3. Materials and Methods

3.1 Tooth Collection

One hundred premolar teeth within six months duration of extraction due to orthodontic reason were collected. Since the teeth were extracted due to orthodontic reasons, it would be reasonable to assume that the development of the dentine was similarly advanced for all teeth. Collected teeth were disinfected using 0.5 % Chloramines Trihydrate solution for one week. The soft and hard deposits were removed with an ultrasonic scaler (Peizon® Master 400, Switzerland).The teeth were then stored in distilled water in a refrigerator ($4^{o}C$) . Distilled water was changed regularly every week.

3.2 Tooth Selection

The selection procedure involved screening the teeth and discarding those teeth with gross caries and fractures. Teeth were examined under a stereoscopic microscope (MEIJI) at x 10 magnification in order to eliminate teeth that displayed cervical caries, radicular cracks or craze lines from the study. All the teeth were tested within six months of the extraction date.

3.3 Preparation of Class V composite restoration

Class V cavity was prepared 2 mm away from cementoenamel junction (CEJ) with 2 mm depth, 3 mm mesiodistal and 2 mm occlusal-cervical. All margins were beveled 45 degrees to the external tooth surface with ½ enamel thickness 1 mm all around (Figure 3.1 and 3.2).High-speed round diamond bur was used under water spray coolant (Figure 3.3). Cavity was finished with pear shaped slow speed bur (Figure 3.4). The depth, length and width were measured with periodontal probe. All preparations were made as

uniformly as possible, in relation to dimension, instrumentation, outline form and depth.

Only one operator involved in preparing the cavity in order to reduce variation.

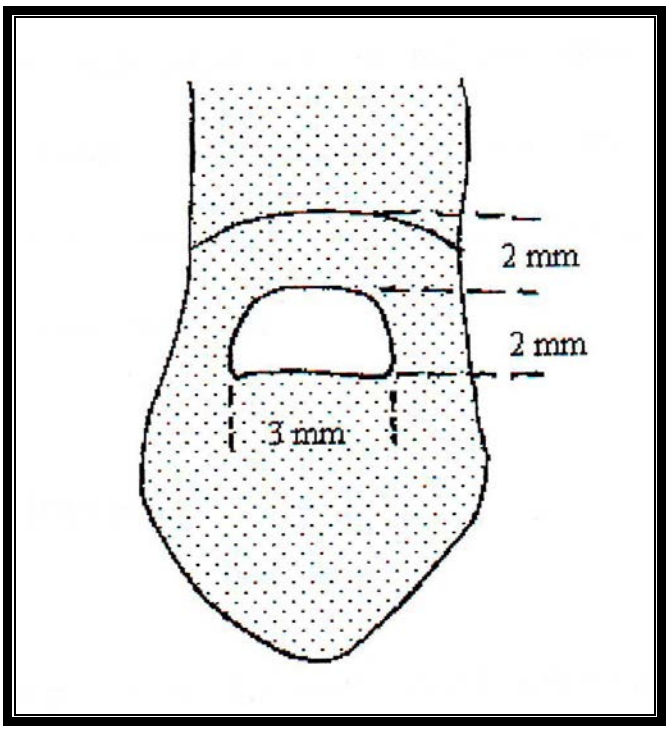


Figure 3.1: Schematic diagrams of Class V composite restoration-buccal view

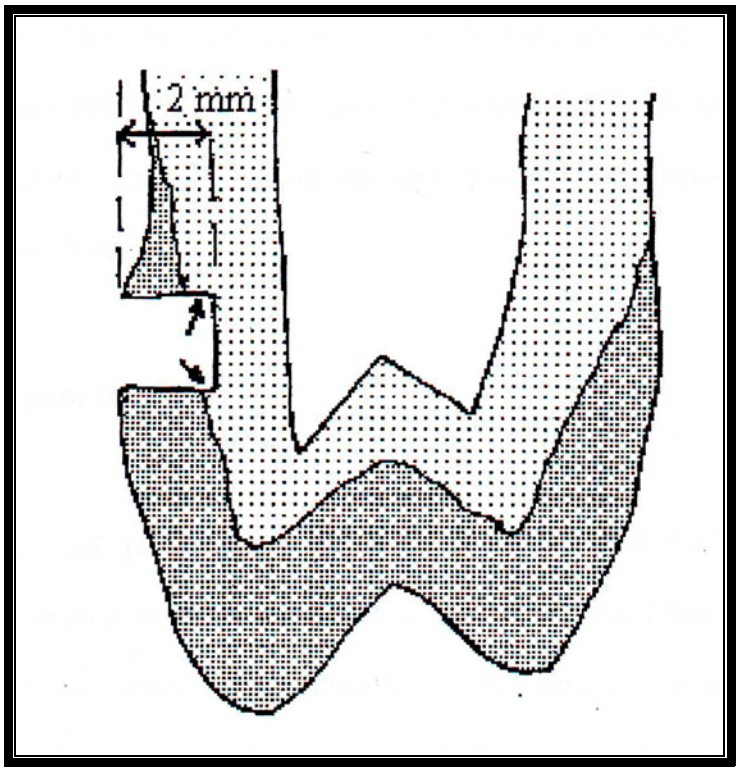


Figure 3.2: Schematic diagrams of Class V composite restoration-Cross sectional view

Figure 3.3: Preparation of Class V cavity

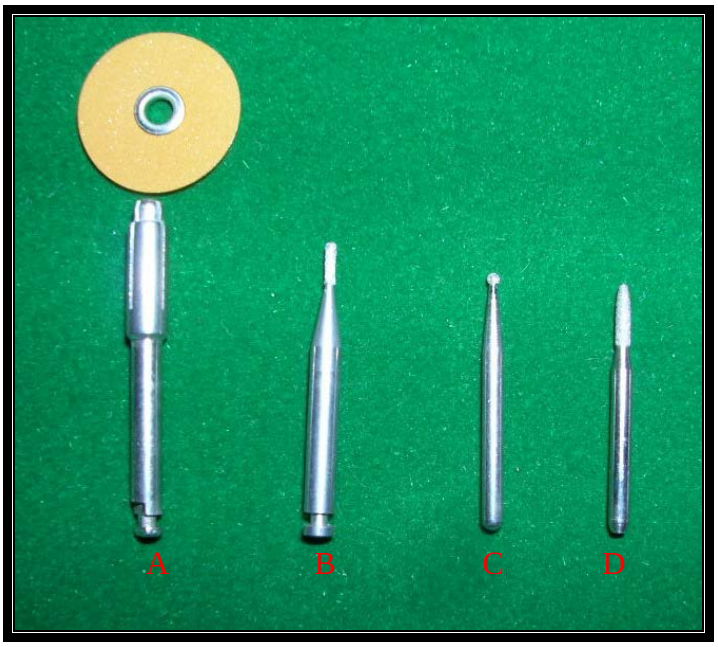


Figure 3.4: Soft lex disc & mandrell (A), slow speed pear shaped bur (B), high speed round bur (C) & high speed flamed shaped bur (D)

After Class V cavity was prepared, tooth was washed and dried 15 seconds with compressed air. Then, cavity was etched with Scotch Bond etching gel (Figure 3.5) for 15 seconds using small brush, washed for 10 seconds and dried with cotton pellet for 15 seconds. Adper Single Bond 2 (Figure 3.7) was applied to the cavity and it's surrounding bevel for 15 seconds, followed by gently air thin for 5 seconds to evaporate solvent and cured for 10 seconds with light cure machine (Starlight Pro, Mectron, Italy). Wave length of the light cure was checked with Curing Radiometer (model 100 P/N 10503, Demetron, KERR, USA) each time before used. The accepted length was above 450 nm. 3M Filtek^{TM} Z250 Universal Restorative Paste composite shade A3 (Figure 3.6) was then packed into cavity with plastic instrument no.1.Cellulose mylar strip was used to cover the material, and with light pressure using the plastic instrument, the material was adapted to the cavity and cured for 20 seconds. Restorations were polished with coarse, medium and fine soft lex discs (Figure 3.8) with 8-10 strokes for every disc using contra angle slow speed handpiece (Figure 3.9 and 3.10). Each disc was disposed off after every ten samples were polished. The polished samples then were stored in distilled water for one week at 37°C and 100% humidity in an incubator for complete polymerization.

Table 3.1: Compositions of Filtek Z250 Universal Restorative Paste

Material used	Manufacturer	Batch Number	Composition
3M Filtek ™ Z 250 Universal Restorative Paste (Shade A3)	3M,U.S.A.St.Paul, MN	370A	Silica (55%-65%) Zirconium oxide (15%-25%) Bisphenol a polyethylene glycol diether dimethacrylate (3%-8%) 11, 14-dioxa-2, 9-diazaheptadec-16-enoic acid, 4, 4, 6, 16-tetramethyl-10, 15-dioxo-2-(2-methyl-1-oxo-2-propenyl-oxy ethyl ester (3%-8%) Bisphenol a diglycidyl ether dimethacrylate (2%-6%) Methacryloxypropyltrimethoxysilane (2%-6%) Aluminium oxide (0.5%-1.5%) Triethylene glycol dimethacrylate (0.4%-1.2%)

Table 3.2: Compositions of Scotchbond Etching Gel

Material used	Manufacturer	Batch Number	Composition
Scotchbond ™ Etching Gel	3M,U.S.A.St.Paul, MN	7523	Water (55-65 %) Phosphoric acid (35-45 %) Amorphous silica (5-10 %)

Table 3.3: Compositions of Adper Single Bond 2

Material used	Manufacturer	Batch Number	Composition
Adper™ Single Bond 2	3M,U.S.A.St.Paul, MN	51202	Ethyl alcohol (30-40%) Bisphenol adiglycidyl dimethacrylate (15%-25%) 2-hydroxyethyl methacrylate (10%-20%) Glycerol 1.3 dimethacrylate (5%-15%) Copolymer of acrylic & itaconic acids (5%-15%) Diurethane dimethacrylate (2%-8%) Water (2%-8%)

Figure 3.5: Scotchbond ™ Etching Gel

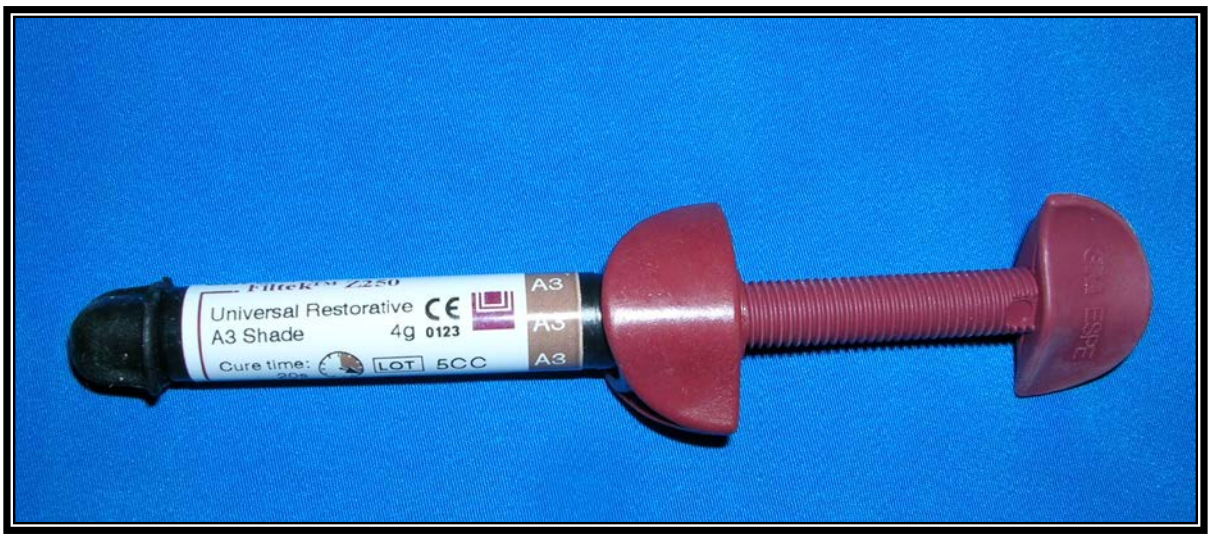


Figure 3.6: 3M Filtek ™ Z 250 Universal Restorative Paste

Figure 3.7: Adper ™ Single Bond 2

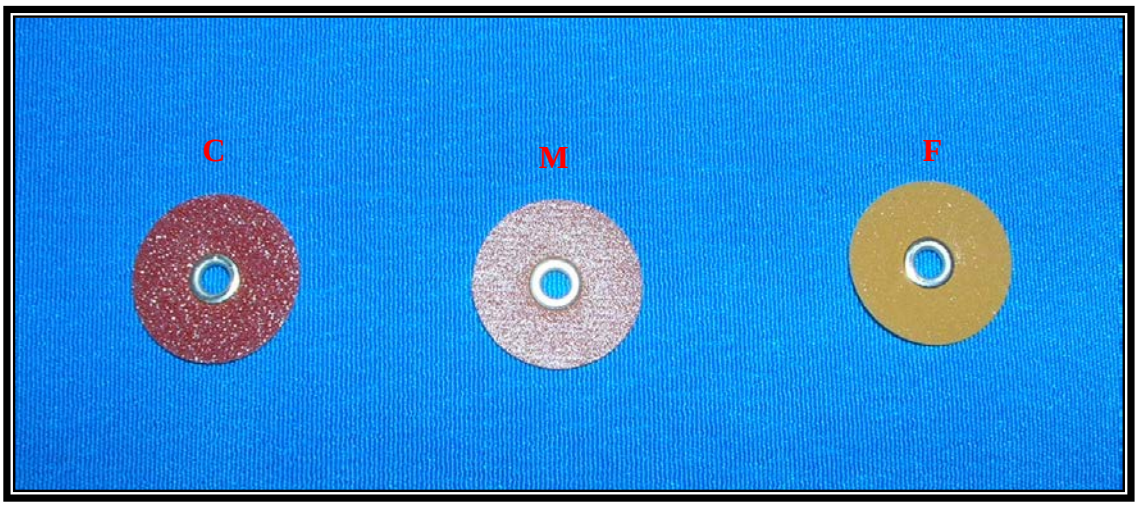


Figure 3.8: Soft Lex Disc: Course (C), Medium (M) & Fine (F)

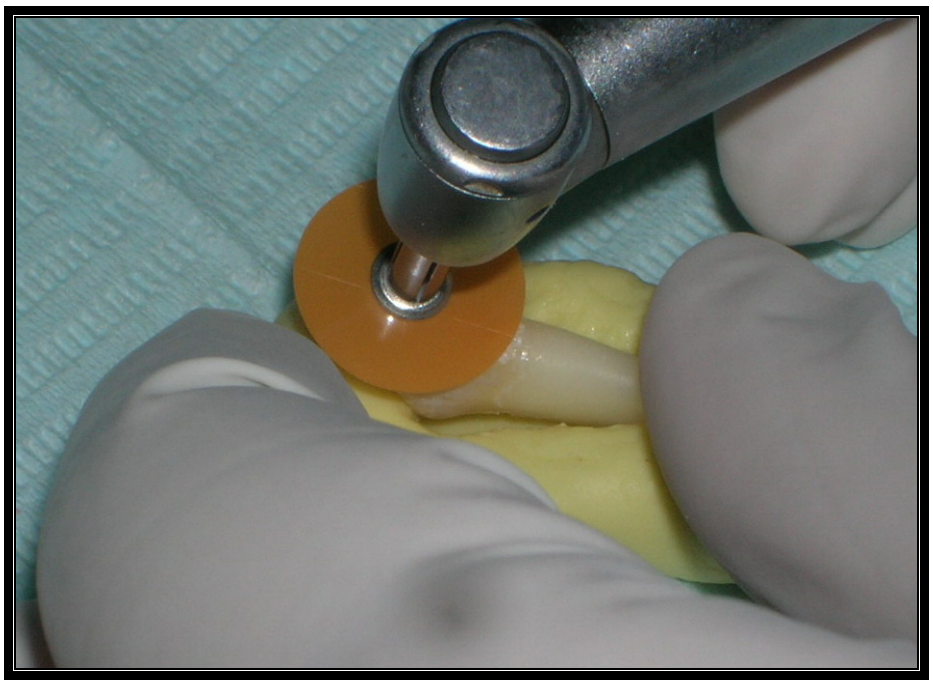


Figure 3.9: Sample during polishing

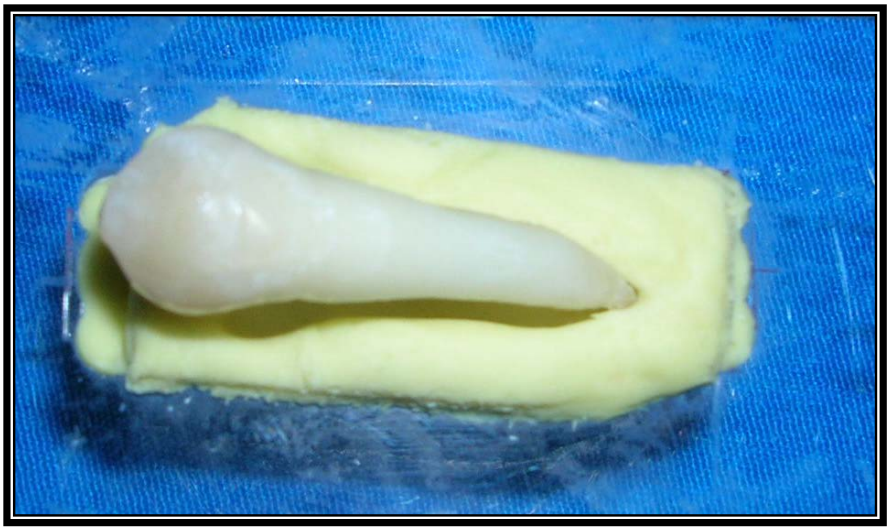


Figure 3.10.: Sample after polishing

3.4 Thermocycling

The thermocycling machine used in this study was Neslab Thermocycler (Neslab Instruments Inc., USA 002013) (Figure 3.12). The two baths of the machine were filled with distilled water. The temperatures were thermo statistically controlled at 55 ^{o}C for the hot bath (Figure 3.13), and 5 ^{o}C for the cold bath (Figure 3.14). The temperatures were maintained at all time at ± 1^{o}C.The specimens of each group were placed in a soft wire mesh (Figure 3.11).The mesh was then secured tightly to the specimen holder with the help of threads. One group of the specimens was thermocycled at each time. 500 cycles were used as recommended by ISO. Exposure to each bath was 15 seconds and the transfer time between the baths was 5 seconds. The temperatures of both baths were checked regularly using two separate thermometers respectively. When thermocycling ended, the specimens were removed from the specimen holder and the wire mesh. The teeth were dried and prepared for immersion in different pH medium.

Figure 3.11: Specimen in a soft wire mesh

Figure 3.12: Specimen in thermocycling machine

Figure 3.13: Sample in hot bath

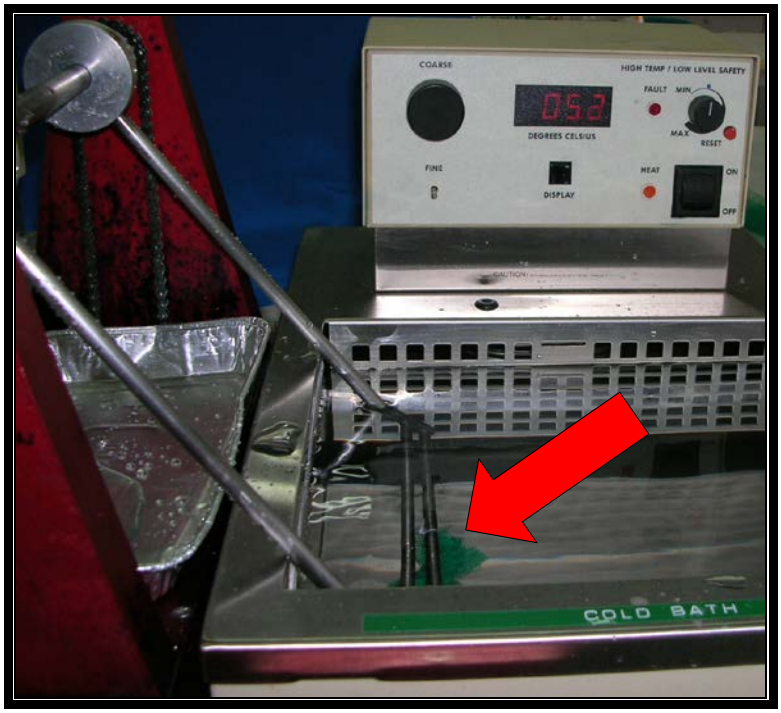


Figure 3.14: Sample in cold bath

3.5 Evaluation of microleakage

3.5.1 Preparation prior to immersion in dye solution

After thermocycled, samples were group according to different pH (Table 3.4) and soaked in the particular medium for ten minutes. 1 M Citric acid (210g/L) and I M Sodium Hydroxide (40g/L) were used to prepare different pH soaking mediums. 100 ml 1 M Citric acid was used as a base solution and titrated with 1 M Sodium Hydroxide solution until the particular pH was achieved. pH of the medium was measured using Microprocessor pH meter (Hanna Inst.) (Figure 3.15). pH meter was calibrated using buffer solution pH 2, 4, 7, 10 and 12 before used. After ten minutes soaking in the particular pH medium, samples were washed with distilled water and dap dried with paper towel.

Table 3.4: Different groups of specimen

Group	pH medium
A	2.5
B	3.5
C	4.5
D	5.5
E(control)	7
F	8.5
G	11.5

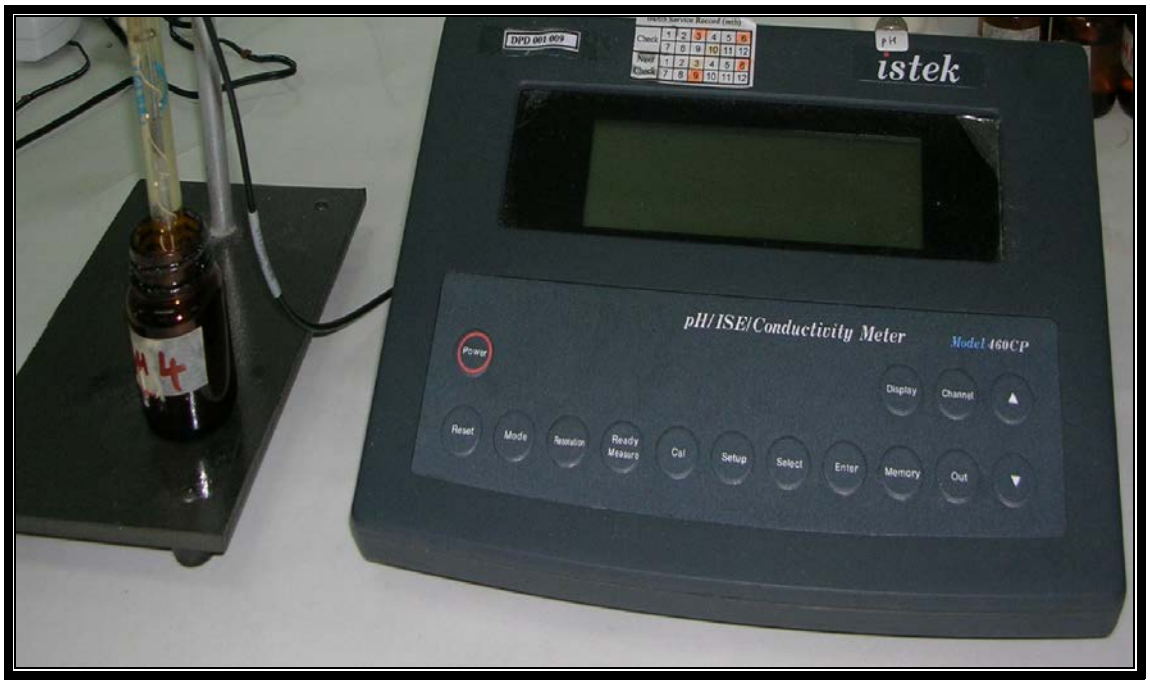


Figure 3.15: pH meter

3.5.2 Microleakage test

After 10 minutes immersion in respective pH solutions, specimens were dried and coated with two layers of nail varnish exposing 2 mm of enamel around the restoration. Root tips of the teeth were sealed with sticky wax (Figure 3.16). Subsequently, the specimens were immersed in 2 % methylene blue dye (R & M Chemical Marketing, UK) for 24 hours.

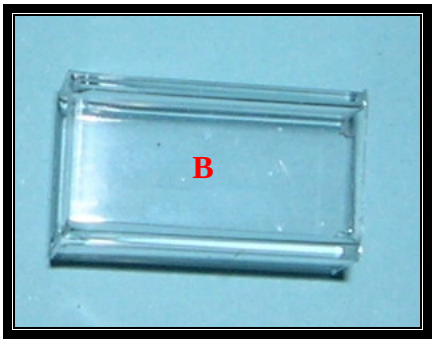
Figure 3.16: Specimen before immersion into dye

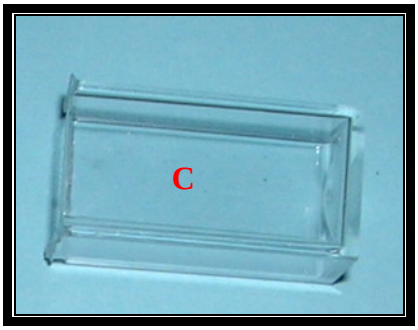
Figure 3.17: Specimen after immersion into dye

3.5.3 Sectioning of specimens

After taking out from the dye solution (Figure 3.17), the samples were washed under tap water and dap dried with paper towel. Samples were mounted in the epoxy resin (Mirapox 950-230) in the plastic Cuvettes (Dispolab Kartell). Each plastic Cuvetts was sectioned into two parts (Figure 3.18). Each part was prepared like a rectangular box with the help of modeling wax before tooth was mounted (Figure 3.19). After 24 hours, when epoxy resin was set, specimens were ready for sectioning (Figure 3.20). Specimens were sectioned longitudinally (buccal-lingual) at the middle of the restoration using Low speed saw (Buehcer, 11-1180 Isomet ^{TM}) (Figure 3.21A and B).

Figure 3.18: Cuvett before section (A), after sectioned into two pieces (B & C)





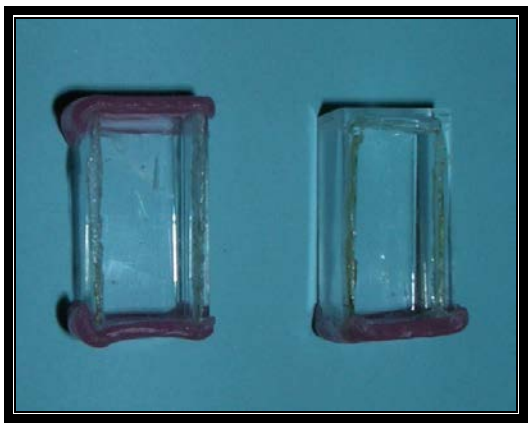
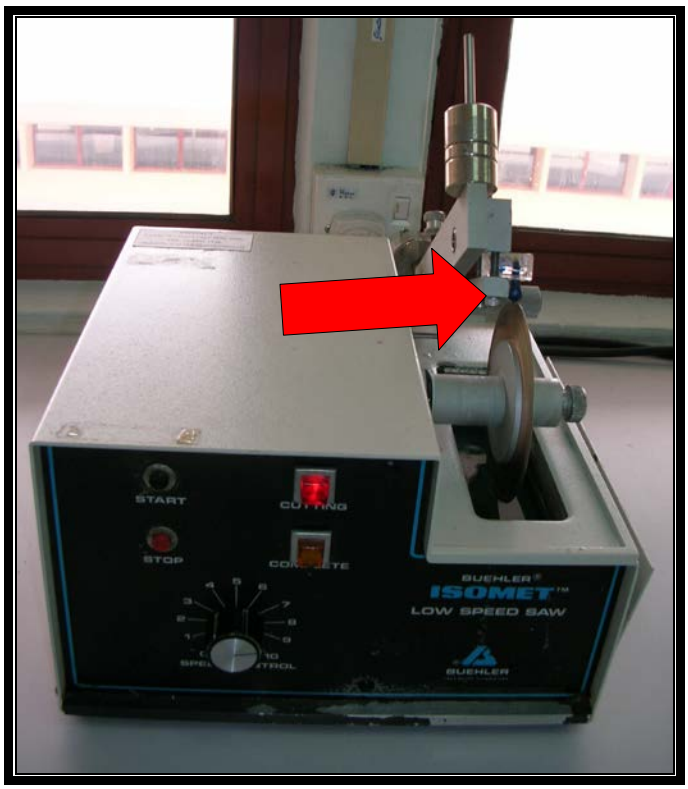


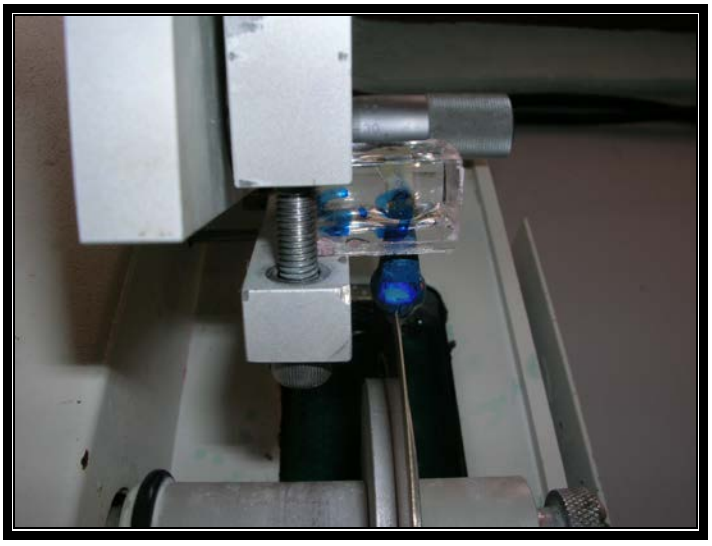
Figure 3.19: Cuvetts ready for mounting the specimen

Figure 3.20: Specimen mounted in epoxy resin



A

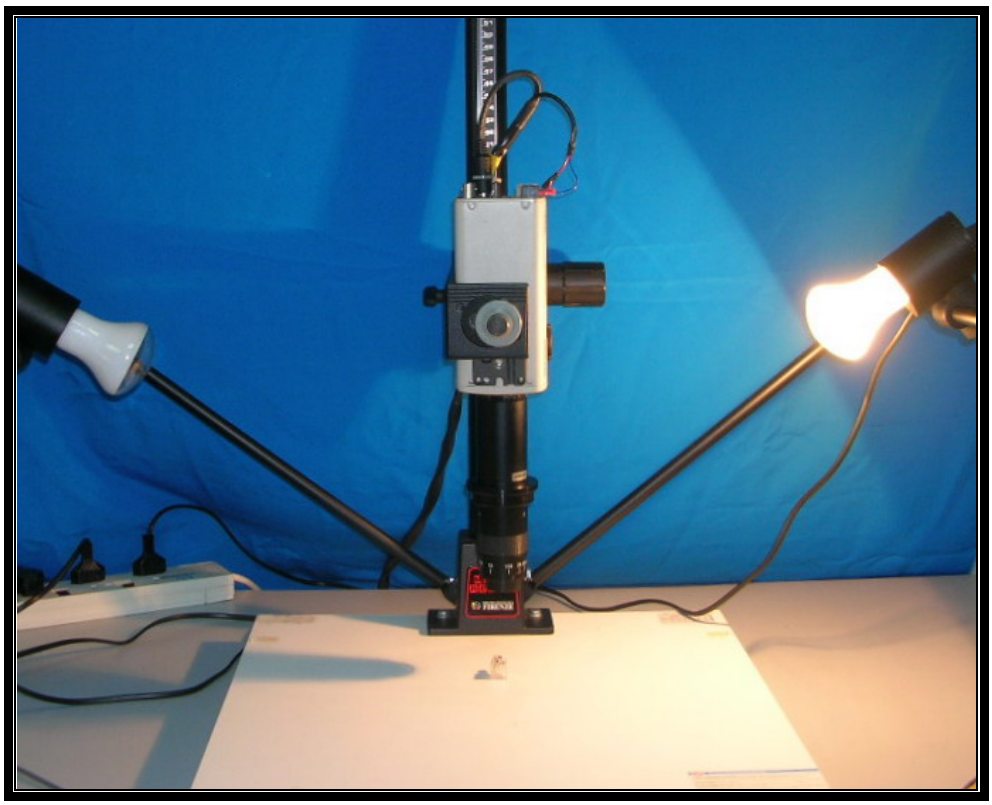
Figure 3.21 A: Sectioning of the specimen with Low speed saw (Buehcer, 11-1180 Isomet ™)



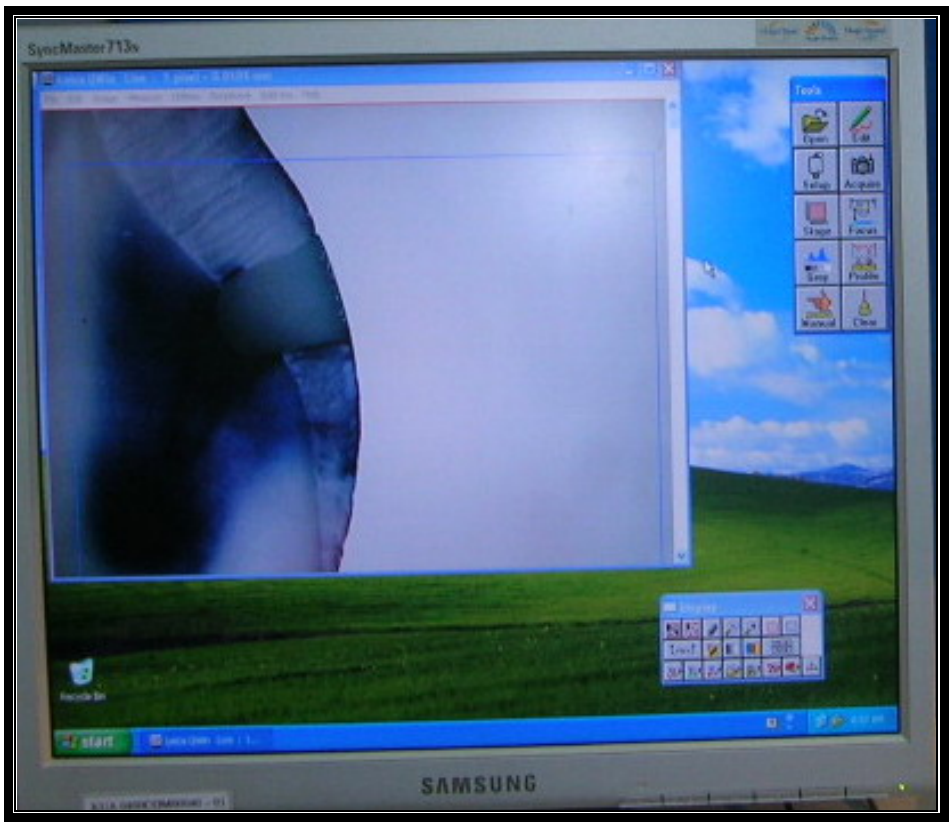
B

Figure 3.21B: Specimen during sectioning (closer view)

Figure 3.22: Specimens after sectioned



A



B

Figure 3.23: Image Analyzer (A) with related soft ware (B)

3.5.4 Microleakage evaluation procedure

Two halves of the specimens were viewed under Image Analyzer (Leica Qwin, Leica Imaging Systems Ltd. Cambridge, England) (Figure 3.23).The half that showed more leakage was chosen for evaluation. All specimens were examined three times for microleakage with three days period between each evaluation. Each evaluation was carried out independently without reference to previous score.

3.5.5 Criteria for microleakage evaluation

Data were collected according to their scores. Occlusal and cervical scores were read separately (Figure 3.24, 3.25 and Table 3.5).

3.5.6 SEM evaluation

One specimen from the most acidic (pH 2.5), neutral (pH 7) and the most alkaline (pH 11.5) group that showed score 3 at the occlusal and cervical margins were viewed under SEM (FEI, FESEM, Quanta 200, USA) for comparison.

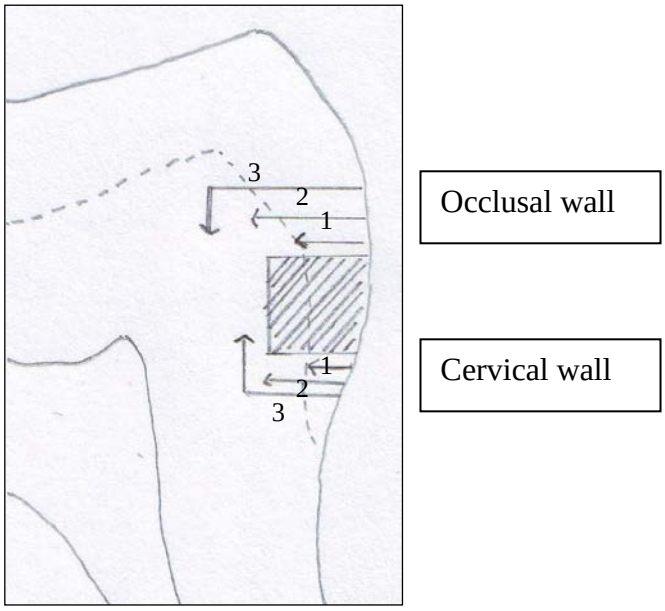
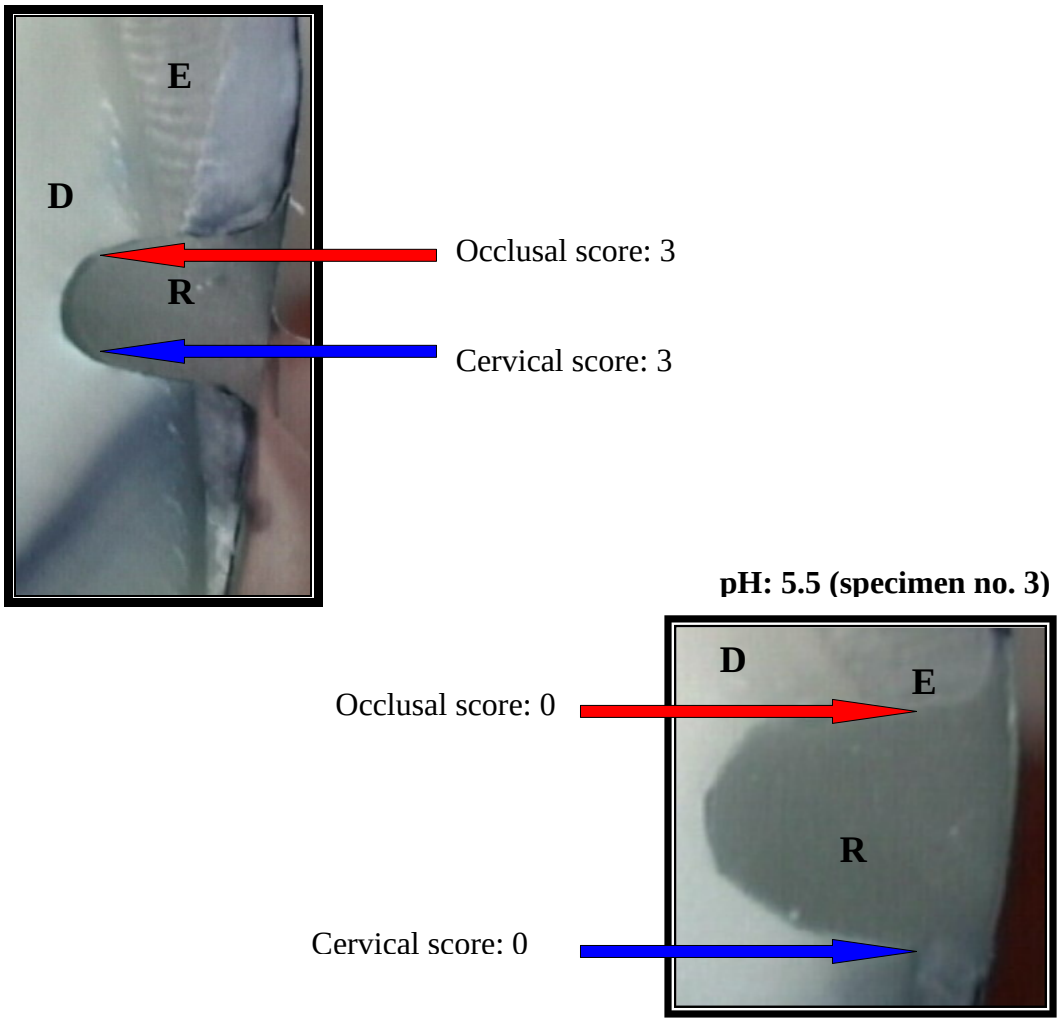


Figure 3.24: Diagram of scoring systems

Table 3.5: Scoring system for occlusal and cervical margin

Score 0	No dye penetration
Score 1	Dye penetration into enamel
Score 2	Dye penetration into the dentine, not including the axial wall
Score 3	Dye penetration into the dentine, including the axial wall

pH: 3.5 (specimen no. 6)



E: Enamel **D**: Dentin **R**: Restoration

Figure 3.25: Examples of specimens viewed under image analyzer