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Edited by Ashwini Dhopte
E-mail: ashwinidhopte@gmail.com
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Medicinal-syrup effects on CAD-CAM zirconia veneer's colour stability - An *in vitro* study

Ayesha Thaivalapil*, Sindhu Sudhakar Kumararama & Krishna Kumar

Department Of Prosthodontics, Rajarajeswari Dental College and Hospital, Bengaluru, India; *Corresponding author

Affiliation URL:
https://www.rrdch.org/

Author contact:
Ayesha Thaivalapil - E-mail: ayeshamohammed1999@gmail.com
Sindhu Sudhakar Kumararama - E-mail: sindhuami@gmail.com
Krishna Kumar - E-mail: ukrishnav@gmail.com

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Abstract:
Repeated exposure to coloured medicinal syrups may compromise the optical stability of zirconia restorations. Therefore, it is of interest to evaluate the iron and cough syrups against artificial saliva. Hence, thirty multilayer CAD-CAM zirconia veneers were baseline-measured spectrophotometrically and randomly allocated to artificial saliva, Dexorange iron syrup, or Ascoril Expectorant (n=10/group), with solutions renewed daily for 7 days at 37°C. Colour change (ΔE*) was calculated from CIE L*a*b* measurements and compared using Kruskal-Wallis testing with Dunn post-hoc comparisons. Mean ΔE* values were 0.357±0.411, 0.612±0.183 and 0.557±0.198 for artificial saliva, iron syrup and cough syrup, respectively. The overall group difference was reported as significant (p=0.02), with the largest colour change after iron-syrup exposure. Thus, data shows that repeated exposure to coloured medicinal syrups, particularly iron syrup, may reduce the colour stability of zirconia veneers.

Keywords: CAD-CAM zirconia, colour stability, iron syrup, cough syrup, spectrophotometry, colour change (ΔE*)

Background:
Aesthetic ceramic restorations are increasingly used because patients expect both durable function and stable appearance **[1].** Zirconia combines high strength, biocompatibility and favourable optical properties, supporting broad restorative applications **[2]**. Conventional monolithic zirconia is comparatively opaque; newer multilayer yttria-stabilized zirconias use graded colour and translucency to improve anterior aesthetics **[2, 3]**. Thin zirconia frameworks can retain adequate mechanical performance, making conservative veneer designs clinically feasible **[4]**. Long-term success, however, also depends on colour stability because ageing; surface finishing and exposure to staining agents can alter optical properties **[5]**. Therefore, it is of interest to evaluate the colour stability of CAD-CAM milled zirconia veneers after exposure to iron syrup, cough syrup and artificial saliva.

Materials and Methods:
This *in vitro* study was conducted in the Department of Prosthodontics Crown and Bridges, Rajarajeswari Dental College and Hospital, Bengaluru. A total of 30 multilayer zirconia veneer were fabricated and divided into three groups (n=10) for further evaluation.

Sample size calculation:
The sample size for the present study was estimated using G*Power software (latest ver. 3.1.9.7; Heinrich Heine University Düsseldorf, Dusseldorf, Germany). The sample size estimation was performed at 5% alpha error (α = 0.05), with an effect size of 0.45 for the mean difference in the Colour Stability (NBS values) b/w groups and the power of the study at 80%, demonstrated that a minimum of 30 samples will be needed for the present study. So, each group will consist of 10 samples (10 samples x 3 groups = 30 samples).

Specimen preparation:
Standardization for preparing zirconia veneer samples:
The fabrication of Zirconia veneer samples for this study was done using multilayer zirconia blanks (Ips E max zircad prime A2 14 mm) encompassing digital design and advanced CAD/CAM milling to ensure consistent dimensions. Utilizing Exocad software, individual samples were fabricated by 3D CAD designing of upper dentulous model with prepared central incisors to receive a zirconia veneer of dimensions 10.5mm in length, 8.5mm in width and 0.4 mm in thickness These designed veneers were later milled (Ivoclar Vivadent Pvt. Ltd., Liechtenstein) To ensure uniformity, all 30 specimens were fabricated from the same blank and the dimensions were verified using a digital calliper. These 30 samples were then subjected to thermocycling and were placed in artificial saliva. (Methyl-p-hydroxybenzoate, sodium carboxymethyl cellulose, KCL, MgCl2). 6H2O, CaCl2. 2H2O K2HPO4, KH2PO4 the pH of artificial saliva was adjusted to 6.75 with KOH. This storage was carried out to simulate the physicochemical environment taking place in the oral cavity.

Spectrophotometric analysis:
Objective measurement of colour was performed using the spectrophotometer (Lamda 1050+, PerkinElmer, USA). Before carrying out the measure, transmittance baseline values were established using a white background standard to reduce measurement errors through instrument calibration. Spectral measurements were done from 450–650 nm, the corresponding CIE L*, a*and b*coordinates were obtained based on the acquired wavelength.

Immersion of the zirconia veneer samples:
The fabricated zirconia veneer samples were randomly divided into three groups consisting of ten samples each: Group A, Group B and Group C. Group A specimens were immersed only in artificial saliva and served as the control group. Group B samples were immersed in iron supplement syrup, Dexorange (WardeX Pharmaceuticals Pvt. Ltd.), while Group C samples were immersed in a cough syrup, Ascoril Expectorant (Glenmark Pharmaceuticals Ltd.). Each group of specimens was stored separately in closed BPA-free containers containing 10 ml of the respective immersion solution to prevent contamination and evaporation. The solutions were freshly replaced every 24 hours to maintain uniform concentration throughout the experimental period. The immersion protocol was continued for duration of 7 days. Following completion of the immersion cycle, all these specimens were again subjected to subject to spectrophotometric analysis after one week to assess colour stability. The mean values of L*, a* and b* coordinates post immersion were calculated and recorded for statistical analysis.

Calculation of the colour difference:
The colour difference between pre-immersion and post-immersion measurements was calculated using the colour difference formula (ΔE*), expressed as:

$$\Delta E = \sqrt{(L_1 - L_0)^2 + (a_1 - a_0)^2 + (b_1 - b_0)^2}$$

This equation quantifies the magnitude of the overall colour change between the pre-immersion and post-immersion measurements.
The obtained ΔE* values were converted to National Bureau of Standards (NBS) units. The NBS system provides a standardized interpretation of colour variation by correlating numerical values with perceptibility levels.

Statistical analysis:
Statistical analysis was performed using IBM SPSS Statistics for Windows, version 22.0. Delta E values were summarized as mean±standard deviation for each group. The Kruskal-Wallis test followed by Dunn post-hoc comparisons was used to compare mean ΔE* values among the three groups. Statistical significance was set at $p<0.05$.

Results:
The mean ΔE* value was 0.357±0.411 in Group A, 0.612±0.183 in Group B and 0.557±0.198 in Group C. The overall difference among the three groups was reported as statistically significant (p=0.02) (**Table 1**). Dunn post-hoc comparisons reported mean differences of -0.255 for Group A versus Group B (95% CI -0.569 to 0.059; p=0.02), -0.200 for Group A versus Group C (95% CI -0.514 to 0.114; p=0.04) and 0.055 for Group B versus Group C (95% CI -0.259 to 0.369; p=1.00) (**Table 2**). Because the first two reported confidence intervals include zero while their p-values are <0.05, these post-hoc statistics should be verified against the original statistical output before submission. These results emphasized that zirconia veneers were most resistant to colour change in a neutral environment, but their stability was compromised when exposed to commonly use medicinal syrups. The findings carried clinical relevance, as they highlighted the potential for iron and cough syrups to affect the long-term aesthetic outcomes of zirconia restorations, particularly in patients with frequent or prolonged exposure to such medications.

Table 1: Comparison of mean Delta E values b/w 3 groups using Kruskal Wallis Test

Groups	N	Mean	SD	Min	Max	p-value
Group A	10	0.357	0.411	0	1.36	0.02*
Group B	10	0.612	0.183	0.32	0.92	
Group C	10	0.557	0.198	0.28	0.85	

Table 2: -Multiple comparison of mean diff. in Delta E values b/w 3 groups using Dunn's Post hoc Test

Group I	Group J	Mean difference (I-J)	95% CI - Upper		p-value
Group A	Group B	-0.255	-0.569	0.059	0.02*
	Group C	-0.2	-0.514	0.114	0.04*
Group B	Group C	0.055	-0.259	0.369	1

Discussion:
In general, zirconia-based dental materials are appealing due to their excellent flexural strength, fracture toughness, biocompatibility, bio functionality and low price. Consequently, alumina-based dental materials have been nearly entirely supplanted by zirconia-based dental materials **[6].** Shamseddine and Majzoub reported that Y-TZP zirconia has excellent toughness and durability for use in fixed dental prostheses and aesthetic restorations. Yet, a significant drawback of conventional zirconia ceramics is their relatively opaque nature when compared with glass-ceramic materials, limiting their application in areas where aesthetics is paramount **[7].** In order to improve the optical characteristics of zirconia restorations, multi-layered ultra-translucent zirconia materials were developed. The author also demonstrated that the translucency parameter of multilayer zirconia was significantly influenced by specimen thickness, with thinner specimens exhibiting higher translucency compared to thicker ones materials are fabricated with gradational translucency and chroma that resemble the natural optical characteristics of enamel and dentin **[7].** Similarly, Khosravani *et al.* reported that ceramic thickness plays a significant role in determining the final colour of multilayer zirconia veneers and influences the optical behaviour of the restoration **[8].** As a result of these developments, multilayer ultra-translucent zirconia has been suggested for conservative aesthetic restorations, like laminate veneers. Previous studies have proposed that zirconia veneers can reach an acceptable level of translucency and clinical performance with a minimum thickness of about 0.4 mm **[7].** On the basis of all these literature, 0.4mm thickness was maintained as multilayer zirconia blank thickness. Anaemia in India is one of the most common systemic conditions. Givens *et al.* reported anaemia burden is still high, especially among children and women of reproductive age and continues to be one of the most important nutrition- and public health-related problems in the country **[9].** Joe *et al.* (2022) determined that the highest coverage of iron and folic acid supplementation occurred among pregnant women (roughly 90.3%), whereas coverage among children aged 6–59 months was markedly lower at approximately 14.9% **[10],** As a reason the iron syrup was chosen as one of the parameters to analyse the colour stability of multilayer zirconia veneer. Other common health-related phenomena are respiratory diseases and cough is among the most prevalent symptoms in clinical practice. Morice *et al.* reported that cough is one of the most frequent complaints for which patients present to medical services and it is a common presenting complaint in both primary and secondary healthcare settings **[11].** Additionally, cough has been reported to affect around 5–10% of the Indian population according to clinical consensus documents, making it one of the most frequently seen symptoms in primary care set-ups. As respiratory tract infections are prevalent in the normal populace, cough syrups are often prescribed or self-administered for symptomatic treatment **[12].** Derafshi *et al.* found that immersion in mouthrinses (commercially available) such as chlorhexidine and Listerine produced measurable colour-change for both materials suggesting restoration materials may be discoloured or

stained when exposed to common oral solutions **[13].** In the same way, Alnassar *et al.* found that zirconia specimens exhibited significantly higher ΔE values after they were immersed in solutions of these beverages, with coffee and soft drinks causing the most pronounced colour alterations **[2].** Colombo *et al.*, Abounassif *et al.* and Soares *et al.* performed an *in vitro* which analysed the colour stability of CAD/CAM zirconia ceramics exposed to acidic drinks and staining solutions like coffee. The authors concluded that immersion in staining agents materially influences the colour stability of zirconia with measurable alterations in ΔE values over time. According to these results, zirconia restorations can develop colour changes after immersion in solutions of the oral environment **[14, 15 and 16].** The color stability of dental restorative materials represents an important determinant of their long-term aesthetic acceptability and clinical performance. Previous investigations have demonstrated that restorative materials exhibit varying degrees of discoloration following exposure to commonly used mouthrinses. Lithium disilicate ceramics have generally shown greater resistance to discoloration than zirconia and polyether ether ketone (PEEK) materials under certain experimental conditions. These findings suggest that the composition and structural characteristics of restorative materials significantly influence their susceptibility to extrinsic staining and color alteration **[17]**. The influence of liquid oral medications on restorative material color stability has also received considerable attention, particularly in pediatric dentistry. Repeated exposure to commonly prescribed medications, including paracetamol and iron-containing preparations, has been associated with measurable alterations in the color characteristics of restorative materials. Differences in staining behavior among composite resins and glass ionomer cements emphasize the importance of material composition and the chemical properties of the exposing solutions when evaluating long-term aesthetic performance **[18]**. The relationship between mouthrinse composition and restorative material discoloration is further supported by comparative laboratory investigations involving different CAD/CAM resin-based materials. Both alcohol-containing and alcohol-free mouthrinses have demonstrated the potential to produce significant color changes, indicating that components other than alcohol may contribute to discoloration. Furthermore, increasing exposure duration has been associated with greater color differences, suggesting a cumulative influence of repeated chemical exposure on the optical characteristics of restorative materials **[19]**. Previous investigations involving pediatric medication syrups have further highlighted the importance of considering frequently administered oral formulations when selecting tooth-colored restorative materials for children. Repeated contact with medicated syrups may influence surface staining and color stability, with the extent of discoloration depending on the restorative material and characteristics of the medication. These observations reinforce the need for appropriate material selection and preventive guidance regarding oral hygiene following medication administration **[20]**. The effect of pediatric multivitamin preparations on restorative material color stability has also been demonstrated in controlled laboratory investigations. Significant differences in color alteration have been reported among reinforced glass ionomer materials, giomers and nanohybrid composites, with both medication type and exposure duration influencing the magnitude of discoloration. These findings indicate that restorative materials do not respond uniformly to staining challenges and that their optical stability should be considered alongside their mechanical and biological properties during clinical material selection **[21]**. Although the mechanical and aesthetic properties of zirconia are excellent, our findings indicate that the colour stability still can be influenced by exposure to different staining solutions found in oral conditions. Thus, the colour stability of zirconia restorations when exposed to different chemical and chromogenic substances continues to be an individual variability phenomenon.

Conclusion:

CAD-CAM multilayer zirconia veneers showed the least colour change in artificial saliva and greater change after exposure to medicinal syrups. Iron syrup produced the highest mean ΔE* value, followed by cough syrup, although the post-hoc confidence intervals and p-values should be verified against the original statistical output. Thus, data support counselling patients with zirconia restorations about potential staining during frequent or prolonged use of strongly coloured medicinal syrups.

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