



**ORIGINAL RESEARCH**

**EVALUATE DEGREE OF CONVERSION OF NEW BIOACTIVE ORTHODONTIC ADHESIVE WITH COLOR CHANGE & FLUORESCENCE PROPERTY**

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**ABSTRACT**

**Objectives:** This study aimed to assess the effect of adding fluorescent dyes & color change dyes in different concentration to bioactive orthodontic adhesive by measure the degree of conversion.

**Materials and Methods:** Bioactive BEAUTIFIL Injectable XSL (S-PRG), from (GIOMER, SHOFU, JAPAN) mixed with color change dye, Black changing to Colorless, (Atlanta chemical engineering, USA), using in 0.02%, 0.2% & 2% of weight concentrations & with Flourcence dye (Strontium aluminate), White Glow in the Dark Powder from (Techno Glow Inc., USA) which glows up for long time, using in 5%, 10% & 15% of weight concentrations. For degree of conversion (DC) 100 disk shape samples prepared & divided into 10 groups with 10 samples as following: Group 1: BEAUTIFIL Injectable XSL Adhesive (control group), Group 2: BEAUTIFIL Injectable XSL with 5% flourcence material. Group3: BEAUTIFIL Injectable XSL with 10% flourcence material. Group4: BEAUTIFIL Injectable XSL with 15% flourcence material. Group5: BEAUTIFIL Injectable XSL with 0.02% color change material. Group6: BEAUTIFIL Injectable XSL with 0.2% color change material. Group7: BEAUTIFIL Injectable XSL with 2% color change material. Group8: BEAUTIFIL Injectable XSL with 0.02% color change material & 5% flourcence material. Group9: BEAUTIFIL Injectable XSL with 0.2% color change material & 10% flourcence material. Group 10: BEAUTIFIL Injectable XSL with 2% flourcence material & 15% flourcence material. The Fourier Transform Infrared spectrophotometer FTIR (ALPHAII PLATINUM-ATR. Bruker. GERMANY) used to determined degree of conversion.

**Results:** The use of dyes with bioactive adhesive show statistically significant difference between groups. but there was decreasing in degree of conversion with increase dye concentration.

**Conclusions:** Acceptable degree of conversion within bioactive adhesive with color change &/or fluorescence properties are obtained but with increase concentration of dyes the degree of conversion decreased.

**Keywords:** Orthodontic treatment, bioactive orthodontic adhesive, fluorescence property

**INTRODUCTION**

Orthodontic treatment involves using fixed or removable appliances to correct the positions of teeth.

Fixed appliance treatment is a traditional and widely used form of orthodontic treatment to correct malpositions of the teeth and occlusal discrepancies <sup>1,2</sup>.

The movement of teeth is achieved by forces generated and directed to the teeth via arch wires and brackets <sup>3</sup>. During the active treatment, the arch wires are changed as the treatment progresses, but the brackets remain attached to the enamel for the whole

active treatment period. A wide variety of orthodontic adhesive are available for the bonding brackets & orthodontic attachments. Resin adhesives are a good choice for orthodontic bonding as they have good mechanical and aesthetic properties and low failure rates. light curing adhesives consider the modern orthodontics adhesive <sup>4,5</sup>. Orthodontic Adhesive should be balance between providing sufficient strength to retain the appliance during the course of treatment, and allowing its easy removal at the end of treatment <sup>6</sup>.

Patients with fixed orthodontic appliances show rise in the risk of white spot lesions (WSL) <sup>7</sup> due to difficulty in preserving oral hygiene which results in amplified bacterial plaque accretion, acid decalcification and enamel demineralization which cause disquiet to patient <sup>(8)</sup>. These bacteria metabolize fermentable sugars and produce organic acids which soften the enamel calcium phosphate mineral resulting in enamel demineralization which saw as WSL <sup>9</sup>.

Efforts were made to reduce enamel demineralization by introducing fluoride releasing adhesives <sup>10</sup> e.g. amorphous calcium phosphate (ACP) containing adhesives <sup>11</sup>, Transbond Plus Adhesive from 3M Unitek company <sup>12</sup>, glass ionomer & resin modified glass ionomer <sup>13</sup>, fluoride varnish <sup>14</sup>, topical fluoride agents <sup>15</sup>. Another solution proposed by research teams is introduction of bioactive glass (BAG) into the composition of orthodontic adhesives <sup>16</sup>.

Bioactive glass material included the GIOMERS, as a surface pre-reacted glass core (S-prg). In the field of orthodontics, it helps prevent and treatment of WSL <sup>(17)</sup>. Giomers prove to be useful in the process of collating orthodontic brackets with mechanical properties similar to composite resins & it offer protection against carious lesions <sup>(18)</sup>. S-prg releases various ions (fluoride, sodium, silicate, aluminium, borate and strontium ions that provide multiple biological functions, including the release & recharge of fluoride, anti-plaque, anti-biofilm effects & pH modulation that providing protection against caries <sup>19</sup>.

Another problem associated with orthodontic adhesive is the presence of excess of adhesive escaping from under the bracket base which promotes the accumulation of food debris and creates favorable area for bacterial collection which led to facilitating the demineralization and formation of WSL <sup>20,21</sup>.

The solution turned out to be introduce adhesive characterized by a contrasting color before cross-linking, which facilitates removal of the excess prior to curing e.g: Transbond Plus (3M Unitek, USA), and Grengloo and Blugloo (Ormco Corporation, USA) <sup>22</sup>. Color change adhesive is manufactured by adding chromatic indicators, which facilitate the visibility of excess orthodontic adhesive around orthodontic brackets before bonding procedure <sup>(23)</sup>. This color characteristic allowed the operator to see the adhesive flash around the bracket base and remove it before it polymerized <sup>24</sup>.

The third problem related with orthodontic treatment are, after end of orthodontic treatment, debonding procedures occur, or in bracket failure, there are great difficulties in removing the remaining adhesive on the tooth surface <sup>25</sup> even with all due precautions, it represents critical actions that affect the integrity of the enamel. leaving the remnant of orthodontic adhesive on the enamel surface facilitates plaque formation and caries <sup>26</sup>.

Also using rotating dental tools to remove the remaining white or transparent adhesive can cause hurt to enamel <sup>27</sup>.

To overcome this problem, fluorescent adhesive has been developed to improve the visibility of the remaining adhesive after debonding by using ultraviolet light <sup>28,29</sup>. Fluorescent additives will facilitate the discrimination between the enamel and remnants of the adhesive. This modification can maximize the preservation of tooth structure after debonding procedure <sup>30</sup>.

The aims of this research is to evaluate the effect of adding color change & fluorescent dyes to bioactive composite to be used as an orthodontic adhesive by evaluate the degree of conversion.

## MATERIALS & METHODS

### Materials

1. BEAUTIFIL Injectable XSL (Self-Leveling) (Surface Prereacted Glass/Pre-Activated Surface Ionomer (GIOMER, SHOFU Inc., Kyoto. JAPAN).
2. Color change dye material (Black changing to Colorless (Atlanta chemical engineering, USA).
3. Fluorescence dye material, White Glow in the Dark Powder Strontium aluminate (which glows up for long time). (Techno Glow Inc, USA).

For color change material use the following concentrations 0.02%, 0.2% & 2% of weight as suggested by Herbert in 1967 <sup>(31)</sup> and for fluorescence dye material use the following concentrations according to company instruction 5%, 10% & 15%. of weight.

### Mixing method

The components were weighed by using a precision scale (Kern ABS analytic balance 120-4N, Kern Corporation, Germany). To achieve homogeneous distribution of the material within the giomer structure, mixing was done by using the electro-mechanical stirrer (Krafit ki-20 dental implant motor, Saeyang Company, Korea) they mixed for 90sec (45sec clockwise & 45sec counter-clockwise) at 2,500 rpm and at room temperature until the mixture becomes homogeneous. During mixing of a material, the material is continuously pushed into the corner between bottom and cup wall, whereas the additional rotation forces move the material towards the center of mixing cup. After that, the mixture extracted from the cup and kept at room temperature until a day of preparing the samples by stored it in lightproof containers to prevent the exposure to ambient light and to allowed it to grow freely until used it <sup>32, 33</sup>.

### Methods

This study was conducted at Mosul university, Dentistry College, Dental Hospital Central laboratory & was approved by the Ethics Committee of College of Dentistry/ Mosul University/ IRAQ (under the code UoM.Dent.23/49).

### Degree of Conversion:

We prepared 100 disc-shaped sample for 10 groups (with 10 sample for each) as following groups:

- Group 1: BEAUTIFIL Injectable XSL Adhesive (control group)
- Group 2: BEAUTIFIL Injectable XSL with 5% fluorescence dye.
- Group 3: BEAUTIFIL Injectable XSL with 10% fluorescence dye.
- Group 4: BEAUTIFIL Injectable XSL with 15% fluorescence dye.
- Group 5: BEAUTIFIL Injectable XSL with 0.02% color change dye.
- Group 6: BEAUTIFIL Injectable XSL with 0.2% color change dye.
- Group 7: BEAUTIFIL Injectable XSL with 2% color change dye.
- Group 8: BEAUTIFIL Injectable XSL with 0.02% color change dye & 5% fluorescence dye.
- Group 9: BEAUTIFIL Injectable XSL with 0.2 color change dye & 10% fluorescence dye.
- Group 10: BEAUTIFIL Injectable XSL with 2% fluorescence material & 15% fluorescence dye.

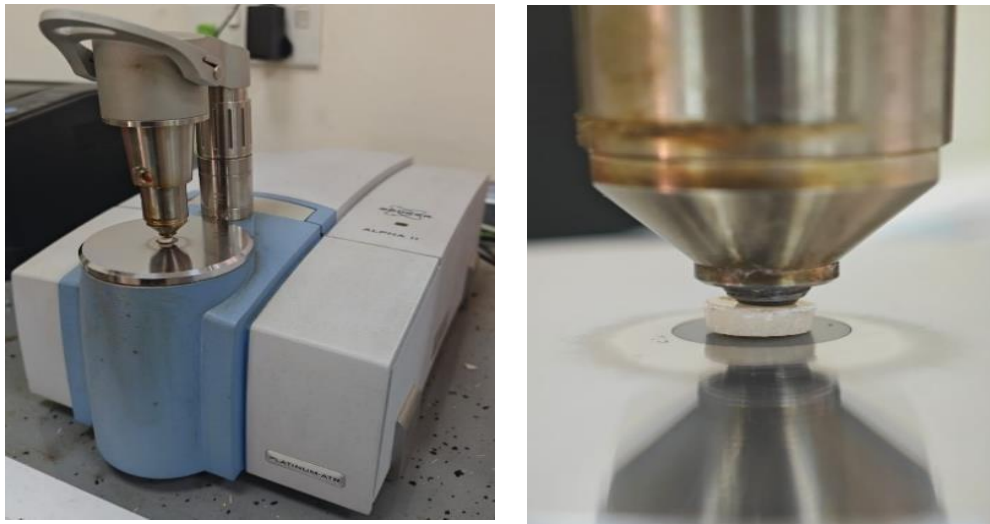
**Specimen Preparation for Test Degree of Conversion:**

To prepare the disc-shaped specimens for testing degree of conversion (DC), The uncured testing materials were poured into plastic molds of 5 mm in diameter and 2 mm in thickness, which was confined from both side by transparent strip and a 1mm in thickness laboratory glass slide. i.e; mold was sandwiched between transparent strips & glass slides, which was pressed on top of the plastic mold. then specimen polymerized using a LED light-curing with at  $1500 \text{ mw/cm}^2$ , the tip of the LED light guide was positioned on the top surface of the material for photo- activation. Irradiation time was as recommended by the manufacturer for each material (20 seconds). A glass slide (1 mm) was used to standardize the distance between the light source and each specimen, also to produce specimens with a flat smooth surface & to prevent oxygen inhibition layer. after curing specimen removed from mold then remove any excess edges from it by scalpel blade(fig1). This process was made at room temperature & repeated for all testing groups, after that & to prevent additional exposure to light exposure, specimens were after that were coded & stored in dry & dark container containing deionized water at room temperature for 24hr prior to testing <sup>34,35</sup>.

**Measuring the degree of conversion**

The DC was determined for each material using Fourier Transform Infrared spectrophotometer FTIR (ALPHA II PLATINUM-ATR. Bruker OPTIK GmbH & Co. GERMANY). FTIR spectroscopy has been proven to be a powerful technique and has been used as are liable method as it detects the C = C stretching vibrations directly before and after curing of composite resins. Each specimen was placed directly in the center of the crystal of the ATR detector's crystal for prompt analysis. The degree of polymerization was monitored by the FTIR spectroscopic analysis. Non-cured resin specimens were also subjected to FTIR spectroscopy, to serve as non-cured references. Between each measurement, the crystal plate was cleaned with a soft absorbent paper and ethyl alcohol and dried with an air blower. The measuring done in the 1<sup>st</sup> Central laboratory in the College of Sciences-Mosul University <sup>36,37</sup>.

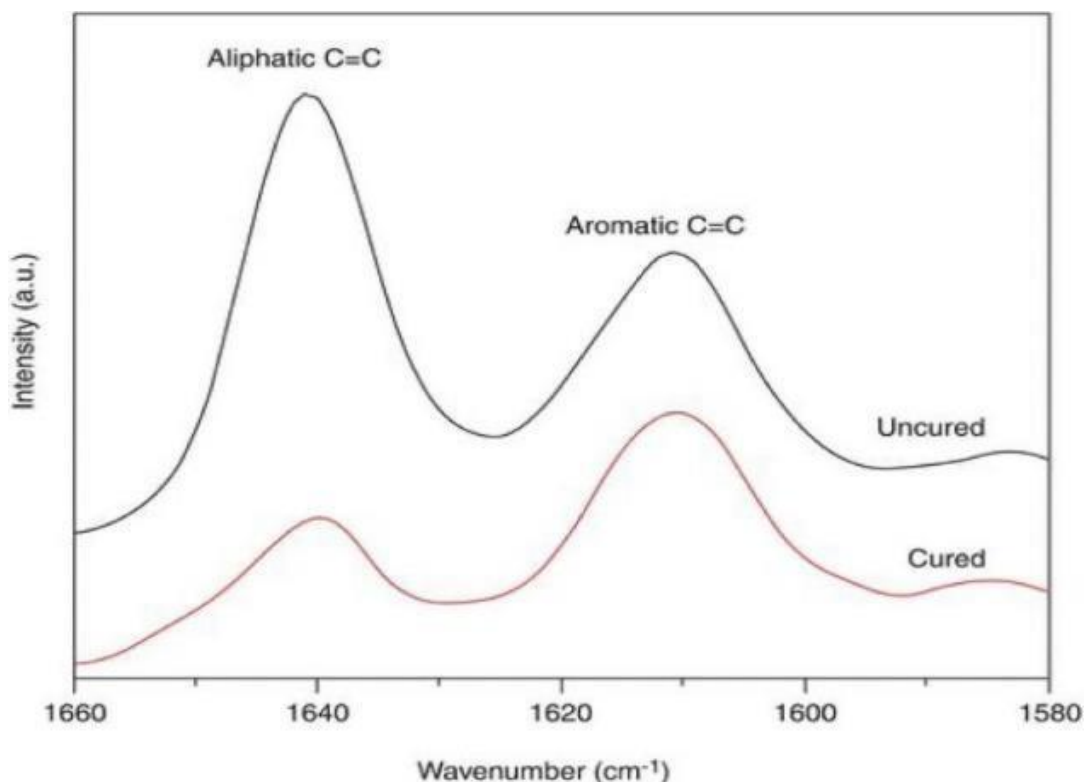
The peri-operative data were gathered and categorized based on the method employed to identify the (RLN). The (SPSS-version 21) was used to examine the data. The data were displayed as a percentage for dichotomous variables, and for continuous variables, the mean with standard deviation. The continuous variables were compared using the T-test. Simultaneously, The category variables were analyzed using chi-square tests. The correlation between different variables was assessed using Pearson correlation and multivariate linear regression tests. The statistical tests were two-tailed, with a  $p < 0.05$  as the predefined statistical significance threshold. Degree of conversion of each specimen was determined by comparison of the aliphatic carbon = carbon with that of the aromatic component for the cured and uncured resins. The DC of each specimen was estimated on a relative percentage basis with the 2-frequency method and the tangent baseline technique. The aliphatic carbon = carbon (C=C) double bond group has a characteristic infrared absorption peak around  $1,636 \text{ cm}^{-1}$  to  $1,608 \text{ cm}^{-1}$ .



**Figure 1.** Measuring the Degree of Conversion.

The aromatic carbon - carbon (C-C) single bond peaks due to the aromatic bonds of the benzene rings in the monomer molecules and its intensity remains unchanged during the polymerization reaction. The DC was calculated by using the baseline technique using the change in the ratio of the aliphatic C=C to the aromatic C=C before and after curing, DC of resin composite was calculated by the following equation:

$$DC\% = 1 - \left[ \frac{C_{aliphatic}/C_{aromatic}}{U_{aliphatic}/U_{aromatic}} \right] \times 100$$
 , DC% = 1 - [Cured aliphatic (C = C)/Cured aromatic (C..C)]/[Uncured Aliphatic (C = C)/Uncured Aromatic (C..C)] × 100. Where, Cured aliphatic (C = C) = Absorption peak at 1,636 cm<sup>-1</sup> of the cured specimen. Cured aromatic (C..C) = Absorption peak at 1,608 cm<sup>-1</sup> of the cured specimen. Uncured aliphatic (C = C) = Absorption peak at 1,636 cm<sup>-1</sup> of the uncured specimen. Uncured aromatic (C..C) = Absorption peak at 1,608 cm<sup>-1</sup> of the uncured specimen<sup>(38,39)</sup>. (Figure 2).



**Figure 2.** Diagram illustrate the Degree of Conversion

The Degree of Conversion (DC), represented in (percent %), which mean the degree of percent of converting resin from monomer to polymer, table (1).

Table 1. Degree of Conversion Value in (%) for all Groups

Groups	1	2	3	4	5	6	7	8	9	10
Control	71	68	71	66	73	67	69	72	69	68
F 5%	71	69	69	67	71	69	69	70	69	69
F10%	71	69	68	69	71	69	68	70	69	66
F15%	70	69	67	67	70	69	67	69	69	65
C 0.02 %	70	68	69	68	70	68	69	69	67	70
C 0.2 %	70	69	66	69	70	69	66	69	69	64
C 2 %	66	70	68	70	65	70	68	65	70	66
5%+0.02%	68	70	68	67	68	70	66	67	65	66
10%+0.2%	70	67	68	67	65	67	68	64	67	66
15%+ 2%	69	66	64	66	67	66	62	68	63	67

The descriptive statistics that includes the minimum, maximum, mean & standard deviation, are given in the table (2).

Table 2. Descriptive Analysis of Degree of Conversion in (%)

Groups	Minimum	Maximum	Mean	Std. Deviation
Control	66.00	73.00	69.40	2.27
F 5%	67.00	71.00	69.30	1.15
F10%	66.00	71.00	69.00	1.49
F15%	65.00	70.00	68.20	1.61
C 0.02 %	67.00	70.00	68.80	1.03
C 0.2 %	64.00	70.00	68.10	2.02
C 2 %	65.00	70.00	67.80	2.14
5%+0.02%	65.00	70.00	67.50	1.64
10%+0.2%	64.00	70.00	66.90	1.66
15%+ 2%	62.00	69.00	65.80	2.20

Statistically the results indicate that the highest mean value of DC appear with control group, which followed by flourcense groups (F5% & F10% & F15% respectively), then followed by color change groups (C 0.02 %, C 0.2 % & C 2 % respectively) with (C 0.02 %, more than F15%) while, the mixed adhesive groups (5%+0.02%,10%+0.2% & 15%+2%) show lowest one. As Figures below (figure 3 to 12).

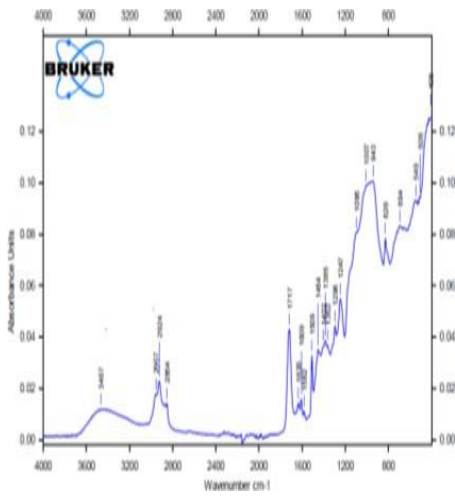


Figure 3. FTIR spectrum of control group

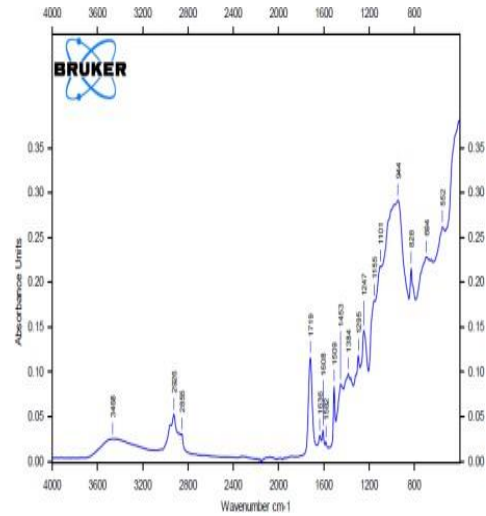


Figure 4. FTIR spectrum of adhesive + flourcense dye 5%

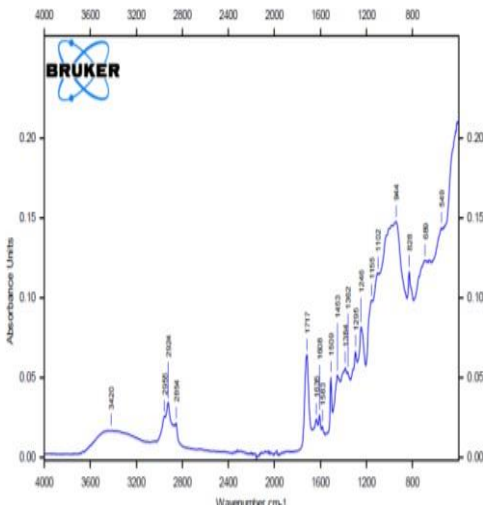


Figure 5. FTIR spectrum of adhesive + flourcense dye 10%

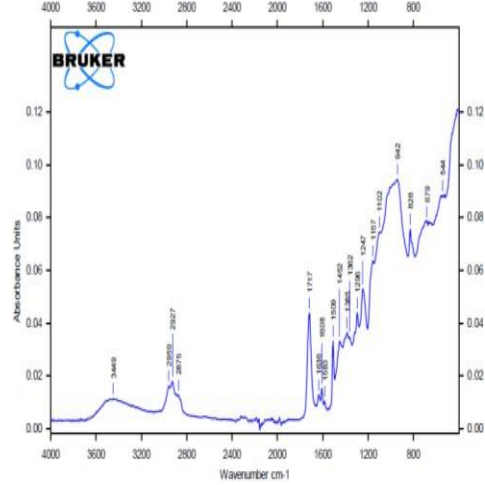


Figure 6. FTIR spectrum of adhesive + flourcense dye 15%

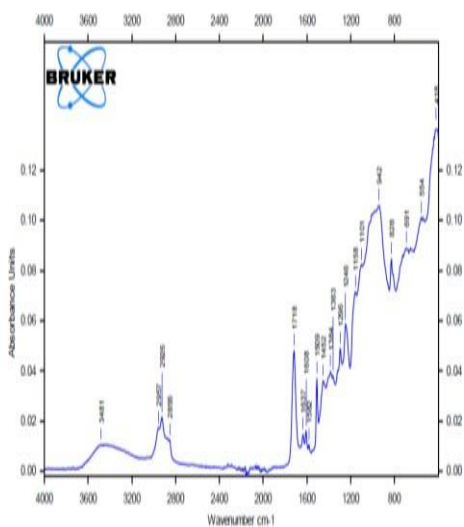


Figure 7 FTIR spectrum of adhesive + color change dye 0.02% change dye 0.2%

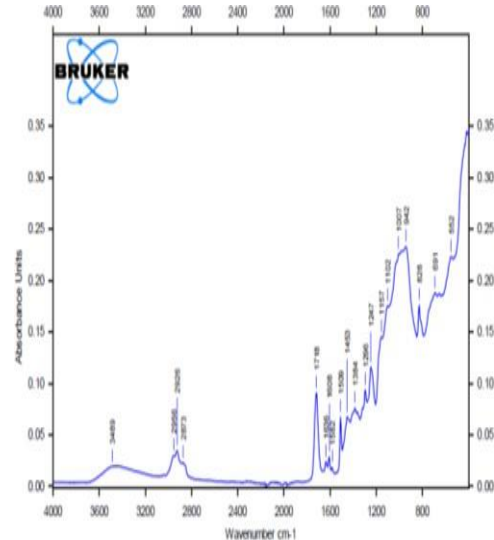


Figure 8. FTIR spectrum of adhesive + color change dye 0.2%

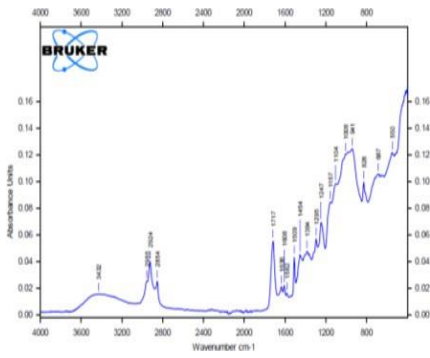


Figure 9 FTIR spectrum of adhesive + color change dye 2% 5% + color change dye 0.02%

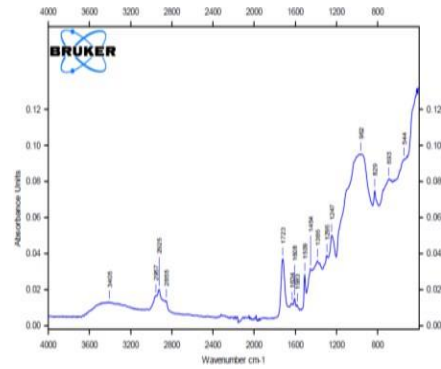


Figure 10. FTIR spectrum of adhesive + fluorescence dye 5% + color change dye 0.02%

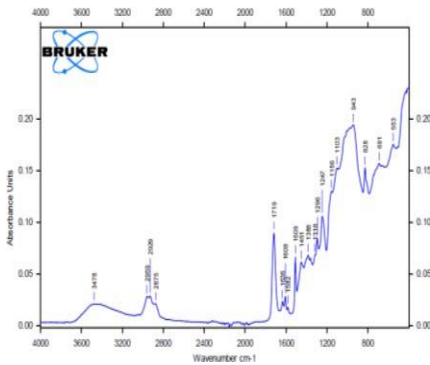


Figure 11. FTIR spectrum of adhesive + fluorescence dye 10% + color change dye 0.2%

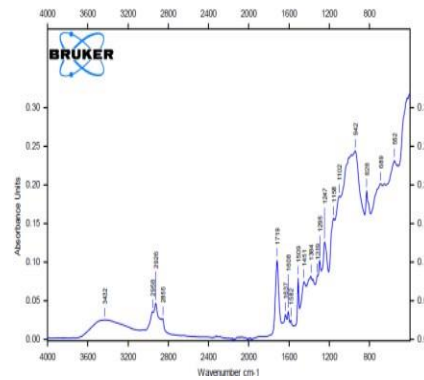


Figure 12. FTIR spectrum of adhesive + fluorescence dye 15% + color change dye 2%

For every group, the analysis of variance of one way (ANOVA) test reveals significant difference ( $p \leq 0.05$ ) between them as illustrated in table (3).

Table 3. Difference Among Degree of Conversion of groups using ANOVA (one way)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	116.160	9	12.907	4.102	.000
Within Groups	283.200	90	3.147		
Total	399.360	99			

The Duncan's multiple range test result in table (4) show significant difference within groups a significant difference ( $p \leq 0.05$ ).

Table 4. Duncan's Analysis for Determine the Significant Difference Between the Groups

Group	Mean	Std. Deviation	Duncan's test
Control	69.40	2.27	a
F 5%	69.30	1.15	ab
F10%	69.00	1.49	ab
F15%	68.20	1.61	abc
C 0.02 %	68.80	1.03	ab
C 0.2 %	68.10	2.02	abc
C 2 %	67.80	2.14	abc
5%+0.02%	67.50	1.64	bc
10%+0.2%	66.90	1.66	cd
15%+ 2%	65.80	2.20	d

\*different letters are significant

## DISCUSSION

The two main components of orthodontic resin-based adhesives are an inorganic filler component and an organic monomer matrix based on functional dimethacrylate, such as triethyleneglycol dimethacrylate (TEGDMA) and bisphenol A glycidyl methacrylate (Bis-GMA). Typically, the polymerization of dimethacrylates results in glassy resins where only a portion of the available double bonds react. The Degree of Conversion (DC) is the number of double carbon bonds (C=C) in the monomers that are changed into single bonds (C-C) to create the polymeric chain during the polymerization process<sup>40,41</sup>.

The DC of the aliphatic C=C in orthodontic adhesive resins is an important factor influencing the mechanical and physical properties such as flexural modulus of elasticity, hardness, tensile strength, compressive strength, color stability, solubility, degradation and biocompatibility. In general, the higher the conversion of double bonds, the greater the mechanical strength. The unreacted double bonds may present in free monomer or on the network. Therefore, it is thought that one of the ideal characteristics of a polymerization system is the reduction of the residual double bonds to the lowest level<sup>42,43</sup>.

The DC of a resin may be examined using a variety of techniques, including direct techniques like Fourier Transform Infrared Radiation (FTIR) spectroscopy and indirect techniques like microhardness studies. Indirect techniques, such as surface microhardness testing, work on the premise that harder materials are produced by converting more resin adhesive monomer to polymer. Alternatively, it is an indirect way to measure DC. However, FTIR analysis offers a more straightforward, accurate & dependable method for evaluate the DC<sup>44</sup>.

Fourier-transform infrared (FTIR) spectroscopy is a widely used technique for investigating materials in the gaseous, liquid, or solid phase. It is based on the interaction between electromagnetic radiation and natural vibrations of the chemical bonds among atoms that compose the matter. FTIR can detect the stretching vibrations of carbon-carbon double bonds involved in polymerization<sup>45</sup>. By comparing the proportion of methacrylate groups left after curing to the amount in the uncured material, the conversion of monomers is ascertained. The variations in conversion values observed may be due to variation in resins composition, type and quantity of load particles, concentration of diluents and initiators. The commonly achieved level of DC% for majority of dental applications is approximately 55-75%<sup>46,47</sup>.

There are different contributing factors that could influence the DC of resin composite such as the

light transmission of light through the material & properties of the matrix, the concentration of initiator/inhibitor in the resin<sup>48</sup> and the type of photoinitiator<sup>(50)</sup>, chemical structure & composition of the resin,<sup>(51)</sup> viscosity of the monomers<sup>52</sup>. Moreover, exposure time, light intensity, exposure time and the resin's absorption of energy<sup>53,54</sup>. Additionally, the filler volume percentage that might cause light reflection from the composite between the light source and the tooth structure and filler particles<sup>42</sup>.

Within this current study, the findings clearly show that DC have little difference between variable groups and the control group which have the highest DC one with 69.4 % while the adhesive with (Adhesive with 15% fluorcent & 0.2% color change) have the lowest one with 65.8 % DC. Anyhow, all DC falls inside the range of 55-75% documented in the literature for composite resins<sup>54</sup>.

In this study we find there was a relation between DC & viscosity, as when add dyes will affect the viscosity of adhesive which later affect the DC %. As in the control group, the samples with the lowest viscosity had the greatest DC mean values, because resins with lower viscosities provide improved monomeric mobility and free radical distribution within the material, which may improve the polymerization process and increase monomer conversion. Also with this principle, low viscosity group allow enhanced diffusion of reactive groups and promote the curing reaction, resulting in a higher DC. These agree with Francescantonio *et al* (2013)<sup>55</sup>. Also agree with de Araujo *et al* (2015) who indicated a relationship between the materials' viscosity and DC. The filler content affect the viscosity of resins & the low viscosity resins had a higher DC. As the viscosity increases, the remaining reactive species become entirely immobile<sup>36</sup>. As a result, the polymerisation stops before the reactants are used up completely<sup>56</sup>. These explain why the control group have the high DC as it don't have dye so it's have low viscosity while other groups have adding dye fillers with different percent. This phenomenon explains why there are decrease in DC when there are increase in dye concentration which in turn increase the viscosity of mixture.

Another contributing factor that could influence the DC of resin is the filler particles type, size and permeability to light. The light scattering within the resin might increase as the particle size of the filler approaches the wavelength of the activating light. The larger particles as in (fluorescent dye) have a greater depth of cure as they are less affected by light scattering while smaller filler particles as in (color change dye) scatter more light than producing less depth of cure. So, this scattering reduces the quantity of light that is transferred through the resin leading to decrease DC. This agree with Craig & Powers (2002) & Asmussen & Peutzfeldt, (2003)<sup>57,58</sup>.

Furthermore, increasing filler proportion, increase the scattered light that will reduce overall light transmittance through the resin material & the more difficult for the light to penetrate resin and consequently decrease the DC. This explain way the DC decrease when the percent of fluorescent or color change within group increase. This agree with da Silva *et al.* (2008) how showed that the DC of P60 (3M Dental Products) hybrid (large filler) composite and of Filtek greater than (3M ESPE) nanofilled (small filler) composite <sup>59</sup>.

Also agree with Rastelli *et al.* (2012), who claimed that the resin's increased tiny filler content would affect the outcomes by encouraging a lower DC. Because it lowers the organic matrix and lessens the production of free radicals, which lowers the DC <sup>60</sup>.

Other factor that affect the DC is the color of dye. The higher DC observed in fluorescent dye, as it has white to clear color, than that in color change dye, which is dark in color, the white color allows more light to pass through the mixture than dark color which in turn lead the increase DC. However, the increased filler content and lower resin translucency probably impaired light transmission through the material and inhibited monomeric conversion (e.g., translucency, size, type and amount of filler) and the shade be able to effect the DC this agree with Gaglianone *et al.* (2012) <sup>61</sup>. I also concur with other writers who claim that the degree of polymerization is influenced by the color of the resin substance like Koupis *et al.* (2006) who investigated that the shade A2 provides greater DC than shade A4 <sup>62</sup>. While Jafari *et al.* (2015) said that this phenomenon was explained by the fact that darker pigments absorb more light, which reduces the depth at which light may penetrate the resin <sup>63</sup>. Other research, however, found that darker materials produced greater polymerization than lighter counterparts <sup>64</sup>. Also this study disagrees with the Amato *et al.* (2014) who suggest that the orthodontic adhesives (Grenghlo and Green Glue) show more DC than the white Transbond XT due to they absorb more light as a result of the chromatic agents in their composition <sup>65</sup>.

Lower DC lead to composites with poor mechanical properties and greater degree of discoloration and degradation and lead to restorations with low wear resistance and poor color stability <sup>66,67</sup>. The lower DC can be the result of a compositional change rather than a sign of incomplete polymerisation leaving monomer behind <sup>54</sup>. According to Saikaew *et al* (2021), there was a link between an increase in colour concentration and a reduction in the degree of monomer conversion. By extending the light-curing time from 20 to 40 seconds, the degree of monomer conversion of the composites mixed with the color modifier was significantly enhanced <sup>68</sup>.

## CONCLUSION

Within the limitation of this study we can conclude that add color change &/or fluorescence dyes produce acceptable degree of conversion of bioactive adhesive, but when the concentration of dyes increase the degree of conversion will decrease.

## DECLARATION

### Competing interest

The authors declare that there are no competing interest.

### Funding

The work was not funded.

### Ethical Approval

“Not applicable”

### Consent for publication

“Not applicable” No funding was received from any financially supporting body

### Competing interests

The authors declare no competing interests.

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