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EVALUATION OF COLOR AND WETTABILITY OF A UNIVERSAL ADHESIVE MODIFIED BY INCORPORATING CHITOSAN CAPPED CERIUM OXIDE NANOPARTICLES

Hisham I. Wali[1*], Sawsan H. Al-Jubori[2]

[1]Department of Conservative Dentistry, Faculty of Dentistry, Tishk International University, Erbil, Iraq
[2]Department of Conservative Dentistry, College of Dentistry, University of Mosul, Mosul, Iraq

Abstract. *Purpose*: To evaluate color and wettability of a universal adhesive modified by incorporating chitosan capped cerium oxide nanoparticles (C-CeNPs) at four different weight percentages. *Methods*: C-CeNPs was synthesized and characterized by FTIR, TEM, FESEM and EDS. Experimental groups; G I, II, III and IV were evaluated for color and wettability. Data were analyzed by ANOVA and Duncan's test. *Results:* FTIR charts confirmed assembly of chitosan and cerium. TEM revealed homogeneous distribution of C-CeNPs with particle size less than 50nm. FESEM showed a foam-like irregular microsphere of C-CeNPs. EDS confirmed the elemental analysis of C-CeNPs. Color absorption peaks for the experimental groups ranged between 430-470 nm wavelength. Mean contact angle values for G I, G II, G III and G IV were 21.1°, 40.43°, 42.35° and 46.07° respectively. *Conclusions*: Incorporating C-CeNPs did not significantly change the color of the experimental groups while it significantly changed their contact angle values.

Keywords: *Cerium oxide, chitosan, color, nanoparticles, universal adhesive, wettability.*

Corresponding Author: *Hisham I. Wali, Department of Conservative Dentistry, Faculty of Dentistry, Tishk International University, Erbil, Iraq, e-mail: hishamibrahim1976@gmail.com*

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1. Introduction

Nowadays, resin composites and dental adhesive systems are most commonly used to restore decayed teeth as primary direct restorative materials (Alzraikat *et al*., 2018; Attia, 2021). Currently the so-called universal adhesives (UAs) could represent a potential new trend for dentists as they provide a more straightforward approach to the traditional concept of adhesive technology by reducing procedure's sensitivity, increasing efficiency and saving time for clinical application (Ahmed *et al*., 2019; Ermis *et al*., 2019). UAs are also called multimode adhesives since they can be used with any adhesive strategy and hence, they are considered as user friendly (Cuevas-Suárez *et al*., 2019; Ahmed & Chakmakchi, 2015). The color and wettability of UAs are considered as paramount pre-requisites to gain an esthetic and well-functioning tooth filling. Being composed of several components, like monomers, solvents and initiators, UAs can affect the color of the resin composite filling restoration (FiDan & Yağci, 2023). Furthermore,

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incorporating nanoparticles into dental adhesive systems can change the color of these systems (Khan *et al*., 2023). The wettability of a dental adhesive system is a crucial factor to ensure its deep penetration into tooth structure and hence ensure good performance of the filling material (Katyal *et al*., 2021). The wettability of a fluid is intimately related to its viscosity where higher viscosity results in lower wettability and adding nanoparticles might change the viscosity of the adhesive system (Safiei *et al*., 2020).

Improving dental adhesives, by adding nanoparticles, have been evaluated by several studies (Porto *et al*., 2023). Cerium dioxide (CeO_2), an earth oxide found in the lanthanide series of the periodic table, has been increasingly used as a nano-therapeutic material (Jairam *et al*., 2023). Cerium dioxide (CeO_2) has been used to increase radiopacity and enhance the ability to detect dental adhesives under resin composites (Garcia *et al*., 2020; Abdulkadir & Nayif, 2013) and to impart antimicrobial action to an orthodontic adhesive (Pourhajibagher & Bahador, 2022). Maintaining particle size is one of the key issues with using nanoparticles in suspension. Nanoparticles constantly interact with neighboring molecules and other particles due to their charged surface and high surface to volume ratio. As a result, nanoparticles frequently form clusters of up to several microns (Grulke *et al*., 2014). By increasing the size of the filler phase from nanometer to micrometer, the agglomeration of nanoparticles may adversely affect the mechanical characteristics of an adhesive system containing nanoparticles. The agglomeration of nanoparticles can be minimized if suitable capping agents are used (Javed *et al*., 2016).

Chitosan is a co-polymer comprising d-glucosamine and N-acetyl-d-glucosamine produced via alkaline deacetylation of chitin naturally occurring in the crustaceans or hydrolysis of chitin by the enzymatic action of deacetylase (Elgadir *et al*., 2015). Chitosan has been used as a capping agent to stabilize the colloidal dispersion and to control the morphology and optical properties of metal nanoparticles including gold (Franconetti *et al*., 2019), silver (Cinteza *et al*., 2018) and copper (Jayaramudu *et al*., 2019). The aim of this study was to evaluate the color and wettability of a universal adhesive modified by incorporating chitosan capped cerium oxide nanoparticles (C-CeNPs). The null hypothesis was that incorporating C-CeNPs would neither significantly affect the color nor the wettability of the modified adhesive when compared with the non- modified adhesive.

2. Materials and Methods

Synthesis of chitosan-capped cerium oxide nanoparticles colloidal suspension (C-CeNPs): The synthesis of C-CeNPs was based upon the procedure implemented by Zhao (2018). The materials used to prepare C-CeNPs were of analytical grade reagent and used as they were purchased and received from Sigma Aldrich.

To synthesize C-CeNPs, 1g of chitosan (Mw=50,000) was first added into 100ml of deionized water. Since chitosan cannot be dissolved at neutral pH, 0.1 M hydrochloric acid was added into the chitosan solution to promote dissolution. When the chitosan is completely dissolved, the pH value was adjusted back to 7 with the incremental addition of 0.03M sodium hydroxide.

After that, 2.66 g of cerium (III) nitrate hexahydrate was added into chitosan solution under stirring. After 2 days of reaction under stirring, the pH value was approximately adjusted to 3 with the addition of hydrochloric acid. Then 1 drop of hydrogen peroxide was added into the solution to form C-CeNPs. The solution was stirred for 3 days and a suspension of C-CeNPs was formed. Then a rotary evaporator was used

to remove the access water from the solution. The resultant solution's volume was reduced from 100 ml to 25ml and thus the solution was made more concentrated. Thenafter, in order to remove microparticles and aggregates from the solution, it was filtered through syringe filters of pore sizes 0.45, 0.20, 0.1 and 0.05 micrometer respectively. The final colloidal solution was stored in refrigrator for characterization.

Characterization of the prepared colloidal suspension

Characterization by Fourier Transform Infrared Spectroscopy (FTIR): FTIR spectroscopy was performed for chemical characterization of the created colloidal suspension to confirm the chemical reaction between the cerium nanoparticles and chitosan. This was done by comparing the FTIR charts of chitosan powder and cerium tri-nitrate hexahydrate powder with FTIR of the created colloidal suspension after being dried. The method of sample preparation was done by placing a thin film of the C-CeNPs colloidal solution between two sample plates for FTIR measurement. The FTIR spectra were measured at a range between (400-4000) cm^{-1} in the transmittance mode. The charts were then analyzed and interpreted by two specialists.

Characterization by Transmission Electron Microscopy (TEM): TEM imaging was performed to confirm the surface coating of cerium nanoparticles by chitosan and to confirm the size of the nanoparticles. The imaging procedure started by dispersing a single drop of the prepared colloidal solution (10μm/mL) onto carbon coated copper TEM-grid (400-mesh). The grid was left for 30 to 45 min to dry at the room temperature and then dried in vacuum to be ready for imaging by using (TEM, Philips CM120) at 120KeV. ImageJ Digital analysis software was used to analyze the particles size distribution from the TEM images.

Characterization by field emission scanning electron microscopy (FESEM) and energy dispersive X-ray spectroscopy with elemental mapping (EDS): FESEM and EDS were performed to determine the morphology and elemental composition of C-CeNPs respectively.

Incorporation of the Capped Nanoparticles into the Universal Adhesives: After characterizing and examining the nanoparticles size distribution, 0, 1, 2 and 3% by weight of the prepared colloidal suspension were incorporated into the universal adhesive. The adhesive agent selected to be used in this study is of commercially available universal adhesive, which is the All Bond Universal adhesive (Bisco, USA).

After incorporation of the capped nanoparticles, each adhesive bottle was shaken with the aid of mechanical mixing device (VELP Advanced Vortex Mixer; Biobase Biomettch CO. Ltd, China) for 2 minutes in a spiral agitation motion at 2000 rpm speed to allow even blending and dispersion of the capped nanoparticles into the universal adhesive (Ge *et al*., 2017).

The experimental groups were:

Group I (GI): All Bond Universal adhesive without C-CeNPs

Group II (GII): All Bond Universal adhesive with 1% wt C-CeNPs

Group III (GIII): All Bond Universal adhesive with 2% wt C-CeNPs

Group IV (GIV): All Bond Universal adhesive with 3% wt C-CeNPs.

Color evaluation: One sample from each experimental group was used to detect its color using a spectrophotometer (UV-1900i UV-Vis spectrophotometer; Shimadzu, Japan) to test the spectral reflectance. The absorbance spectra were recorded with the wavelength ranging between 340-900 nm. The band gap values of experimental groups were calculated according to the following equation:

$$E=hc/\lambda$$

Where E represents the energy (band gap), h represents the Planck constant, c represents the velocity of light and λ represents the wave length corresponding to the absorption peaks.

Wettability evaluation: Eight samples from each experimental group was used to detect the wettability which was determined by measuring the contact angle of the adhesives. This was done by placing 20 µl of each sample on a smooth flat aluminum plate, covered by polytetrafluoroethylene tape and positioned on the sample table of a homemade contact angle goniometer (Figure 1 A and B). Then, the contact angles were measured using contact angle goniometer's image J software program (Figure 1 C and D).

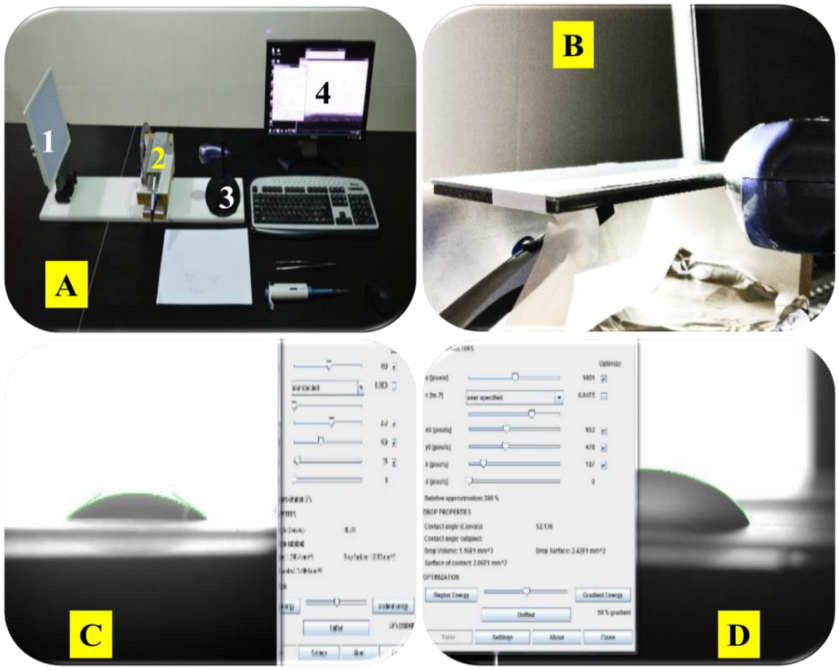

Figure 1. A, Home-made contact angle goniometer used to measure the contact angle of All Bond Universal Adhesive (non-modified) and modified with 0.5% and 1% C-CeNPs colloidal suspension. 1: LED light source, 2: Level-adjustable sample table, 3: High resolution camera, 4: Image J software program shown on computer monitor. B; A 20 µl adhesive drop sample (arrow) placed on aluminum sample table covered with polytetrafluoroethylene tape. C and D; Image J software program interface while measuring contact angles

Statistical Analysis:

The statistical analysis was performed using SPSS 25. Data were analyzed by analysis of variance (one- way ANOVA) and Duncan's test.

3. Results

Characterization results of the prepared chitosan-capped cerium oxide nanoparticles (C-CeNPs):

The results of the characterization of the prepared colloidal suspension of C-CeNPs by Fourier transform infrared spectroscopy, field emission scanning electron microscopy with energy dispersive X ray spectroscopy mapping and transmission electron microscopy are as follows:

Characterization results by Fourier transform infrared spectroscopy (FTIR):

The FTIR spectra of chitosan, cerium tri-nitrate hexahydrate and C-CeNPs (Figure 2). Regarding chitosan, sharp transmittance bands are present at 1025 and 1059 cm^{-1} which correspond to C-O stretching. Axial deformation of C=O bond of NH_2 group is present at 1558 cm^{-1}, C-H symmetric stretching is present at 2919 cm^{-1} and O-H group stretching is present at 3307 cm^{-1} (Figure 2A). Regarding cerium tri-nitrate hexahydrate, stretching and bending vibrations of water molecules are present at 3380 and 1631 cm^{-1} while nitrate ion vibrations are present at 1431, 1293 and 816 cm^{-1} (Figure 2B).

The FTIR chart of C-CeNPs shows transmittance bands in the range of 420-667 cm^{-1} which indicate Ce-O bond stretching, rocking vibrations of NH_2 peaks of chitosan present at 894 and 945 cm^{-1}, Stretching vibrations of C-N and C-O bonds at 1026, 1076 and 1149 cm^{-1} confirming the presence of chitosan, stretching vibrations of the main chitosan chain at 1253 and 1381 cm^{-1}, stretching vibrations of C-H bond at 1545 and 1539 cm^{-1}, a sharp transmittance peak at 1643 cm^{-1} which designates the carbonyl (C=C) bond of chitosan, carbonyl group (C=O) stretch at 1743 cm^{-1}, stretching vibration of C-H bond at 2951 cm^{-1} and a wide band at 3448 cm^{-1} which represent stretching vibration of N-H group bonded to O-H group in chitosan confirming the assembly of chitosan and cerium tri-nitrate hexahydrate (Figure 2C).

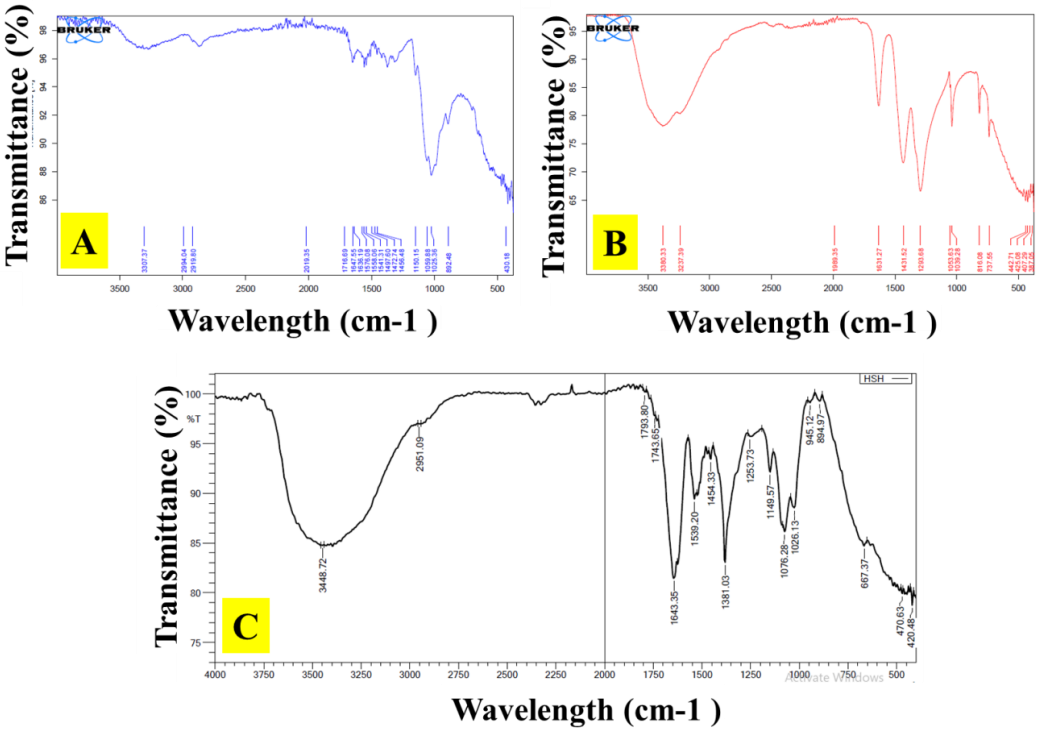

Figure 2. FTIR chart of the studied materials. (A) pure chitosan, (B) Cerium tri-nitrate hexahydrate and (C) C-CeNPs

Characterization results by Transmission Electron Microscopy (TEM):

TEM micrographs (Figure 3 A and B) show homogeneous distribution of C-CeNPs within the colloidal suspension and that the size of the particles is much less than 50nm. The results of the TEM micrographs for the capped nanoparticles demonstrated some areas of small agglomerated clusters, as well as individually dispersed spherical shaped cerium nanoparticles cores that are capped by chitosan shell around the nanoparticles

(Figure 3C). Chitosan capping have also prevented cerium nanoparticles from aggregating with each-other.

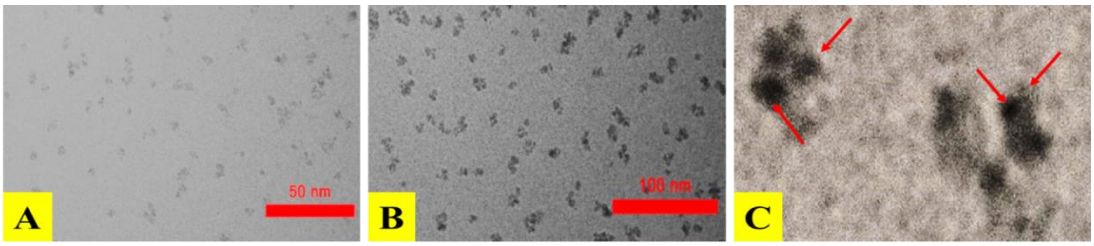

Figure 3. TEM of C-CeNPs. A and B, homogeneous distribution of C-CeNPs within the colloidal suspension. C, TEM micrographs for the capped

Characterization results by field emission scanning electron microscopy (FESEM) and energy dispersive X-ray spectroscopy with elemental mapping (EDS):

FESEM micrograph (Figure 4A) shows an irregularly-shaped microsphere with a very high porosity and foam like morphology. The microsphere does not have the characteristic appearance of chitosan which confirms the union of cerium oxide with chitosan in one structure. EDS micrograph (Figure 4B) shows all the elements of C-CeNPs with a densely packed and homogenously distributed cerium nanoparticles surrounded by carbon and nitrogen nanoparticles which are the main components of chitosan. While micrographs (Figure 4C to H) show carbon, cerium, chloride, nitrogen, sodium and oxygen elements respectively.

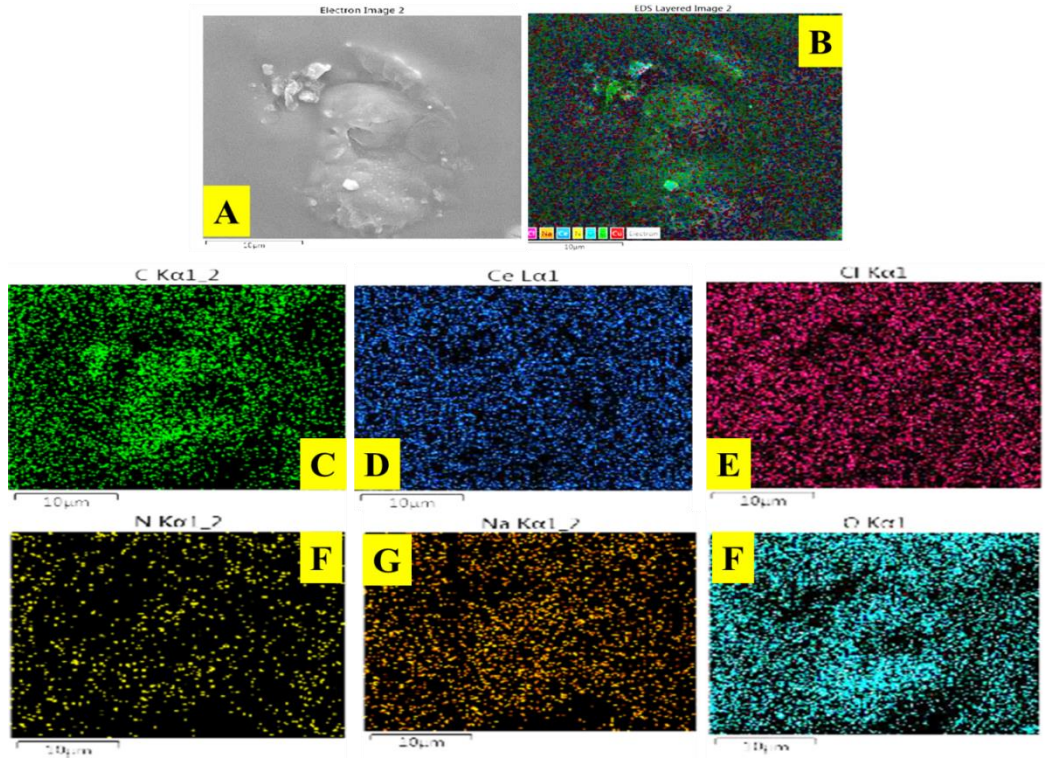

Figure 4. A, FESEM micrographic image showing an irregularly-shaped microsphere. B, elements of C-CeNPs microsphere. C to H show carbon, cerium, chloride, nitrogen, sodium and oxygen elements, respectively

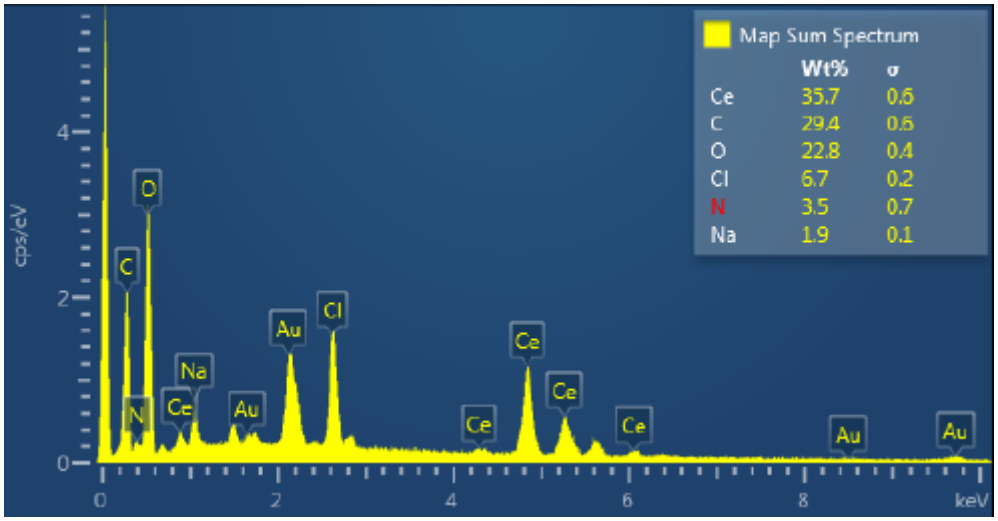

Figure 5. EDS mapping chart showing the chemical elemental analysis and ionic distribution of C-CeNPs colloidal solution

The EDS mapping characterization chart (Figure 5 and Table 1) show the chemical elemental analysis and ionic distribution of C-CeNPs colloidal solution. Additionally, the chart clearly shows that components of chitosan, which are carbon and nitrogen atoms, are homogeneously well dispersed in relationship to cerium and oxygen atoms confirming capping of cerium nanoparticles with chitosan.

Table 1. EDS mapping table showing the chemical elemental analysis and ionic distribution of C-CeNPs colloidal solution

Element	Line Type	Apparent Conc	K ratio	Wt%	Wt$ Sigma	Standard Label	Factory Standard
C	K series	0.3	0.00297	29.45	0.61	C Vit	Yes
N	K series	0.14	0.00026	3.54	0.66	BN	Yes
O	K series	0.85	0.00285	22.75	0.42	SiO_2	Yes
Na	K series	0.08	0.00033	1.94	0.13	Albite	Yes
Cl	K series	0.27	0.00235	6.66	0.17	NaCl	Yes
Ce	L series	1.19	0.01107	35.66	0.59	CeO_2	Yes
Total				100			

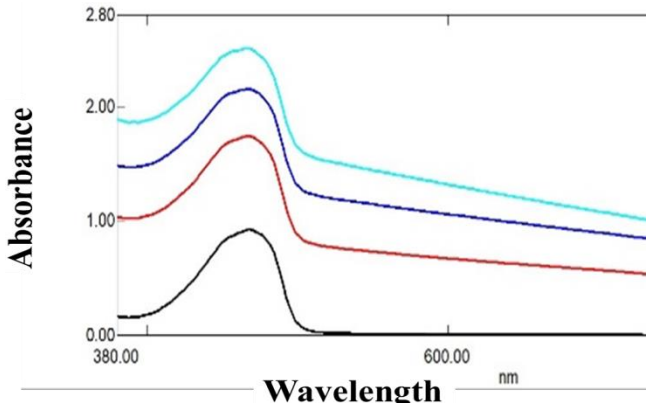

Figure 6. The UV-Vis spectrophotometer charts showing the absorption peaks for the GI (Black curve), G II (Red curve), G III (Violet curve) and G IV (Blue curve)

Color evaluation results: UV-Vis spectrophotometer charts shows the absorption peaks for the experimental groups ranging between 430-470 nm wavelength indicating that their colors are in the yellow region (Figure 6). The band gap values of G I, G II, G III and G IV were 2.82, 2.80, 2.79 and 2.77 eV, respectively.

Wettability evaluation:

The highest mean contact angle was for 3% C-CeNPs incorporated adhesive while the lowest mean contact angle was for non-modified adhesive. One-way ANOVA showed significant differences between the experimental groups while Duncan's test showed that the mean contact angles of experimental groups increased in line with increasing the mass of the incorporated prepared C-CeNPs colloidal suspension (Tables 2 and 3).

Table 2. The differences of contact angle values among experimental groups

Treatment	Mean±SD (n=8)	Min.-Max.
Non-modified	21.1075±0.89554d*	19.88-22.76
Modified 1%	40.4375±1.39852c	38.13-42.11
Modified 2 %	42.3538±1.52182b	40.14-44.54
Modified 3 %	46.0725±1.12902a	44.66-47.44
Different letters indicate significant differences among groups at $p<0.05$ using Duncan's test.		

Table 3. The effect of the mass of C-CeNPs incorporated on the contact value angle values of experimental groups

	Sum of Squares	df	Mean Square	F-Value	P-Value
Between Groups	2995.115	3	998.372	629.048	0.0001
Within Groups	44.439	28	1.587		
Total	3039.554	31			

One-way ANOVA test

4. Discussion

UAs are relatively new category of dental bonding agents. These adhesives have versatile applications in conservative dentistry since they can be used for direct and indirect restorations. Furthermore, these adhesives are compatible with all adhesion strategy like etch and rinse, self-etch and selective enamel etching. The color and viscosity of UAs are properties of paramount importance when restoring teeth. The first has a direct effect on the final outcome of the final restoration since it is associated with the esthetic result and affects the color of the restoration while the latter is related to the wettability of the adhesive and its ability to penetrate the partially demineralized tooth structure (Katyal *et al*., 2021).

In this study the color of the G I did not differ from that of the other groups regardless of the percentage of C-CeNPs added to them. This could be attributed to the color of chitosan and cerium tri-nitrate hexa-hydrate materials used to prepare C-CeNPs. The color of chitosan was faint beige to beige while the color of cerium tri-nitrate hexa-hydrate was white according to specification products sheets PRD.2.ZQ5.10000020770 and (EC) No.1907/2006 respectively. When chitosan and cerium tri-nitrate hexa-hydrate were mixed with other constituents to prepare C-CeNPs colloidal solution, the latter had a nearly translucent color which did not impair color of the modified adhesive.

Being a resin based material, UAs and resin based composites share nearly the same chemistry where both of them have components like monomeric resin matrix, fillers and curing agents. These constituents guarantee effective bonding to tooth structure and between UAs and resin based filling material (Dressano *et al*., 2020).

The wettability of an adhesive is considered the initial step in bonding procedure. An adhesive with good wettability can flow and spread on all the irregularities of the tooth structure ensuring good bonding with that surface. Wettability of a liquid on a solid can be characterized by the contact angle that forms between a liquid and solid, as measured within the liquid. Categories of wettability include "mostly nonwetting" (>90°), "absolutely no wetting" (180°), "mostly wetting" (<90°) and absolute wetting (0°) (Dahl & Stenhagen, 2018).

One of the most important factors that affects the wettability of a fluid is its viscosity (Vafaei *et al*., 2011). Nanoparticles' inclusion in a liquid increases its viscosity (Mishra *et al*., 2014). The viscosity of an adhesive can be affected by several factors like type of monomers, refrigeration time, type of solvents and type, shape, distribution, loading and salinization of fillers (Zhang *et al*., 2021).

Despite of abundance of research about the effect of fillers on viscosity of resin based composites (Habib *et al*., 2018; Lee *et al*., 2006; Shen *et al*., 2014; Zandinejad *et al*., 2006), few articles discuss this issue regarding adhesives (Al-Saleh *et al*., 2021; Fathi, 2015; Rao *et al*., 2023). However, adhesives that contain nanoparticles can be considered as nanofluids. A nanofluid is composed of nanoparticles, which are characterized by their sub-nanometer size. Fluids are transported via a suspension of fluids in colloidal form. Nanoparticles within nanofluidic systems are derived from a variety of sources, including metals, oxides, carbides and carbon nanotubes (Shah *et al*., 2024). There are several factors that strongly influence the viscosity of nanofluids including solid volume fraction, temperature, particle size, particle shape, different base fluids, surfactants addition, ultrasonication, nanoclustering and pH value (Habib *et al*., 2018).

The wettability of the experimental groups has linearly decreased as the percentage of added colloidal solution increased from 0 to 3%. G II, III and IV showed higher viscosity than G I since they incorporate C-CeNPs. However, the measured contact angles where all less than 90° which means that all the experimental groups were in the mostly wetting category. This could be attributed to the capping effect of cerium nanoparticles by chitosan which prevented the agglomeration of nanoparticles with each other since this agglomeration leads to dramatic increase in viscosity and hence decreased wettability of the adhesive (Elgadir *et al*., 2015).

Al-Saleh et al. (2021) and Binhasan et al. (2022) also evaluated the change of viscosity after adding nanoparticles to adhesives. However, their results disagree with this study. In the Al-Saleh et al. (2021), who have added TiO_2 and ZrO_2 nanoparticles to an experimental adhesive made of blend of monomers and measured its viscosity by a rheometer while in the Binhasan et al. (2022) study, cylindrical and hexagonal shaped carbon nanoparticles were added to the prime and bond universal adhesive which has different chemistry to the adhesive used in the current study. The results of this study agrees with Rao et al. (2023) who have added mesoporous bioactive glass nanoparticles of 0.2, 0.5, 1 and 2% wt to a universal adhesive. The authors attributed the increase of viscosity to increased contact angle of the adhesives.

5. Conclusion

Incorporating C-CeNPs into All Bond Universal adhesive has not result in a significant change in the color while it resulted in significant changes in the wettability of the experimental groups. However, the contact angle values of the experimental groups were much lower than 90° which puts them in the mostly wetting category.

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