

Fourier Transform Infrared Spectroscopy (FTIR) of Remaining Dentine after Caries Removal with Newly Prepared Experimental Chemomechanical Caries Removal Agent

Prof. Assist. Ali M. Al-Naimi¹, Assist. Prof. Dr. Makdad Chakmakch²,
Prof. Dr. Amer A. Taqa³

^{1,2}Dept. of Conservative Dentistry, College of Dentistry, University of Mosul, Iraq

³Dept. of DBS, College of Dentistry, University of Mosul, Iraq

ABSTRACT

Aim: Aim of this study was to determine the chemical characteristic of remaining dentine after caries removal with either experimental chemomechanical caries removal agent (ECMCRA) or Carisolv[®].

Materials and Methods: Twenty extracted human third molars were embedded individually in slow curing epoxy resin, the teeth were decoronated and ground flat the occlusal surfaces. A $280 \pm 20 \mu\text{m}$ thick layer of partially demineralized dentine was created on the occlusal dentine surface by pH cycling. After that, the teeth distributed to two groups and sectioned longitudinally. The first half caries was excavated and a thin layer of dentine was gently scraped off with surgical scalpel blade and subjected to FTIR analysis which is considered as a remaining dentine without any CMCR agents. The second halves were obtained after carious tissues removal with either ECMCRA or Carisolv[®] (MediTeam Dental, Sweden) respectively according to the manufactures instructions and this is considered as a remaining dentine after caries removal with either agents. The FTIR spectra of each sample was obtained by ALPHA FTIR spectrometers (Bruker, Germany) with 4.0 cm^{-1} resolution, with the range of $500\text{-}4000 \text{ cm}^{-1}$. To evaluate the integrity of the collagen triple helix, peak absorbance ratios of $1235 \text{ cm}^{-1}/1454 \text{ cm}^{-1}$ were considered.

Results: No disappearance or shift of bands were evident with regard to the mineral and organic content of remaining dentine. ECMCRA did not promote collagen denaturation.

Conclusions: The chemical analyses in the present study for the remaining dentine after caries removal with either ECMCRA or Carisolv imply that remaining dentine after removal with either methods of CMCR are insignificant differ from control dentine.

Keywords: Chemomechanical caries removal, remaining dentine, caries removing agent. FTIR of dentine.

INTRODUCTION

These days the prime objective in treating carious lesions is to remove the outer carious dentine, which is infected and non-remineralizable, while leaving the inner carious dentine intact for remineralization^(1,2).

In recent years, the concept of minimal invasive dentistry (MID) and Atrumatic Restorative Dentistry (ARD) has gained popularity with the development of new dental restorative materials, a better understanding of the caries process and the tooth's potential for remineralization⁽¹⁾. The chemomechanical caries removal methods (CMCR) appeared as an alternative, overcoming some of the inconvenient aspects of drilling, such as pain and discomfort, eliminating or diminishing the need for local anaesthesia, and eliminating the noise during carious tissue removal^(3,4,5).

Recently, vibrational spectroscopy, both infrared (IR) and Raman, has been extensively used to study mineralized tissues. These tissues may have formed of physiologic or pathologic processes and analyses of changes in mineral and matrix (organic phase) in healthy and diseased tissue provide significant information to asses that tissue quality⁽⁶⁾.

The study of chemical changes produced in the collagen structure, the ATR-FTIR technique is highly indicated⁽⁷⁾. The infrared spectrum is characteristic of every molecule, and certain groups of atoms give rise to bands that occur close to one and on the same frequency, irrespective of the structure of the molecule. It is precisely the presence of these characteristic bands of groups that allow one to obtain useful structural information⁽⁸⁾. The purpose of this study was to evaluate the effects of newly prepared papain-based CMCR on human remaining dentine in terms of chemical structure by use of noninvasive FTIR methods

MATERIALS AND METHODS

The newly prepared ECMCRA composed of papain enzyme as a main active ingredient, chloramine, potassium oxalate, toluidine blue, sodium methyl paraben and sterile water. Twenty teeth extracted human third molars were embedded individually in slow curing epoxy resin (Epofix resin kit, Struers, Denmark) with the aid of rubber mold, then the teeth were decoronated 2-3mm below the cervical line by the minitom (Struers, Denmark). The occlusal surfaces were ground flat using 180, 320 and 600grit silicon carbide paper under running water to remove the enamel and expose a flat dentine surface. The enamel boundaries of occlusal surfaces together with the surrounding epoxy resin were covered with two coats of nail varnish to avoid the penetration of the solutions' molecules in any marginal gaps that could exist between the tooth and the epoxy. A $280 \pm 20 \mu\text{m}$ thick layer of partially demineralized dentine was created on the uncoated surface by pH cycling as described in the previous earlier^(9,10).

The teeth distributed in two groups (10 teeth per group). The teeth sectioning longitudinally by minitom. The first half after artificial lesion creation, the caries was excavated with sharpe dental excavator and surgical scalpel blade. The remaining dentine was confirmed with the use of a sharpe dental probe. After that a thin layer of dentine was gently scraped off with new surgical scalpel blade and subjected to FTIR analysis and this is considered as a remaining dentine without any CMCR agents. The second halves were obtained after carious tissues removal with either ECMCRA or Carisolv[®] (Medi Team Dental, Sweden) respectively according to the manufactures instructions. The remaining dentine removed in the same manner of mention earlier and this is considered as a remaining dentine after caries removal with either agents. The FTIR spectra of each sample was obtained by ALPHA FTIR spectrometers (Bruker, Germany) with 4.0 cm^{-1} resolution, with the range of $500\text{-}4000 \text{ cm}^{-1}$.

To evaluate the integrity of the collagen triple helix, peak absorbance ratios of $1235 \text{ cm}^{-1}/1454 \text{ cm}^{-1}$ were considered⁽¹¹⁾. A ratio close to 0.5 denotes compromised integrity of the collagen triple helix, whereas a ratio closer to 1 denotes the maintenance of the integrity of amide III and the C-H bond of the pyrrolidine ring of the type I collagen triple helix⁽¹¹⁾. The compositional analysis of all samples was performed immediately after treatments using ATR-FTIR spectroscopy.

RESULTS

The main infrared spectra of bands for remaining dentine after carious dentine removal with ECMCRA or Carisolv were shown in (Table.1).

Table (1): Summarized the typical IR spectrum of remaining dentine of teeth, relative band position, and after application of ECMCRA or Carisolv.

Compounds	Remaining dentine Peak(cm^{-1})	After ECMCRA	After Carisolv
ν_2 of B2-type Carbonate	871	872	872
ν_1 of PO_4^{-2}	960	958	961
H PO_4^{-2}	1002	1002	1001
H PO_4^{-2}	1022	1022	1026
amide III	1235	1236	1239
proline and hydroxyproline	1454	1456	1460
amide II	1551	1557	1558
amide I	1635	1634	1636
Asymmetric andsymmetric stretching	O-H 2500–3700	----	----

The most intense bands, corresponding to the mineral phase $\{PO_3^{-3}, CO_3^{-2}\}$ of carious dentine samples, were found at $1100-900cm^{-1}$ ⁽¹²⁾. Bands of the phosphate (PO_4^{-3}) incorporated within the hydroxyapatite crystals were observed near $960cm^{-1}$, and the $\nu_3 PO_4$ region which appears as a very strong broad asymmetric band at $\sim 1022cm^{-1}$ ^(13,28)(Fig.1).

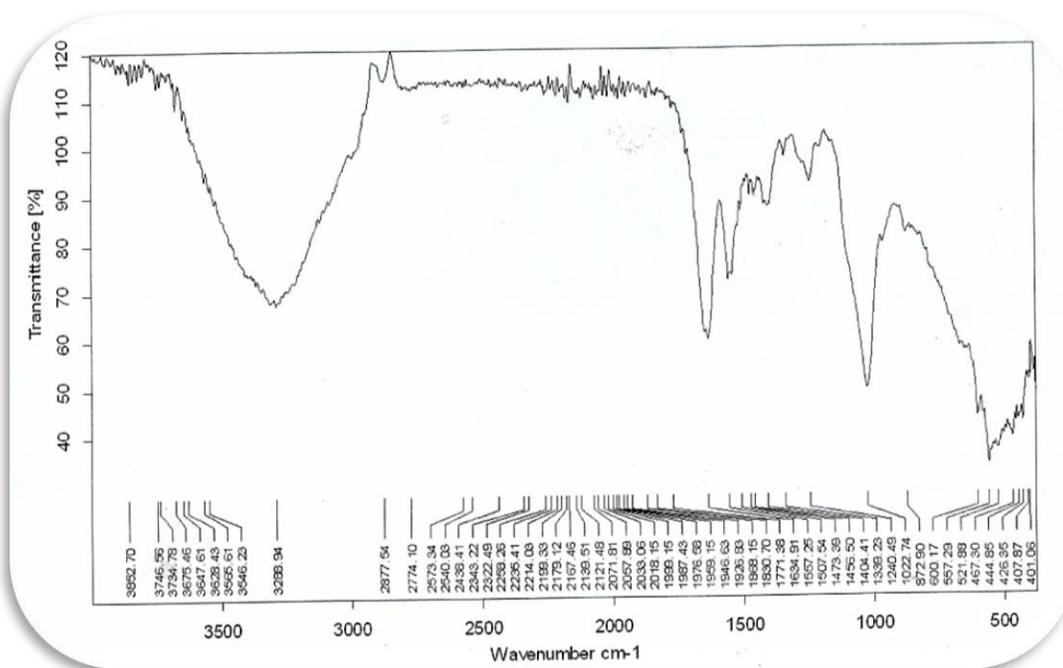


Figure (1): Shows the 500-4000 cm^{-1} region of the IR spectra of remaining dentine, and the relative band assignments and position.

Strong peaks assigned to the B-type carbonate substitution (carbonate for phosphate ion) are observed at $872cm^{-1}$ ($\nu_2 CO_3$ mode). The weak bands in the $\nu_3 CO_3$ region are attributed to CO_3^{-2} replacing PO_4^{-3} ions without an adjacent OH ion⁽¹⁴⁾.

The amides bands have been observed in the IR spectra of tooth samples above $1500cm^{-1}$ ⁽¹⁵⁾. The region of the IR active bands associated with the dentine proteins, are represented by the spectra presented the three main bands of the collagen fingerprint: amide I ($1635 cm^{-1}$) due to carbonyl stretching C-H, amide II ($1551cm^{-1}$) due to the vibrations in the plane of N-H bond and C-N stretching and amide III ($1235 cm^{-1}$) due to C-N stretching and N-H deformation⁽¹⁶⁾.

Other bands corresponding to dentine proteins are the CH_2 bending vibrations at $1450cm^{-1}$. The peaks identified in $1454cm^{-1}$ and in the region between $1417cm^{-1}$ and $1360cm^{-1}$ correspond to the stereochemistry of the pyrrolidine rings of proline and hydroxyproline respectively⁽⁶⁾.

A broad water band near $2500-3700cm^{-1}$ which corresponds to asymmetric and symmetric O-H stretching. The region ($2500-3800cm^{-1}$) shows overlapping of water, organic material and OH bands. Some observed bands are not exactly assigned otherwise, the amide A and amide B bands show weak and broad bands near 3066 and $3318cm^{-1}$, respectively⁽⁶⁾.

Figure (2) displays the mean infrared spectrum of remaining dentine after removal of carious tissues with ECMCRA, showing corresponding infrared bands of collagen, it was observed that there were no alterations in the bands relative to amides I, II, and slight increase in amide III and It is clear to observe that the main absorption peaks of collagen were preserved in all samples. Thus, the analysis of integrity of collagen triple helix showed that ECMCRA did not promote collagen denaturation, because the means bands ratios in $1235cm^{-1}$ (amide III) and $1450cm^{-1}$ (pyrrolidine ring) were 0.9 and thus close to the one.

Also, no new bands, disappearance or shift of bands were evident with regard to the mineral content. However, there is an increase in the band area at $1022cm^{-1}$ related to the HPO_4^{-2} .

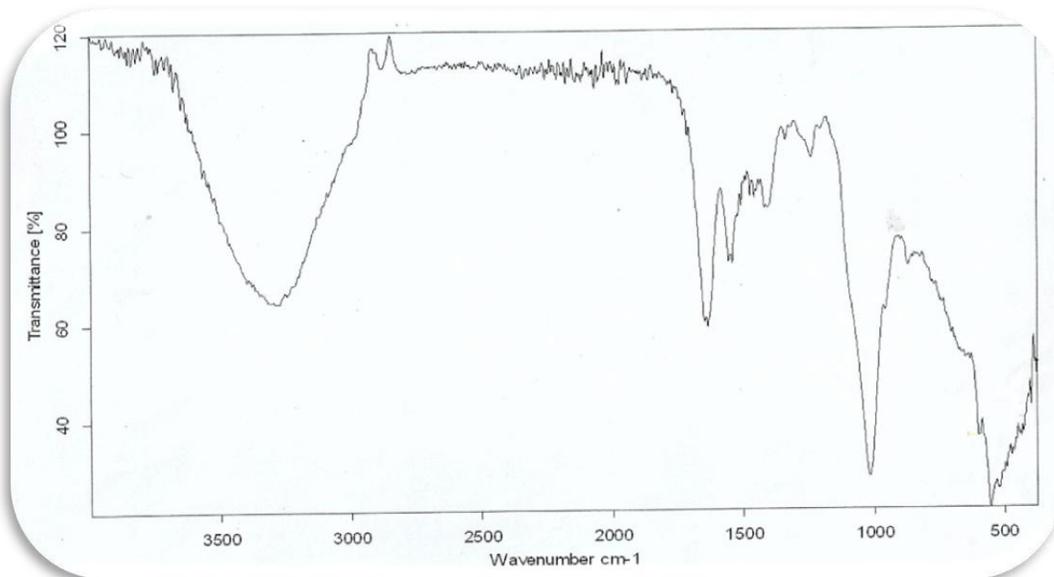


Figure (2): Displays the mean infrared spectrum of remaining dentine after removal of carious tissues with ECMCRA.

Figure (3) displays the mean infrared spectrum of remaining dentine after removal of carious tissues with Carisolv, It can be noted that the two samples present the bands in the same position, no new bands or the disappearance of bands were evident. But, there is displacement of absorption bands about 2-4 degree and peaks reduction in amide I, II and III. Also, reduction and displacement in mineral peaks at 1026cm^{-1} and 960cm^{-1} . The analysis of the integrity of collagen triple helix value is 0.7.

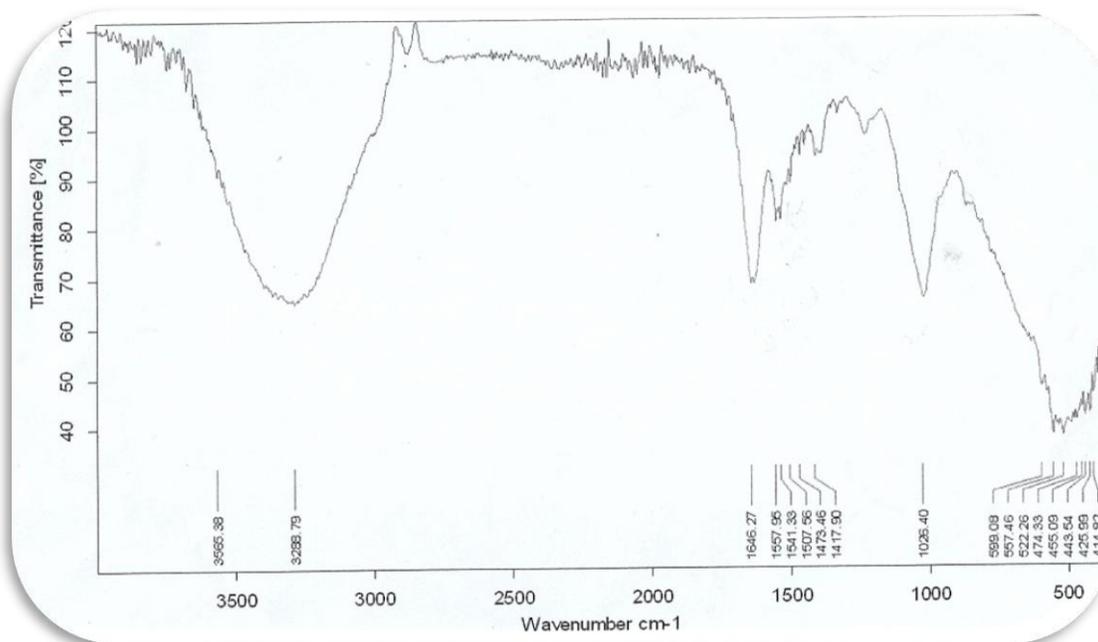


Figure (3): Displays the mean infrared spectrum of remaining dentine after removal of carious tissues with Carisolv.

DISCUSSION

FTIR analysis is a powerful tool for dental researchers. It has been frequently used to observe chemical alterations after different treatments, such as mechanical, chemomechanical and etching with or without further adhesives exposed to dentine and carious tissue ^(15,17,18).

For different chemical groups, the wavelengths absorbed and the natural frequency of the vibrations are unique and depend on the existent bond type (C=C, C-H, C=O, N-H, and O-H)⁽¹⁹⁾. This technique enables analysis of protein linkage to the crystals without further purification. The method used in the present study by FTIR has been shown to be useful for the analysis of chemical alterations in sound and carious dentine, without extensive sample preparation and protein purification^(16,20).

Nine characteristic bands are termed amide A, B, and I–VII. Current understanding of IR spectra of proteins⁽²¹⁾. Amides I and II are the major bands in the protein IR spectrum. Amide I absorption originates from the C=O stretching vibration (70–85%) of the amide group, which gives rise to IR band(s) in the region between ~1600 and 1700 cm⁻¹⁽²²⁾.

Amide II comes from the N–H bending (40-60%) and C–N stretching vibrations (18-40%) and is conformationally sensitive. Amides III and IV are very complex bands resulting from mixtures of several coordinate displacements. Out-of-plane motions are found in amides V, VI, and VII. Because of technical and theoretical limitations, only amide bands I–III are used for investigating protein secondary structure^(22,23).

According to above results, it was confirmed that the application of ECMCRA does not produce any chemical change in the microstructure of collagen, nor does it interfere in its integrity. In addition, it was shown a slight increase on relative intensity of some absorption bands of collagen after ECMCRA application, which suggests that this substance can interact chemically with collagen. However, the increase on relative intensity of some peaks, including the phosphate ones, indicates the interaction of this substance with collagen.

This interaction is owing to proline being an amino acid that has an atom of oxygen from the functional carboxylic group; the proximity to an atom of nitrogen of the adjacent amino acid favours the hydrogen bridge bond type. Therefore, the proline-rich proteins, such as type I collagen, form highly stable bonds⁽²⁴⁾.

Evaluation of the integrity of the collagen triple helix was performed by analysis of the ratio of the absorbance of bands 1235cm⁻¹ (amide III) and 1450cm⁻¹ (stereochemistry of the pyrrolidine rings). Although the first band is sensitive to the presence of the secondary structure of collagen, the second is independent of the ordered structure of collagen⁽¹¹⁾. For type I collagen fibers, the integrity of the secondary structure is demonstrated when the value of the 1235/1450cm⁻¹ ratio is close to 1. According to Sylvester et al. (1989), values close to 0.5 denote a change in the three-dimensional structure of the type I collagen triple helix. Changes in this absorption ratio indicate significant structural alterations in the collagen triple helix. In this study, the value found was approximately 0.9 after application of ECMCRA; significantly higher than that which would be observed for denatured structures, whose value would be around 0.5⁽¹¹⁾.

Fortunately, the presence of native inter- and intermolecular crosslinks of the triple helix of dentine collagen structure provides the basis for the stability and strength of the collagen fibrils which is responsible for maintaining its mechanical properties and intended a great influence on the success of the dental restorative procedure on demineralized dentin.

In FTIR analysis, the intensities of amides I and II are related to the helical structure of collagen and the intensity of amide I could be used for characterizing the secondary structure of proteins⁽¹⁹⁾. Amides I and II are the main infrared absorption bands of the peptide group in collagen. Both bands absorb in different regions depending on whether or not they participate in hydrogen bonding interactions. Hydrogen bonds affect the vibration frequencies of the participating atoms. The presence of hydrogen bonds is an indication of polypeptide chains, assuming regular secondary structures. In fact, both amide I and II bands absorb in slightly different regions if they are in an A-like helix or in b-sheets⁽²⁵⁾. Thus, amide bands can be used to probe the structure of collagen⁽¹⁹⁾.

After the application of ECMCRA, an increase in adsorbed water was found, as evidenced by the increase at the 3300 cm⁻¹ peak⁽²⁶⁾. This adsorbed water comes from the water that constitutes the gel of ECMCRA. However, a decrease on the intensity of 1630 cm⁻¹ peak was also found, which indicates a slight proteolytic action of ECMCRA, reducing the content of amide I. In this case, the effect of absorbed water (which would increase the intensity of 1630cm⁻¹ peak) was not observed, but rather the action of papain on the structure of collagen was evident by the amide I peak, prevailing in relation to the water adsorption effect.

CONCLUSION

The results of our study showed no significant difference between both the groups in their organic or inorganic contents after caries excavation, which is in agreement with the previous study by Arvidsson on chemical and topographical analysis, which showed no major differences between cavities excavated with burs or Carisolv^(26,27).

REFERENCES

- [1]. Jain K, Bardia A, S Geetha, Goel A (2015) Papacarie: A Chemo- mechanical Caries Removal Agent. *IJSS Case Reports & Reviews*. 9 (1):57-60.
- [2]. Piva F, Pereira JT, Luz PB, Hashizume LN, Hugo FN, de Araujo FB (2017) A Longitudinal Study of Early Childhood Caries and Associated Factors in Brazilian Children. *Brazi. Dent. J.* 28(2): 241-248.
- [3]. Rafique S, Fiske J, Banerjee A (2003) Clinical trial of an air-abrasion/ chemomechanical operative procedure for the restorative treatment of dental patients. *Caries Res.* 37: 360-364.
- [4]. Bassadori S K, Silva L R, Guedes CC (2005) Utilization of papacarie for chemomechanical removal of decayed tooth In: *Atraumatic restorative technique(ART). Minimal intervention techninque for treatment of deep dental decay.* Mosby. 391-400.
- [5]. Pleszczynska M, Wiater A, Bachanek T, Szczodrak J (2016): *Enzymes in therapy of biofilm-related oral diseases.* Union of Biochem.and Molec. Biol. Volume 00, Number 0, Pages 1–10.
- [6]. Movasaghi Z, Rehman S, Rehman I (2008) Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tiss. *Appl. Spect. Rev.* 43(2): 134–179.
- [7]. Silverstein RM, Bassler G, Morrill TC, Sawicki D (Editors) (1991) *Spectrometric identification of organic compounds (5th edn.)* NewYork: John Wiley & sons Inc; Pp. 233.
- [8]. Stuart BH (2004) *Infrared Spectroscopy: Fundamentals and Applications– Analytical Techniques in the Sciences.* New York: John Wiley & Sons, Ltd. Pp. 88.
- [9]. Ten Cate JM, Buijs MJ, Damen JJ (1995) pH-cycling of enamel and dentin lesions in the presence of low concentrations of fluoride. *Eur. J. Oral Sci.* 103: 362–367.
- [10]. Yi-pin Q, Nan Li , Li-na Niu , Carolyn M., Primus D, Jun-Qi Ling , David H. Pashley E, Franklin R. (2012) Remineralization of artificial dentinal caries lesions by biomimetically modified mineral trioxide aggregate. *Acta. Biomat.*8: 836-842.
- [11]. Sylvester MF, Yannas IV, Salzman EW, Forbes MJ (1989) Collagen banded fibril structure and the collagen–platelet reaction. *Thromb. Res.* 55: 135–148.
- [12]. Moloney E, Varanasi S, Meyers I, Rintoul L, Symons A (2014) The Effect of Remineralisation Treatments on Demineralised Dentine, an In Vitro Study. *J. of Dent. and Oral Med.* 2(1): 1-8.
- [13]. Amer A. Taqa, Nadira A. Hatim and Zena (2014) Study the FTIR of Hydroxyapatite Additive to Heat Cured Acrylic Resin. *Al-Rafidain Dent J.*;14(1), 32-36.
- [14]. Verdelis K, Crenshaw MA, Atti E, Boskey AL (2003) Spectroscopic imaging of mineral maturation in bovine dentine. *J. of Dent. Rese.* 82: 697-702.
- [15]. Gotliv BA, Veis A (2007) The composition of bovine peritubular dentine: matching TOF-SIMS, scanning electron microscopy and biochemical component distributions. New light on peritubular dentine function. *Cells Tiss. Organs.* 189: 12-19.
- [16]. Potter K, Kidder LH, Levin IW, Lewis EN, Spencer RG. (2001) Imaging of collagen and proteoglycan in cartilage sections using Fourier transform infrared spectral imaging. *Arthritis & Rheumatism.* 44: 846-855
- [17]. Magne D, Guicheux J, Weiss P, Pilet P, Daculsi G (2002) Fourier transform infrared microspectroscopic investigation of the organic and mineral constituents of peritubular dentine: A horse study. *Calcif. Tiss. Inter.* 71: 179-185.
- [18]. Eugenio S, Sivakumar M, Vilar, R, Rego AM (2005) Characterisation of dentine surfaces processed with KrF excimer laser radiation. *Biomater.* 26: 6780-6787.
- [19]. Karoui R, Downey G, Blecker C (2010) Mid-infrared spectroscopy coupled with chemometrics: A tool for the analysis of intact food systems and the exploration of their molecular structure–quality relationships- A review. *Chem. Rev.* 110: 6144–6168.
- [20]. Spencer P, Wang Y, Katz JL, Misra A (2005) Physicochemical interactions at the dentine/adhesive interface using FTIR chemical imaging. *J. of Biomed. Optics.* 10 (3): 031104-031111.
- [21]. Dousseau F, Pezolet M (1990) Determination of the Secondary Structure Content of Proteins in Aqueous Solutions from Their Amide I and Amide II Infrared Bands: Comparison Between Classical and Partial Least-Squares Methods. *Biochem.* 29: 8771–8779.
- [22]. Barth A, Zscherp C (2002) What Vibrations Tell Us About Proteins? *Quart. Rev. Biophys.* 35 :369–430.
- [23]. Bandekar J (1992) Amide Modes and Protein Conformation. *Biochim. Biophys. Acta* 1120: 123–143.
- [24]. Han B, Jaurequi J, Tang BW, Nimni ME (2003) Proanthocyanidin: A natural crosslinking reagent for stabilizing collagen matrices. *J. Biomed. Mater. Res.* 65: 118–124.
- [25]. Williams RW, Dunker A K (1981) Determination of the secondary structure of proteins from the amide I band of the laser Raman spectrum. *J. Mol. Biol.* 152: 783–813.
- [26]. Pai VS, Nadig RR, Jagadeesh TG, Usha G, Karthik J, Sridhara KS (2009) Chemical Analysis of Dentin Surfaces after Carisolv treatment. *J. Conserv. Dent.* 12: 118-122.
- [27]. Arvidsson A, Milleding P, Wennerberg A (2001) The influence of chemo-mechanical caries removal solution on the topography of dental ceramic materials. *Biomater.* 23: 3977-3983.
- [28]. Amer A. Taqa, Nadira A. Hatim and Zena J. Hussan, (2013), FTIR and NMR of Hydroxyapatite incorporation with Heat Cured Acrylic, *Journal of Dental Research*, Vol. 92, Special Issue A.