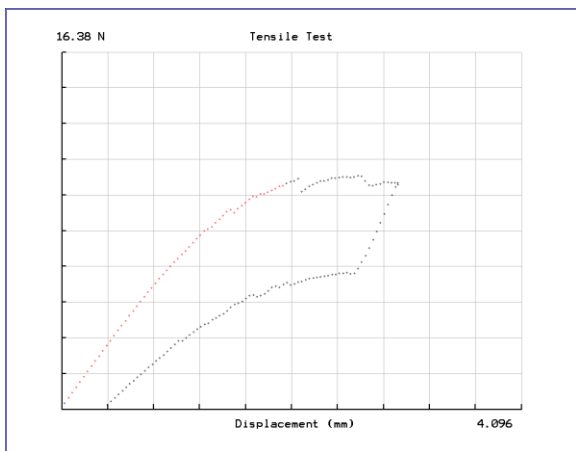
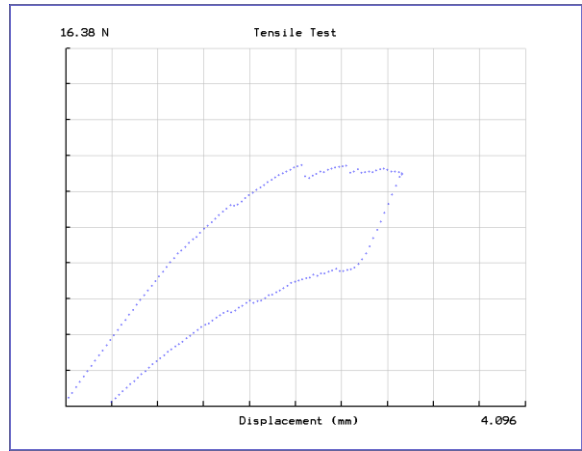
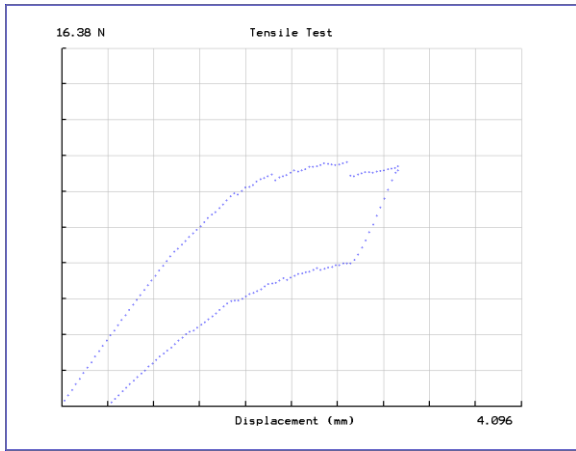
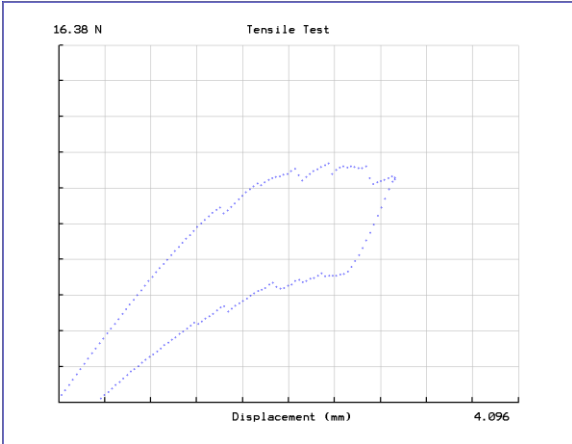
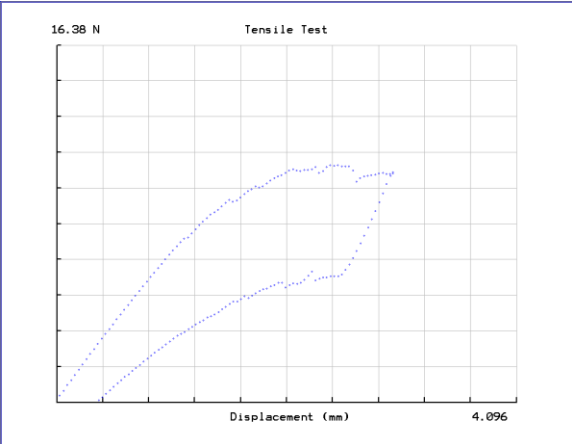
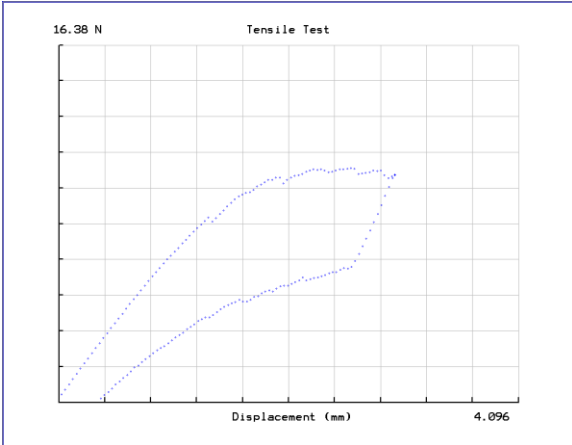
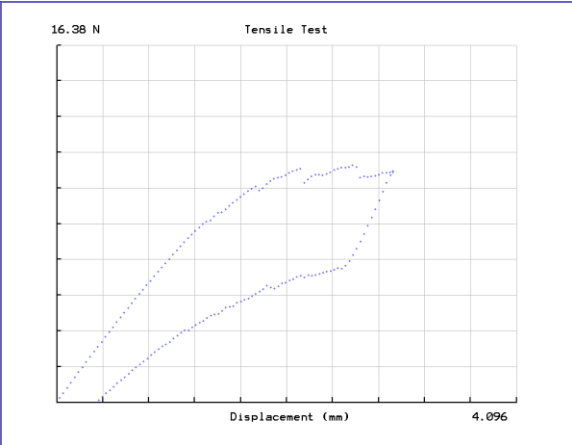
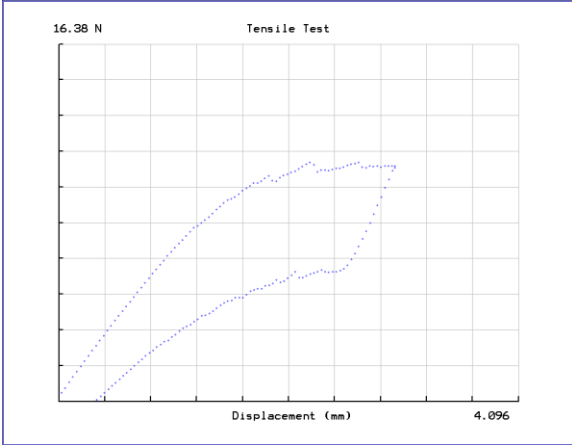
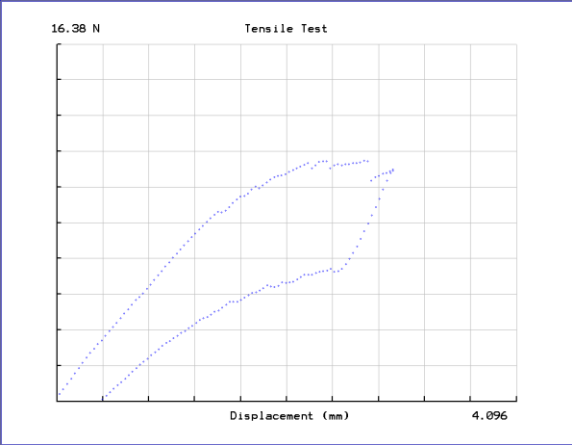


Figure 17. Load deflection curves for ten control Opal wires:



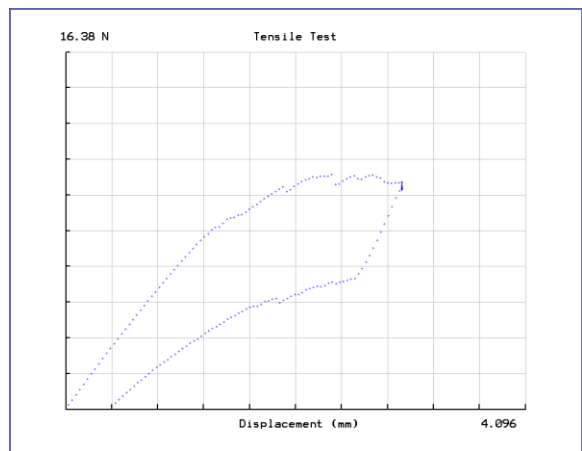
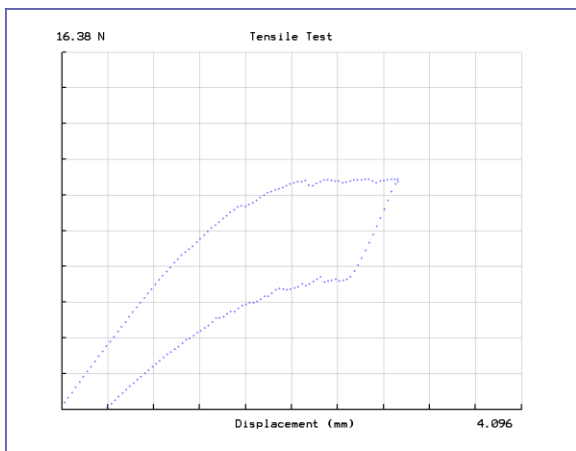
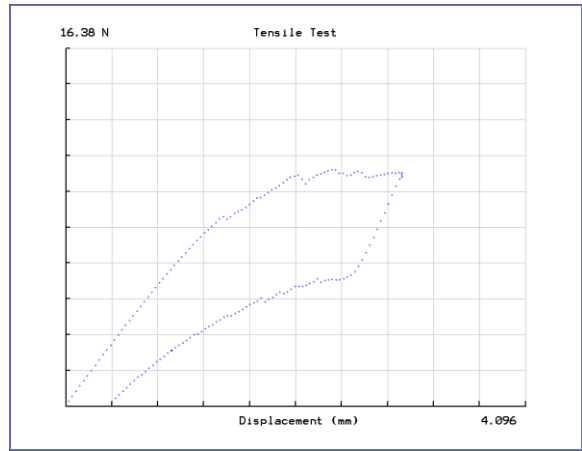
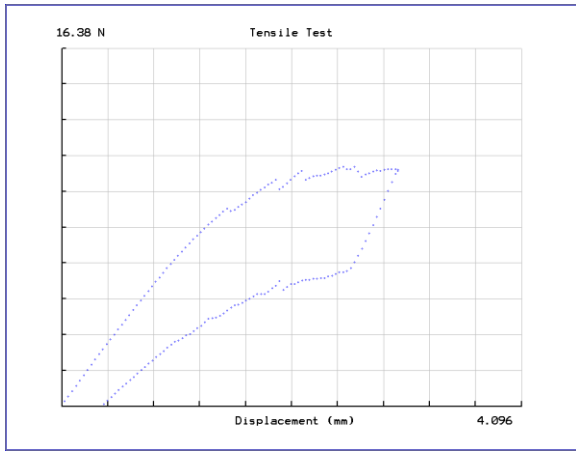
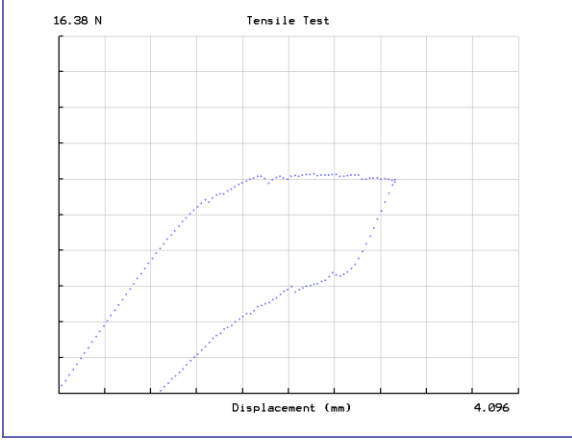
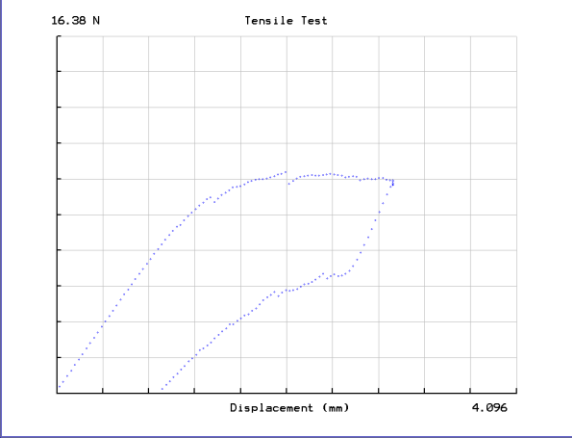
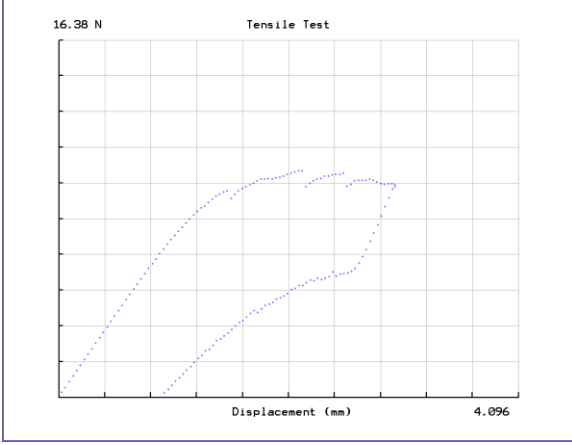
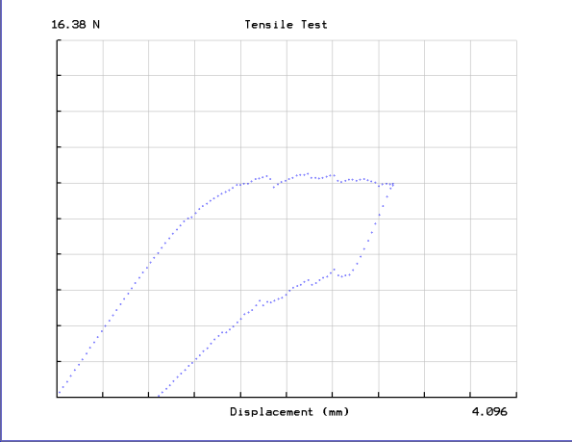
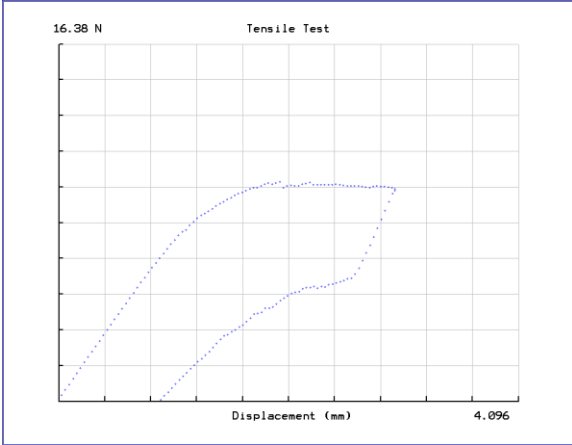
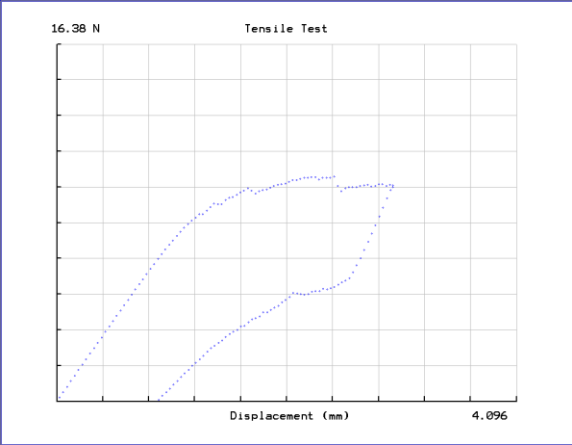


Figure 18. Load deflection curves for ten control Ormco wires:



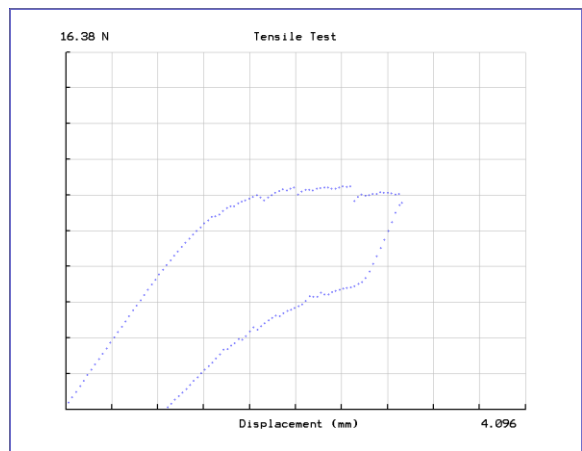
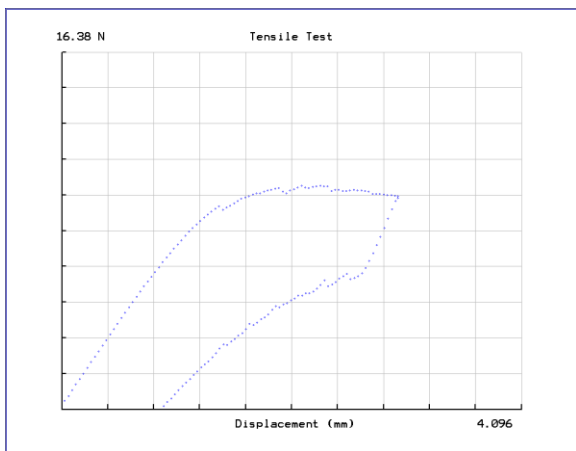
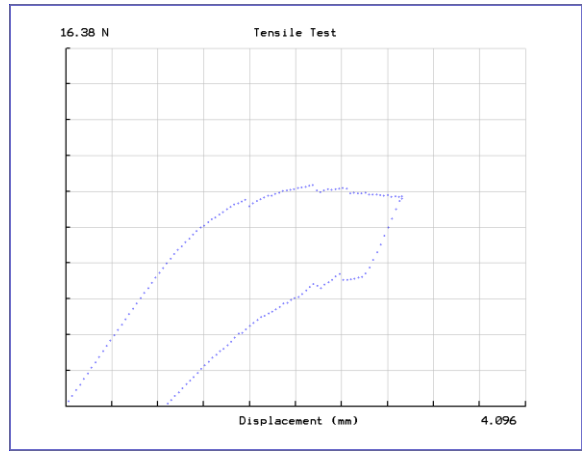
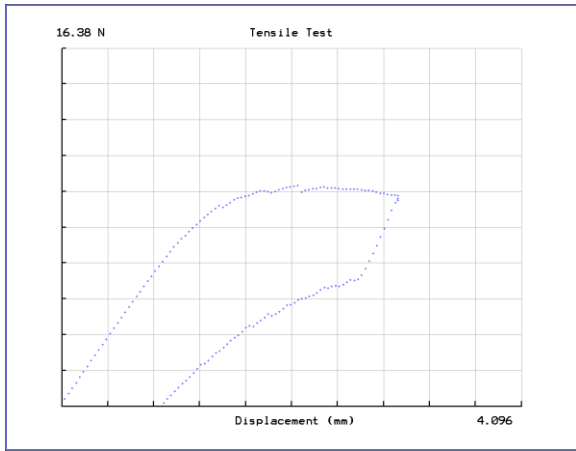
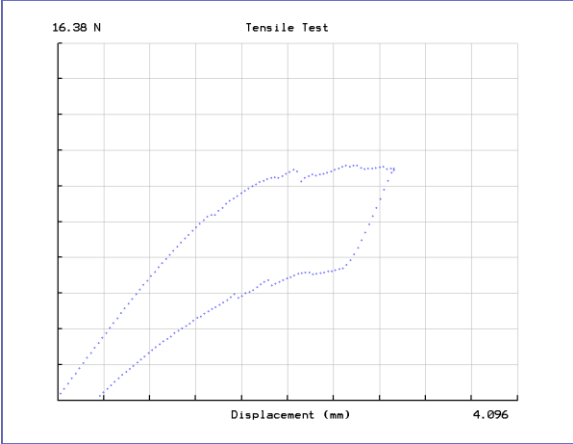
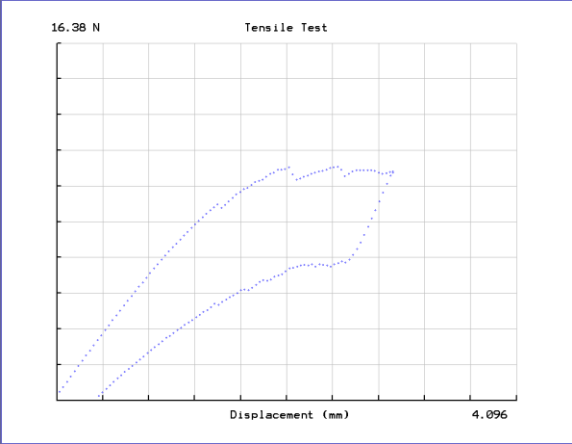
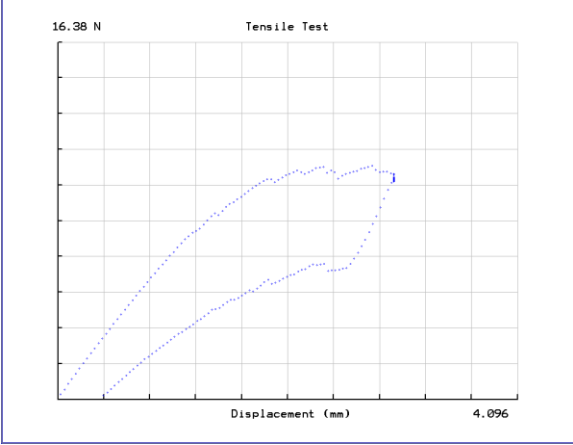
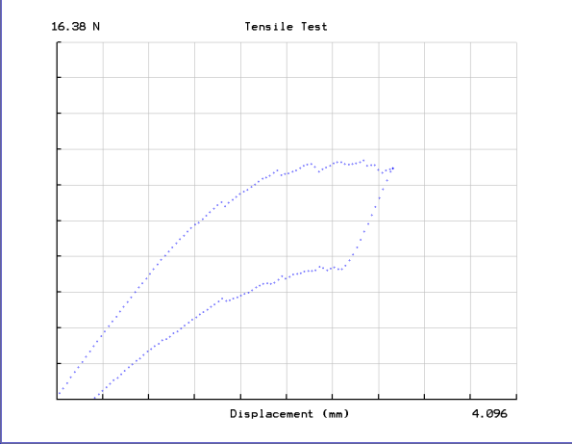
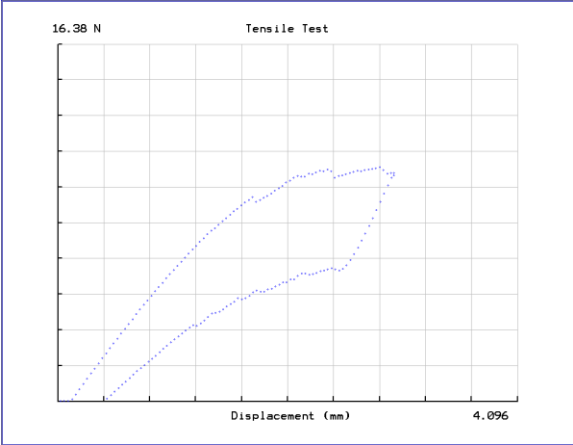
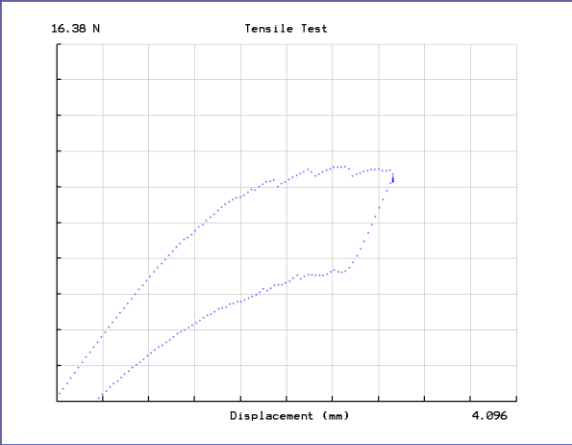


Figure 19. Load deflection curves for ten MI Paste 3M Unitek wires:



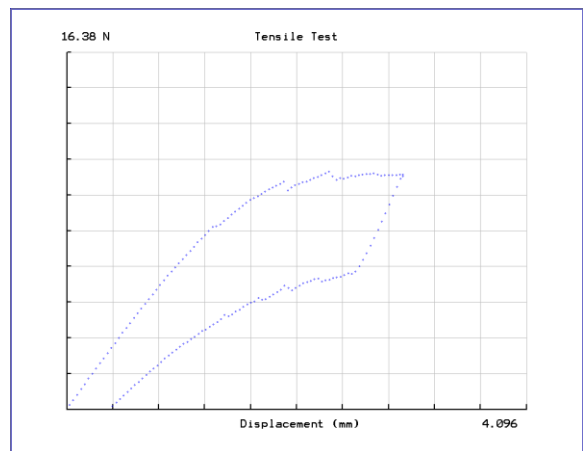
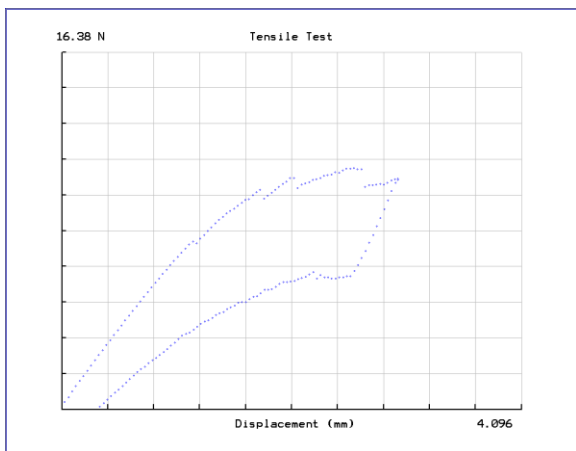
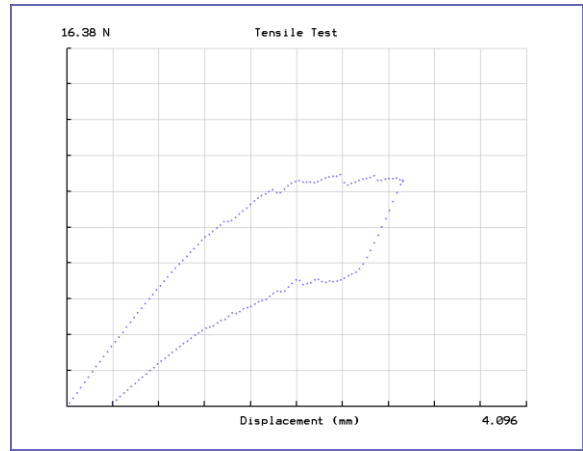
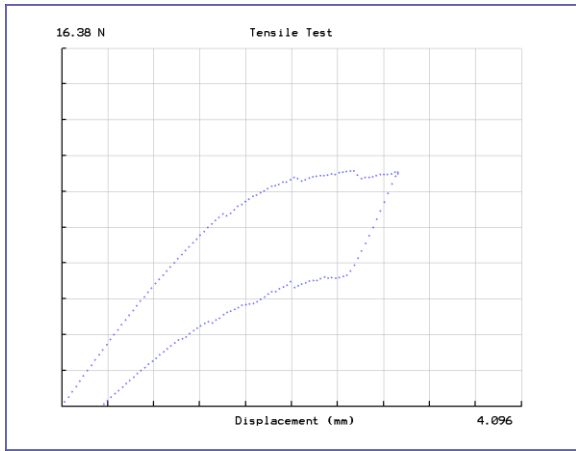
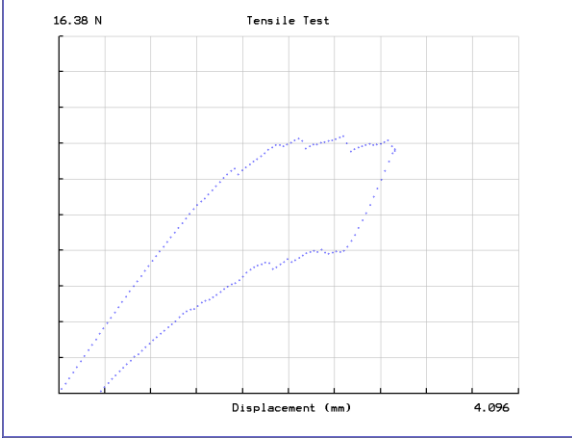
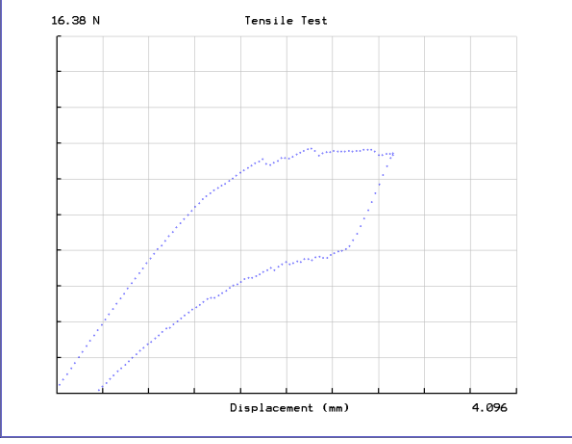
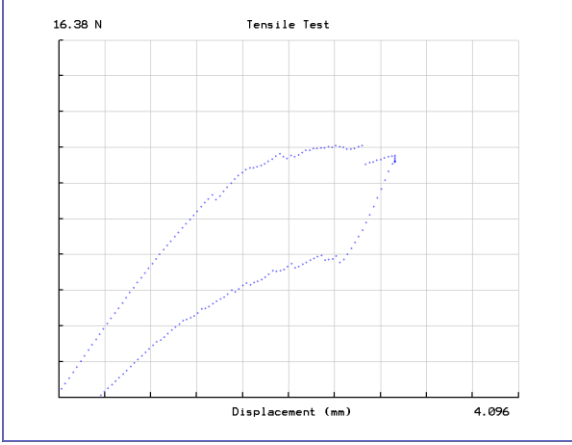
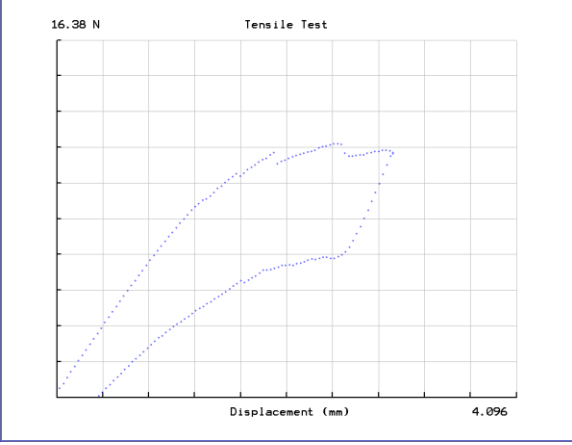
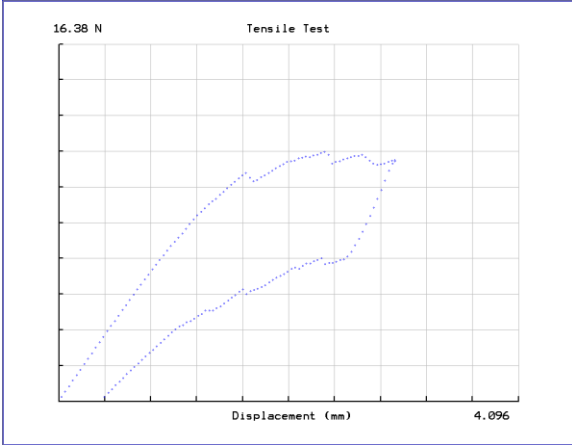
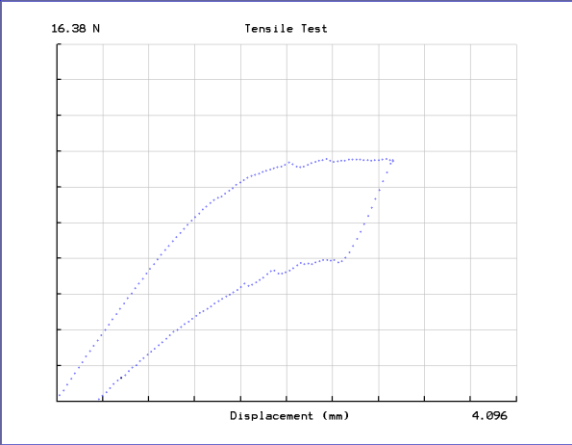


Figure 20. Load deflection curves for ten MI Paste American Orthodontics wires:



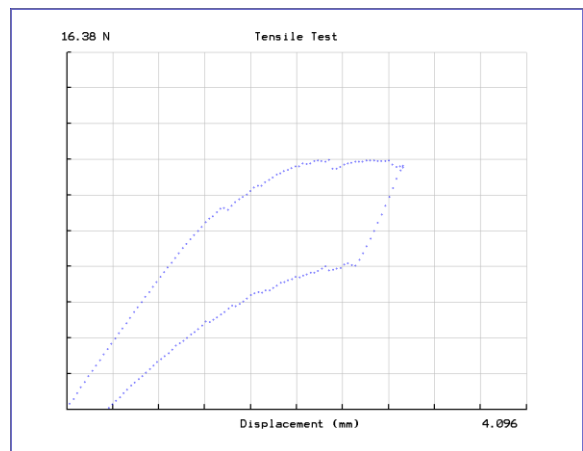
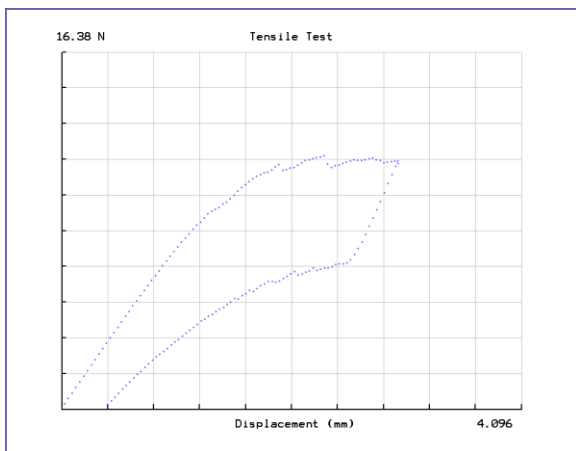
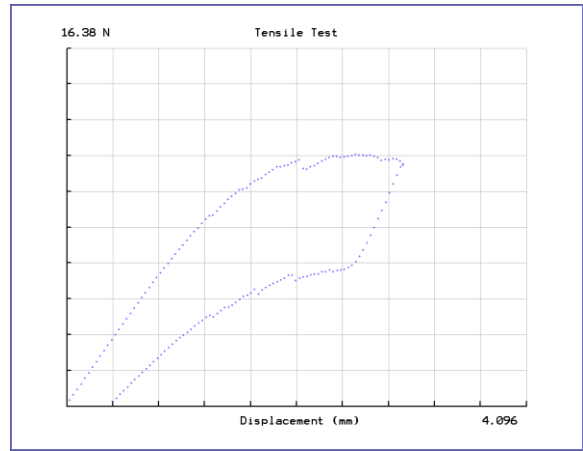
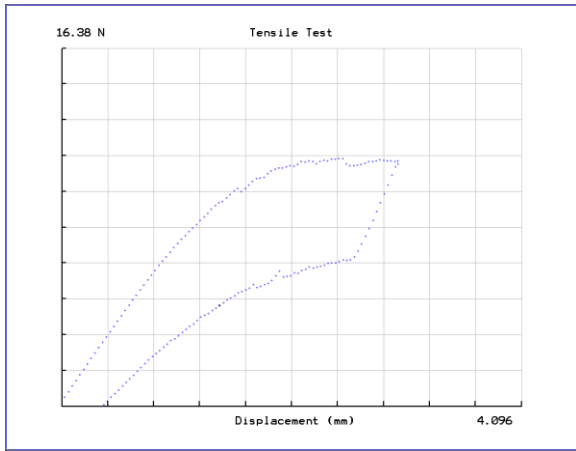
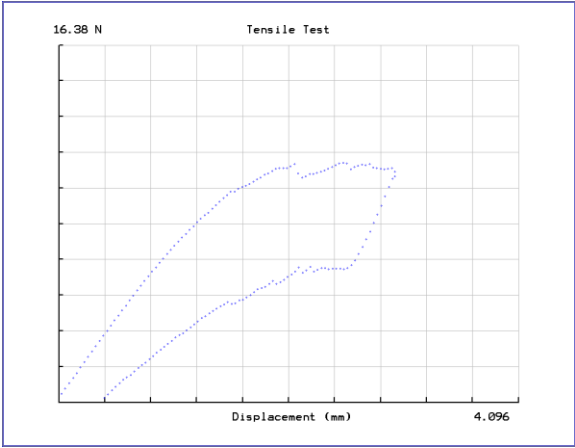
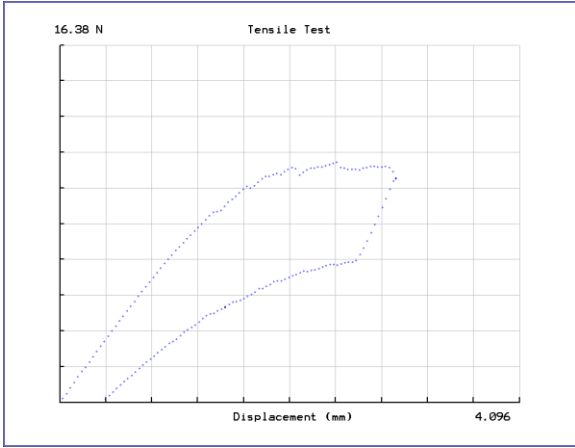
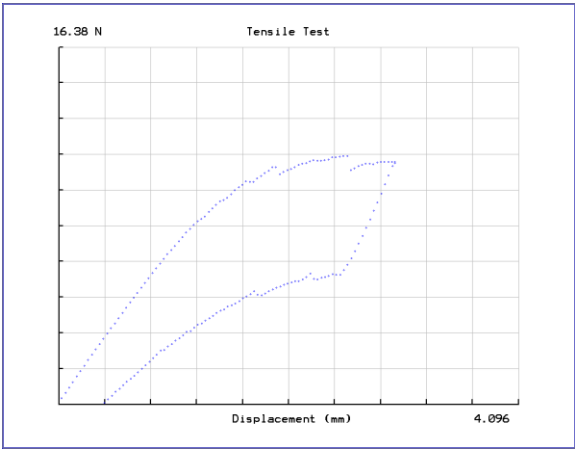
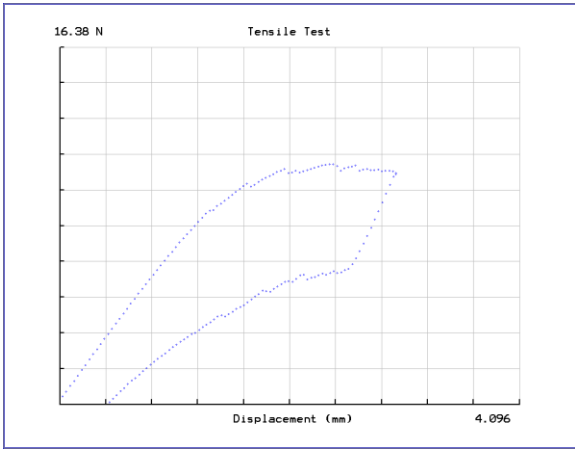
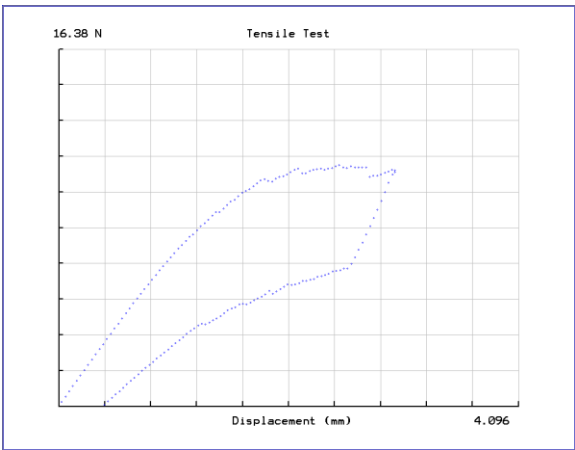
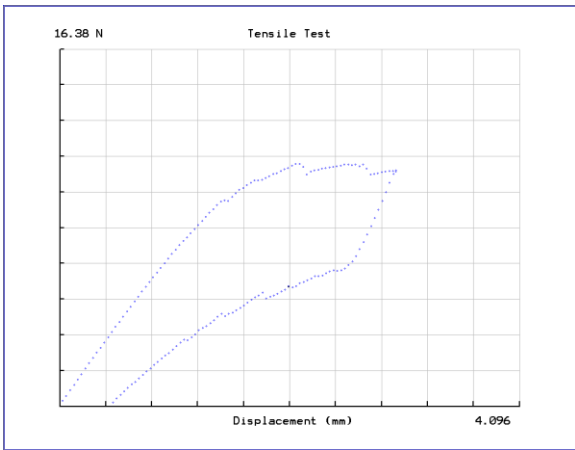


Figure 21. Load deflection curves for ten MI Paste GAC wires:



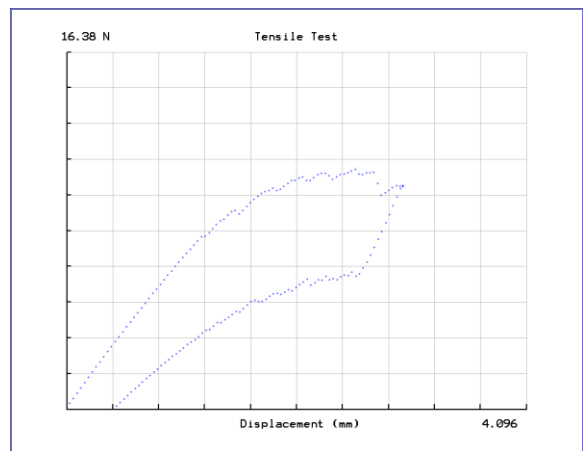
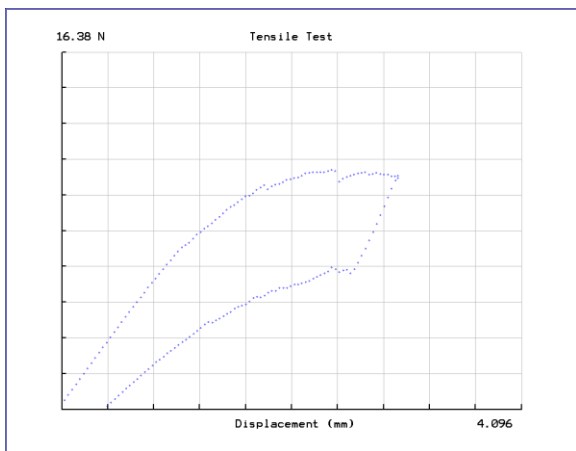
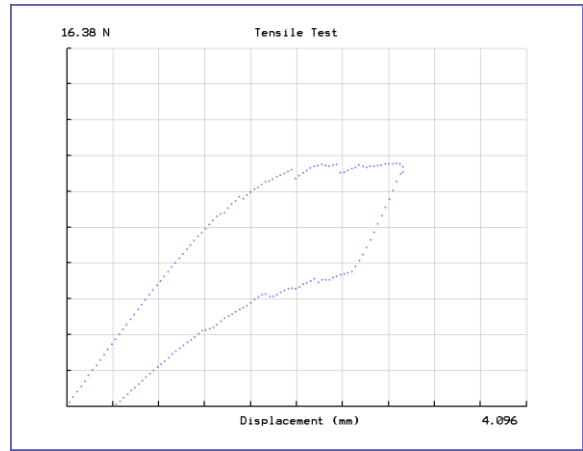
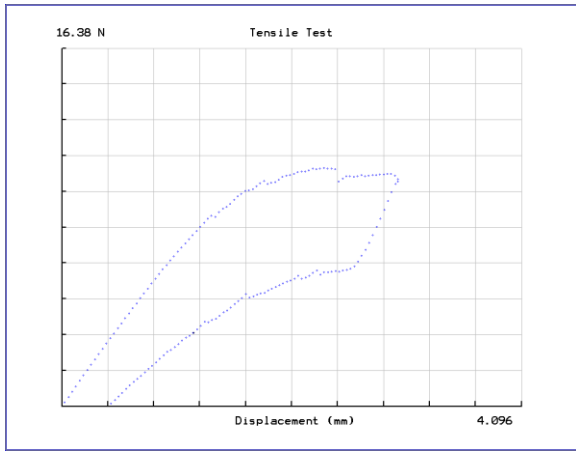
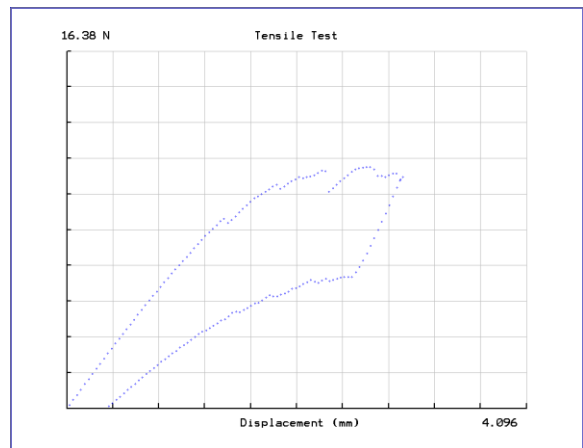
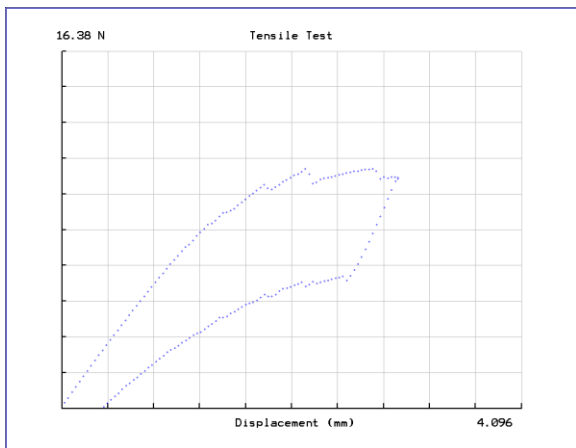
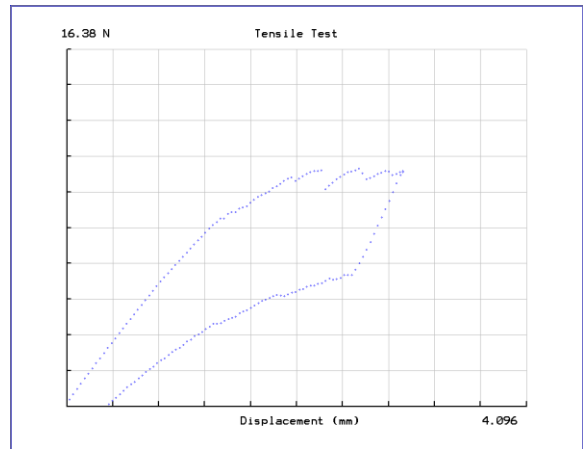
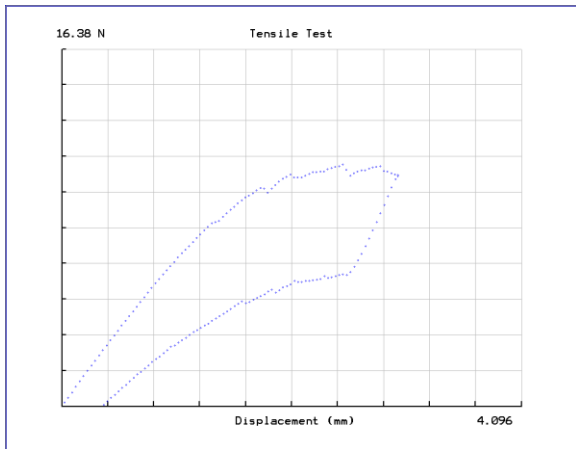
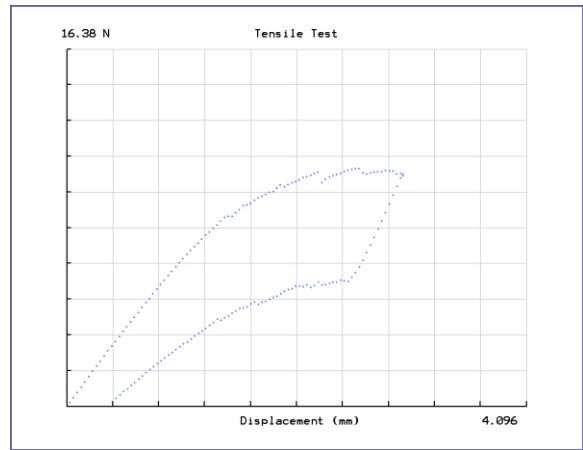
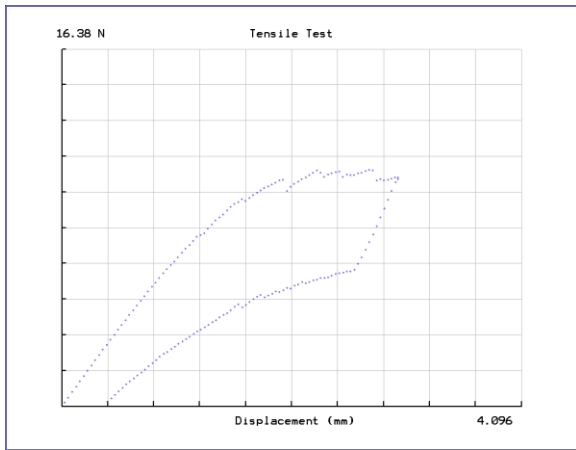


Figure 22. Load deflection curves for ten MI Paste Opal wires:



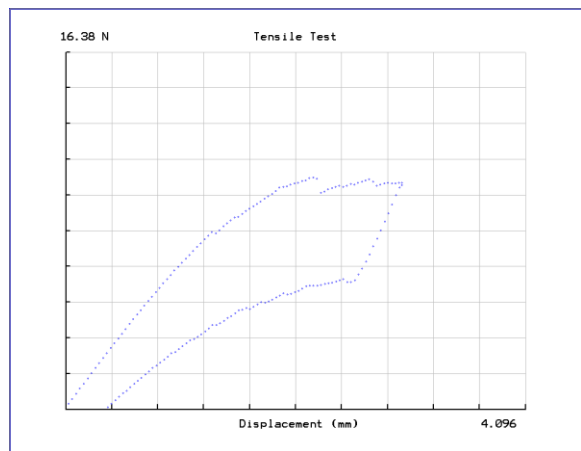
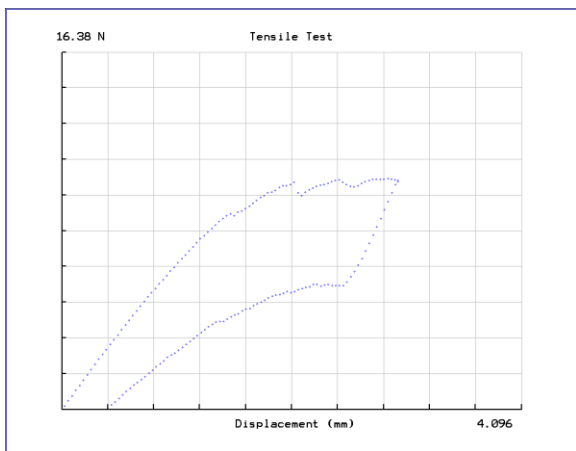
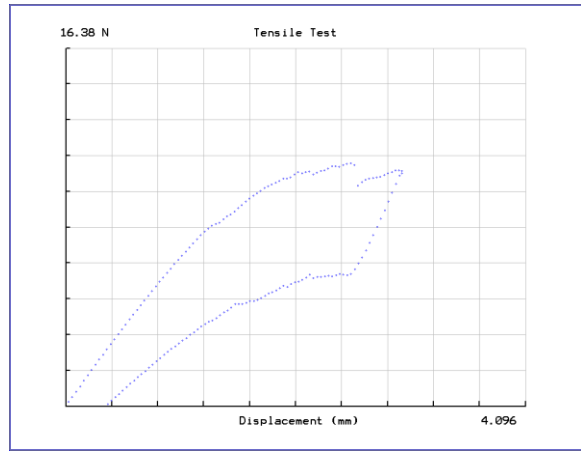
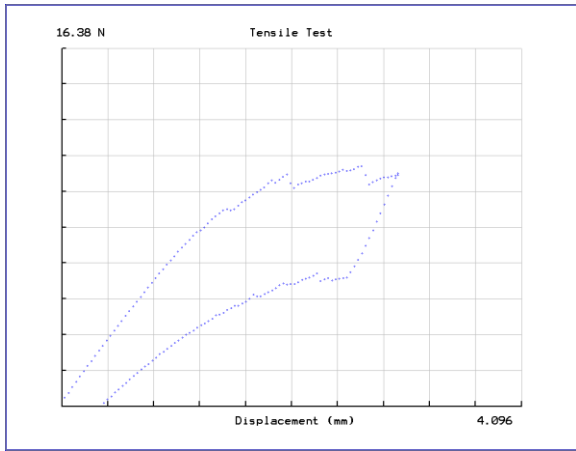
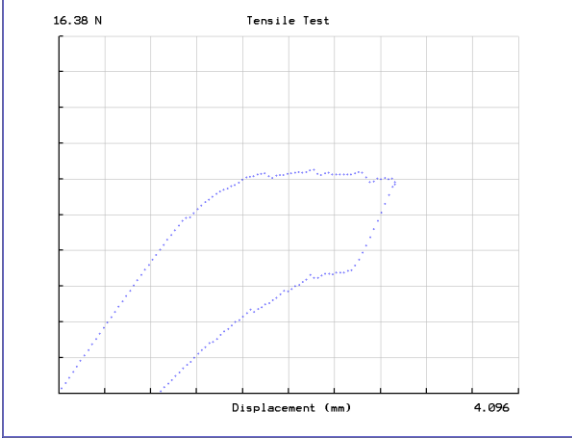
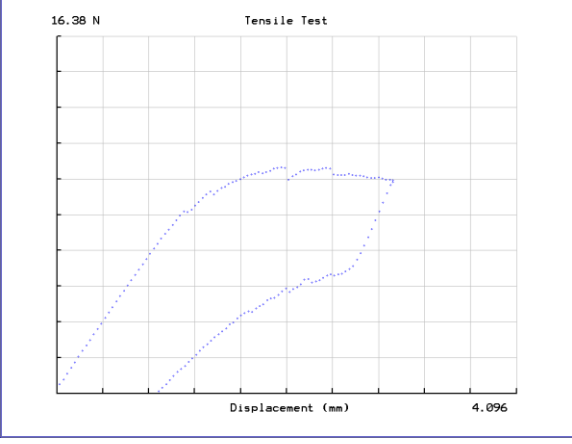
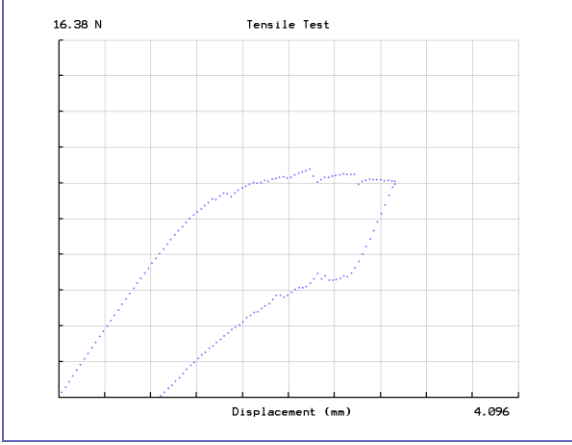
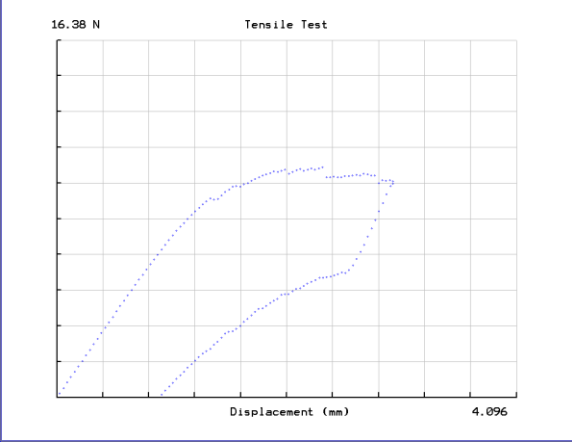
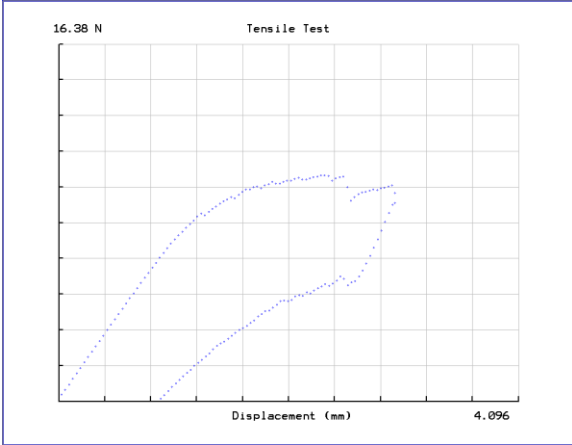
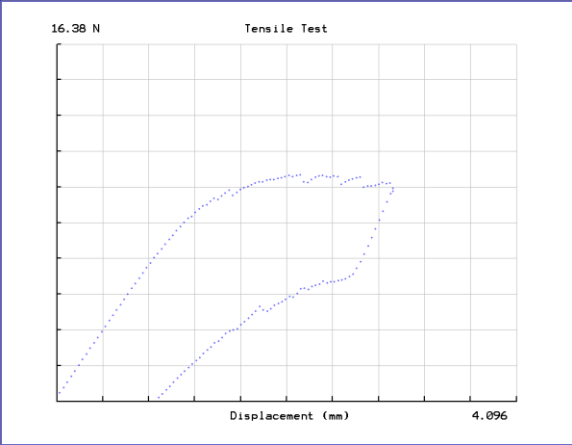
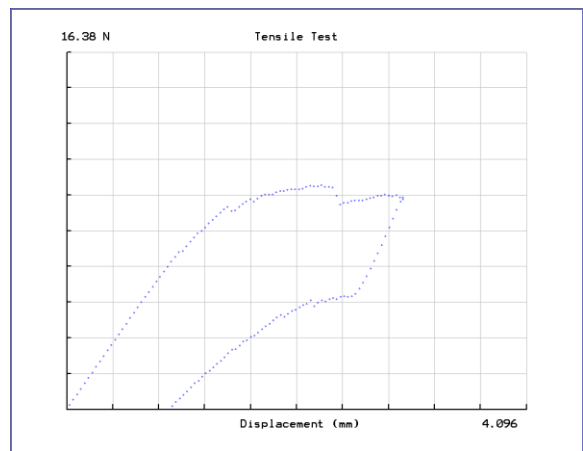
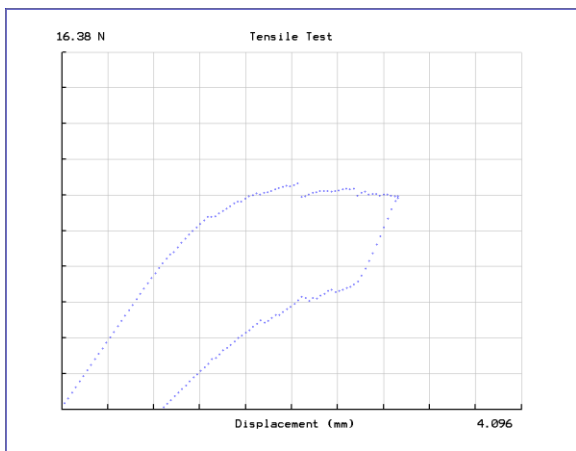
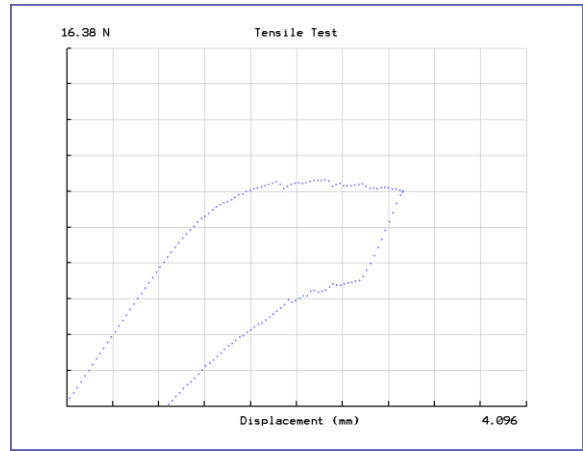
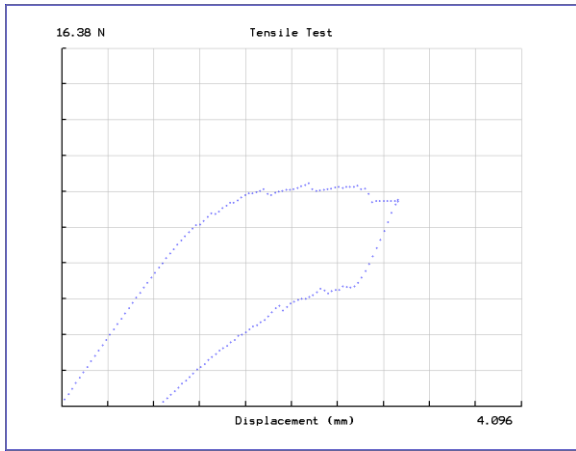


Figure 23. Load deflection curves for ten MI Paste Ormco wires:





APPENDIX B

FORMULAS

Formula used for calculating Modulus of elasticity:

$$E = \frac{Fl^3}{4dbh^3}$$

Where F is force, l is the length of archwire between supports, d is displacement, b is width of the archwire, and h is the thickness of the wire.

Formula used for calculating Yield Strength or Stress:

$$\sigma = \frac{3Fl}{2bh^2}$$

Formula used for calculating 0.2% strain offset (solve for d):

$$\text{Strain} = .002 = \frac{6dh}{l^2}$$

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