

## Spectrophotometric colour stability analysis of Hesperidin incorporated Total-etch dentin adhesive- an invitro study

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### ABSTRACT

**Background:** Hesperidin has been investigated for its ability to promote collagen cross-linking, enhancing the stability and mechanical properties of collagen fibers. By incorporating hesperidin into the total-etch dentin adhesive, it is believed that it may contribute to improved bonding and longevity by strengthening the collagen matrix and reducing the risk of collagen degradation. The rationale behind incorporating these additives is to potentially enhance adhesive properties, improve bonding effectiveness, and address specific challenges related to color stability and moisture control.

**Materials and methods:** In this study, the total etch adhesive used was Adper single bond 2 (3m ESPE). 2% Hesperidin dissolved in Dimethyl sulphoxide was incorporated into total etch adhesive. Color stability was checked using Spectrophotometer after polymerisation of adhesives using a glass slide mould. The initial color of the specimens were measured according to the CIELAB color system. The L\*, a\*, and b\* values were recorded before and after ageing process at 7 days and 1 month. Color difference DE\* was calculated using the following equation:  $DE^* = \sqrt{DL^*^2 + Da^*^2 + Db^*^2}$  ]1/2.

**Results:** The color difference values for control group at 7 days and 1 month were 0.18 and 2.12 respectively and for the test group were 5.96 and 7.49 respectively. Color instability was more in test group compared to the control group. The colour difference is often not perceived visually and is regarded as clinically appropriate if the computed E value is less than 10. Thus the test group values are still clinically acceptable.

**Conclusion:** Overall, the findings of this study suggest that hesperidin can be a promising natural additive for improving the bonding ability of dentin adhesive without compromising its color stability. However, further studies are needed to evaluate the long-term durability and clinical efficacy of the hesperidin-incorporated dentin adhesive in vivo.

**Clinical Significance:** Adding Hesperidin as a natural collagen cross linker to total-etch dentin adhesives may benefit in improving the longevity of composite restorations by stabilising the dentin hybrid layer without compromising the color stability.

**Keywords:** *Hesperidin, Collagen-crosslinker, Total etch dentin adhesive, Micro-organisms, Quality of life*

## INTRODUCTION

Dental adhesives play a vital role in modern dentistry, as they are used to bond restorative materials to tooth structure<sup>1</sup>. However, one of the major challenges in this field is to achieve long-term durability and color stability of the adhesive-restoration interface. The ability of a material to withstand colour changes over time brought on by diverse environmental variables is referred to as colour stability. Color instability of dental adhesives can negatively affect the esthetics of the restoration and compromise the overall clinical success of the treatment<sup>2</sup>. Various factors like exposure to light, moisture, and oral fluids, as well as chemical and mechanical pressures, might impact the colour stability of dentin bonding agents. These elements may cause the bonding agent to deteriorate, change colour, or lose its translucency, which may impair the overall aesthetic appeal of the restoration. Manufacturers may employ a number of approaches to increase the colour stability of dentin bonding agents, including introducing light stabilisers or antioxidants into the formulation, enhancing the composition of material, or utilising sophisticated processing methods<sup>3</sup>. Dentin bonding agents can also keep their colour stability with careful handling and storage. For instance, it is important to utilise these items before their expiration date and store them in a cool, dry location away from heat sources like the sun. In general, colour stability of dentin bonding agents is a crucial factor for producing durable, aesthetically appealing dental restorations. To achieve the best performance and long-term durability, dentists and dental technicians should pick and handle these materials carefully.

Dentin adhesives, such as total-etch and self-etch, are frequently used in dentistry to bond restorative materials to tooth structure. In total-etch adhesive systems, the dentin surface is treated with an acid etchant, then rinsed, dried, and then the adhesive resin is added. Collagen fibres are exposed when the smear layer is removed by the etchant, providing micromechanical retention<sup>4</sup>. Total-etch adhesives frequently offer good bond strength, but they might be more laborious and technique-dependent. Self-etch adhesive methods, on the other hand, integrate the priming and etching processes into a single application<sup>5,6</sup>. These adhesives have acidic monomers that etch the tooth structure while also producing a resin tag

for bonding. In comparison to total-etch systems, self-etch adhesives are typically thought to be less technique-sensitive and potentially simpler to use. There are some distinctions between these two adhesive systems in terms of colour stability<sup>7</sup>. The biggest concern with colour stability is the possibility of adhesive deterioration or discoloration over time, which could change how aesthetically pleasing the restoration appears. The chemical composition and pH of the adhesive solution, as well as the presence of additional materials employed in the repair, can all affect colour stability<sup>8</sup>. It has been discovered that total-etch adhesives offer good colour stability over time. The acid etching procedure generates a spotless and ready-to-bond surface, which extends the life of the adhesive bond. It is crucial to remember that discoloration or degradation may happen if the adhesive layer is exposed to oral fluids because of marginal gaps or other problems. In comparison to total-etch adhesives, self-etch adhesives, particularly the older types, have been linked to slightly inferior colour stability<sup>9</sup>. The mild acidity of self-etch adhesives can make them less successful at eliminating the smear layer and possibly leading to a less durable adhesive surface, is a part of the reason behind this. However, more contemporary formulations now offer better colour stability due to the developments in self-etch adhesive technology. Due to the possibility of acid-induced hydrolysis or deterioration of the resin components, adhesives with lower pH values may be more susceptible to colour changes. Reduced colour stability could result from inadequate polymerization caused by high light exposure or poor curing. Poor polymerization can result in unreacted monomers or free radicals that can interact with staining substances and eventually cause discoloration<sup>10</sup>. The adhesive layer may inflate and expand as a result of water absorption, which could allow staining chemical substances to enter and cause discoloration. For greater colour stability, adhesives with reduced water sorption and solubility qualities have been found to be beneficial.

In recent years, natural compounds have been explored as potential additives to improve the color stability of dental adhesives<sup>11</sup>. To lessen the impacts of oxidative processes, which might result in colour alterations or material degradation over time, antioxidants are frequently added into dental materials. Antioxidants can be added to dentin adhesives to

assist prevent or lessen discolouration that results from the degradation of resin matrix components or by the interaction of adhesive with oral fluids. The potential for flavonoids, a class of naturally occurring compounds present in a variety of plants, to reduce colour instability in dentin adhesives has been studied. Flavonoids have antioxidant capabilities that can help fight against oxidative processes that cause colour fading and material deterioration in dental materials<sup>12</sup>. Hesperidin, a bioflavonoid found in citrus fruits, has shown promising results in this regard. Hesperidin has antioxidant properties and has been reported to inhibit the discoloration of dental materials<sup>13</sup>. Although there is a theoretical basis for considering hesperidin as a potential antioxidant in dental adhesive systems, little published research has explicitly looked at how efficient it is at enhancing colour stability in dental adhesives. Therefore, the present study aims to evaluate the color stability of hesperidin-incorporated total-etch dentin adhesive. The results of this study could provide valuable insights into the potential use of hesperidin as a natural additive to enhance the color stability of dental adhesives. Our team has extensive knowledge and research experience that has translate into high quality publications<sup>14-23,24-28</sup>

## MATERIALS AND METHODS

### *Preparation of Test solution*

In Total-etch dentin adhesive, 2% of hesperidin (HPN) was incorporated (2 mg of HPN powder in 98 mg of bonding agent). In this study, the total etch adhesive used was Adper single bond 2 (3M ESPE). Pure form of HPN powder from Sigma-Aldrich with more than 90% purity was used for this preparation. Hesperidin was solubilized using dimethyl sulphoxide, a small amount of which was utilised as a solvent. Adper Single Bond 2, an over-the-counter total etch dentin adhesive (3 M ESPE), served as the parent substance. For getting 2% concentration, 20 mg of hesperidin (Sigma-Aldrich) powder was directly dissolved in 0.025 ml of pure dimethyl sulfoxide. The final concentration of 2% hesperidin in the total etch adhesive used was obtained by incorporating the Hesperidin/Dimethyl sulfoxide into Adper single bond 2 at the right ratio (20 mg of HPN(0.0025ml DMSO) in 1 ml of bonding agent).

### *Control group*

Flavonoid(HPN)free adhesive

### *Test group*

Flavonoid(HPN) incorporated adhesive ( 20mg HPN+0.025ml DMSO+ 1ml of Adper single bond 2)

### *Sample Preparation*

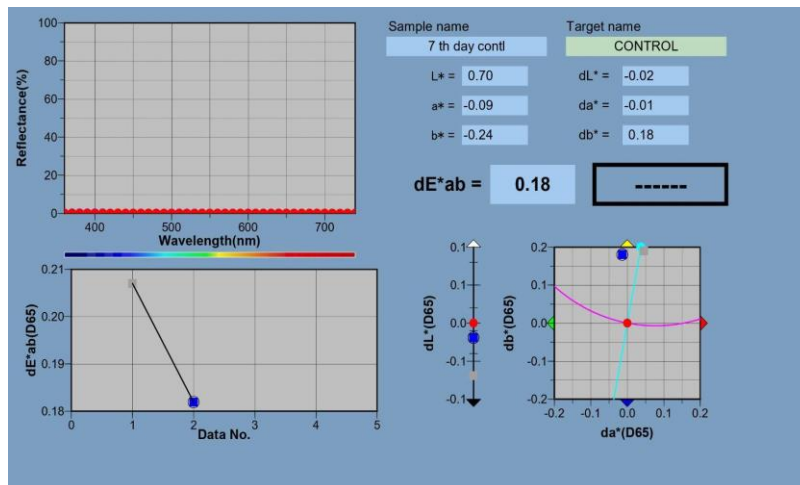
The bonding agents were applied in a glass slide with a mould (6mm in diameter and 1mm in thickness) according to their groups. It was made sure that the adhesive coating was homogeneous and free from any flaws or air bubbles. Each bonding agents were placed in a transparent plexiglass mould, sandwiched between two sheets of glass, and photopolymerized for 20 seconds using a light curing unit(Woodpecker). To create a clear and homogenous specimen, the margins of extra bonding agent was removed by trimming the glass slide margins.

The first colour values were measured on the direct photopolymerized surfaces of both the Groups 1 and 2 specimens after they had been stored in distilled water at 37 degrees Celsius, in complete darkness, for 24 hours. For the measurements of colour change, these original colour values served as the standard. Each specimen was kept in deionized water for 30 days at a temperature of 37 degrees Celsius in the dark. Colour measurements were conducted after the initial baseline measurements at 7, and 30 days of dark/water storage and then after ageing by storage in a water bath at 37°C (98.6°F). Colour change values (DE\*) were determined and expressed as a distance of two colour positions in the three-dimensional colour space between the baseline measurement and subsequent measurements using the following equation:  $DE^* = [DL^*^2 + Da^*^2 + Db^*^2]^{1/2}$ . It is a three-dimensional color model that represents colors based on their perceived lightness (L\*), chromaticity (a\*), and hue (b\*). All color change values were measured using a Spectrophotometer (KONICA MINOLTA SPECTROPHOTOMETER CM-5).

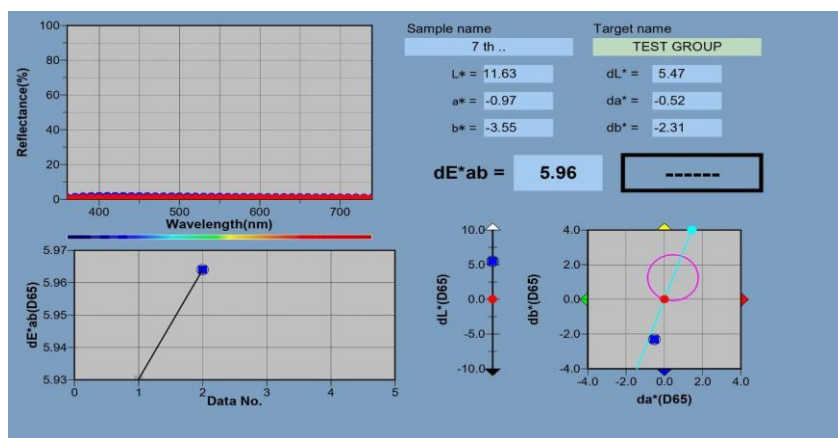


**FIGURE 1:** Konica Minolta – Spectrophotometer Cm-5

**RESULTS**



**FIGURE 2 :** Spectrophotometer analysis of Control group (7 days)



**FIGURE 3 :** Spectrophotometer analysis of Test group (7 days)

**TABLE 1:** Spectrophotometer color difference values for Control Group and Test group

Sample No	Pre			Post			dE*ab
	L*	a*	b*	L*	a*	b*	
<b>Control 7<sup>th</sup></b>	0.72	-0.08	-0.42	0.70	-0.09	-0.24	0.18
<b>Control 1mo</b>	0.72	-0.08	-0.42	2.75	-0.18	-1.02	2.12
<b>Test 7 th</b>	6.16	-0.45	-1.25	11.63	-0.97	-3.55	5.96
<b>Test 1 month</b>	6.16	-0.45	-1.25	13.54	-0.75	-2.51	7.49

## DISCUSSION

Resin-based adhesives have the inherent characteristic of polymerization shrinkage. Polymerization shrinkage can cause marginal gaps and voids at the adhesive interface in total etch adhesives<sup>29</sup>. These gaps may collect stains and cause colour fading over time<sup>30</sup>. Frequently, a wet dentin surface is necessary for the most effective bonding of total etch adhesives. On the other hand, excessive moisture might result in problems with colour stability over time<sup>31</sup>. The color change of the adhesive and deterioration due to moisture absorption could provide a less aesthetically attractive restoration. In order to avoid colour instability and increase the durability of restorations bonded with total etch adhesives, collagen cross-linkers are essential<sup>32</sup>. Collagen cross-linkers lessen the effects of moisture, deterioration, and other elements that may lead to colour changes and adhesive breakdown by strengthening the stability and durability of collagen fibres within the dentin. The stress caused by the polymerization shrinkage can be reduced using collagen cross-linkers<sup>12</sup>. They offer structural support by strengthening the collagen network and lessen the occurrence of voids and gaps at the adhesive interface. By doing this, microleakage-related stain accumulation and colour shifts are reduced. Collagen cross-linkers can be added to the total-etch adhesive formulation to increase the stability and durability of the adhesive-dentin interface, which will improve colour stability and cosmetic results for restorations<sup>33</sup>. The possible function of flavonoids, a class of naturally occurring substances present in a variety of plants, as collagen cross-linkers has been investigated. While flavonoids are mostly recognised for their anti-inflammatory and antioxidant effects, some study indicates that they may also help cross-link

and stabilise collagen fibres. It has been discovered that flavonoids have chemical structures that interact with collagen molecules to encourage cross-linking. This cross-linking takes place when lysine and hydroxylysine, two amino acid residues found in collagen, create covalent connections with flavonoid molecules. These cross-links contribute to the integrity and strength of the collagen matrix. By interfering with enzymes like matrix metalloproteinases (MMPs), flavonoids have been found to impede the enzymatic degradation of collagen<sup>34</sup>. Flavonoids assist in maintaining the stability and integrity of collagen fibres by preventing the action of these enzymes, hence lowering the susceptibility of collagen fibers to deterioration and disintegration<sup>35</sup>. Also Flavonoids are effective against cariogenic microorganisms<sup>36,37</sup>. Flavonoids have strong antioxidant properties, which may help protect collagen. Collagen deterioration and damage can be caused by oxidative stress and free radicals. Free radicals are scavenged by flavonoids, which lowers oxidative stress and stops collagen deterioration. The stability of collagen can be improved and its cross-linking can be indirectly supported by this antioxidant impact. The synthesis and remodelling of collagen can be interfered with by inflammation, which has a detrimental effect on the stability of collagen fibres. Flavonoids have anti-inflammatory qualities and have the ability to control inflammatory reactions<sup>38</sup>. Flavonoids assist in creating an environment that is favourable for collagen formation and appropriate cross-linking by lowering inflammation. It's important to note that certain flavonoids, including proanthocyanidins from grape seed extract and epigallocatechin gallate (EGCG) from green tea, have demonstrated promising results in improving collagen cross-

linking and stability<sup>39,11</sup>. A flavonoid called hesperidin is mostly present in citrus fruits including oranges, lemons, and grapefruits. It has been researched for potential health advantages in a number of contexts, including its function in collagen stability and cross-linking. Additionally, Hesperidin has anti-inflammatory, analgesic, antibacterial, antioxidant, bone loss prevention, promotes remineralization and inhibits demineralization, anti-caries impact (prevents caries progression due to collagen cross linking property) and MMP inhibition apart from collagen cross linking property<sup>40</sup>. The chemical composition of hesperidin, which consists of a flavanone backbone with many hydroxyl groups, is what is thought to be responsible for its antioxidant activity. Free radicals and reactive oxygen species, which are engaged in oxidative reactions that can cause tooth materials to deteriorate and discolour, may be scavenged by the hydroxyl groups<sup>41</sup>. Hesperidin may assist in preventing or reducing the oxidation of resin matrix elements, such as methacrylate monomers, which are vulnerable to deterioration upon exposure to oxygen and other reactive species, by neutralising free radicals. This might help sustain the dentin adhesives and their ability to retain their colour over time. Dental adhesives have used the solvent DMSO to assist regulate moisture levels in the dentin because of its strong penetrating characteristics<sup>42</sup>. Too much moisture might weaken the bond strength of the adhesive and cause colour instability. It is believed that adding DMSO will help to remove water from the dentin, enhancing the entry of adhesive monomers and bonding effectiveness overall. DMSO was used as a solvent to dissolve Hesperidin in this study. This study evaluated the color stability of Hesperidin incorporated total-etch dentin adhesive. The present study also evaluated the effect of aging on the color stability of the hesperidin-incorporated dentin adhesive. The color difference values as represented in Table 1 for control group at 7 days and 1 month were 0.18 and 2.12 respectively and for the test group were 5.96 and 7.49 respectively. Color instability was more in test group compared to the control group. The "E 10" or "E10" criterion refers to the interpretation that regards a colour difference value (E) less than 10 as clinically acceptable. To decide if colour discrepancies between dental restorations, dental materials, or changes in tooth colour are acceptable, this criterion is frequently employed in dental

research, dental material evaluation, and clinical practise. The E10 criterion offers a threshold value that can be used to determine whether a colour change is within a permissible range for therapeutic applications. The colour difference is often not perceived visually and is regarded as clinically appropriate if the computed E value is less than 10. Thus in our present study the test group values are still clinically acceptable. The fact that this was an in vitro investigation and glass slides were used instead of natural teeth may have contributed to the high colour instability values that were achieved. In this study glass slides were used because glass slides offer a reliable and uniform substrate for testing colours. Natural teeth can vary in dentin colour, enamel thickness, and surface morphology, all of which can affect how colours are measured and add new variables. On the other hand, glass slides have a homogenous surface, enabling more precise and trustworthy colour comparisons. The purpose of ageing at 37 °C is to mimic the oral environment and the environmental exposure of dental materials. This temperature is chosen because it simulates a situation that is practical and clinically applicable for assessing the colour stability and other qualities of dentin adhesives.

Overall, the findings of this study suggest that hesperidin can be a promising natural additive for improving the bonding ability of dentin adhesive without compromising its color stability. However, further studies are needed to evaluate the long-term durability and clinical efficacy of the hesperidin-incorporated dentin adhesive in vivo. Furthermore, other natural compounds with antioxidant properties should also be investigated for their potential as additives in dental adhesives to improve their color stability. Hesperidin is thought to improve colour stability in part because of its antioxidant qualities. However, more investigation is required to validate these results in clinical settings and assess the long-term colour stability of Hesperidin incorporated total-etch dentin adhesive.

## CONCLUSION

In conclusion, the present study provides evidence that the incorporation of hesperidin which has potential applications in dental restorations can provide clinically acceptable color stability of dentin adhesive.

**Clinical Significance**

Adding Hesperidin as a natural collagen cross linker to total-etch dentin adhesives may benefit in improving the longevity of composite restorations by stabilising the dentin hybrid layer without compromising the color stability.

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