Original Article

Evaluating the Color Stability of Ocular Prosthesis after Immersion in Three Different Immersion Media: An *In Vitro* Study

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ABSTRACT

Background: Immersion media such as ophthalmic irrigation solution, eye lubricant solution, and neutral soap solution will be the best, which will help in the longevity as well as the color stability of ocular prostheses. **Aim:** The aim of this study was to evaluate and compare color stability of ocular prosthesis after immersion in three different immersion media. **Materials and Methods:** A total of 90 samples of scleral acrylic resin were prepared, which were painted with natural dry earth pigment NEAS and then were divided into three groups. These samples were checked before and after immersion in respective media after 8 weeks. To assess the color change, a spectrophotometry test was performed and the results were statistically analyzed by one-way analysis of variance and *post hoc* Tukey's test. **Results:** Among all the three groups, the samples immersed in neutral soap solution proved to be most color stable, followed by eye lubricant solution, and the least color stable among the three was ophthalmic irrigating solution. **Conclusion:** The neutral soap solution proved to be the most color stable.

Keywords: *Color stability, custom-made ocular prosthesis, ocular pigment*

Introduction

In response to congenital or acquired defects, human beings are constantly in progression to deal with their debilities by using their intellect and the material resources available for restoration.

The maxillofacial prosthesis was introduced and encountered as a consequence of individuals' need to hide and disguise their maxillofacial defects, to appear and materialize into normal and natural. The rehabilitation treatment of patients with facial defects helps them to improve their looks, appearance, and personal well-being.[1]

Owing to repetitive use of the prosthesis, the basic iris color may fade off, but it can be prevented to an extent if we can identify how to store and preserve the ocular prosthesis. To avoid these likely issues, the ocular prostheses should be stored in an appropriate medium, to retain the color of the iris for a longer period.[2]

Therefore, this study was carried out to evaluate which of the following immersion media such as ophthalmic irrigation solution, eye lubricant solution, and neutral soap solution will be the best, which will help in the longevity as well as the color stability of ocular prostheses. The selection of these media was performed based on the frequency of their usage and also as there is no study in the literature comparing these three media. The objective of the study was to evaluate and compare color stability of ocular prosthesis after immersion in three different immersion media.

Materials and Methods

A cross-sectional study was performed for 1 year from May 2015 to May 2018, at Sharad Pawar Dental

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College & Hospital (SPDC) after the ethical clearance from the Institutional Ethics Committee (DMIMS (DU)/IEC/2016–17/5086).

Materials

Heat-cured scleral resin (J-510, Factor II, Arizona, USA), metallic circular mold (diameter: 1.5 cm and thickness: 2 mm), neutral soap solution (Johnson & Johnson, Mumbai, India), eye lubricant solution (Hypromellose Eye Drops BP and Lacryl Forte eye drop, Entod Pharmaceuticals, Mumbai, India), ophthalmic irrigating solution (normal saline), iris pigment (NEAS, pigment for tinting ocular prosthesis; shade: burnt umber), clear acrylic, and auto polymerizing monomer.

Methodology

Fabrication of customized mold

A metallic circular mold with an internal diameter 1.5 cm and thickness of 2 mm was used to make circular-shaped specimens. Two screws were fabricated on the lower member with cover nuts attached to the upper member. The material was then sandwiched between the upper member and the lower member. The screws helped in complete seating of the assembly [Figure 1].

Preparation of scleral resin samples (by using factor 2)

The heat-polymerized factor 2 scleral resin dough was prepared. Packing was carried out carefully into the custom-made metallic mold space (internal diameter: 1.5 mm and thickness: 2 mm) and trial closure was carried out. After the packing of the scleral resin, it was then subjected to polymerization using a short curing cycle. The heat-cured scleral resin samples after retrieval were inspected carefully and finishing and polishing was carried out. The samples were sandblasted after selection (diameter: 1.5 mm and thickness: 2 mm) [Figure 2].

- After sandblasting, iris painting was carried out using the pigment (ocular prosthesis stain, NEAS). The pigment used was ocular prosthesis stain by NEAS. These pigments are natural dry earth pigments that are made by adding monopoly syrup [Figure 3].
- It was applied layer by layer, allowing the first layer to dry completely. Three to four layers were applied and they were allowed to dry for the next 24 h.

Figure 1: Mold

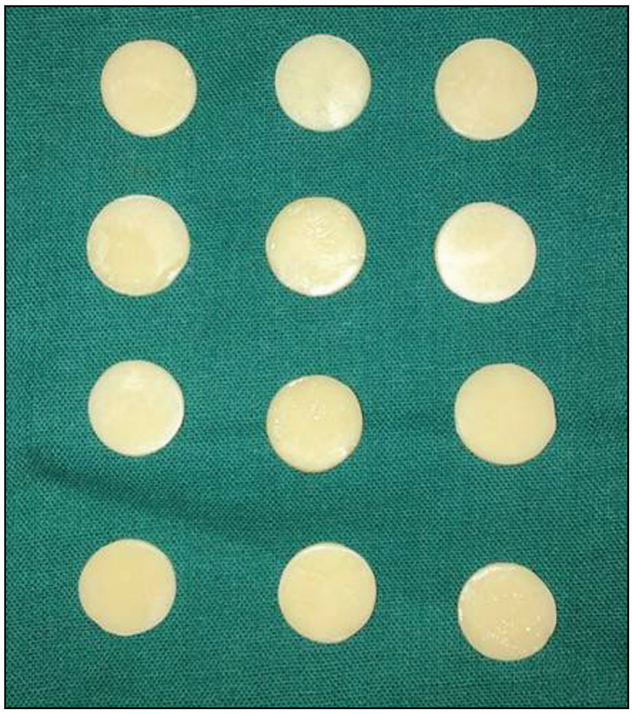

Figure 2: Scleral resin samples

Figure 3: Pigmented samples (NEAS stain)

- After this, a layer of varnish made up of clear acrylic and auto polymerizing monomer (monopoly syrup NEAS) was applied over the iris-pigmented samples [Figure 3].
- These samples were allowed to dry for the next 24 h.
- Grouping of the 90 samples was carried out, and they were immersed for 8 weeks [Figure 4].
- Samples of each group were washed daily with normal water and were cleaned using manual friction and dried with gauze. The samples immersed in the neutral soap solution, eye lubricant solution, and ophthalmic irrigation solution groups were cleaned daily [Figure 5]; cleaning involved immersing them in a solution of distilled water at an initial temperature of 37°C for 15 min, followed by rinsing in running water for 30 s, using manual friction with gauze for 1 min.

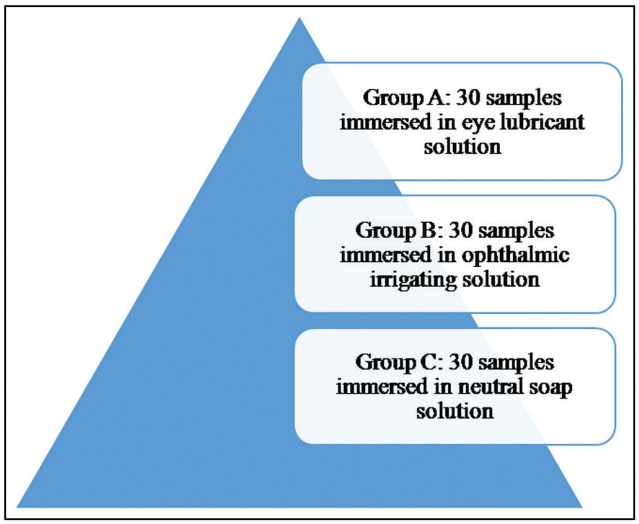

Figure 4: Grouping of samples

- After immersion for 8 weeks, the samples were tested for color stability using spectrophotometry (i7 reflective spectrophotometer).
- After this, the values obtained by spectrophotometry (L, a, b, ΔL, Δa, Δb, and ΔE) were analyzed statistically, using various statistical methods.

L value is known as whiteness or brightness of the sample; a value represents the quantity of red color (+ve value) and green color (–ve value); b value represents yellow color (+ve value) and blue (–ve value); ΔE is for color change; and ΔL, Δa, and Δb stand for their mean.

Statistical analysis

In this research study, descriptive and analytical statistics were performed. The normality of data was analyzed by the Shapiro–Wilk test. As the data followed normal distribution, parametric tests were used to analyze the data. The one-way analysis of variance test was used to check the mean differences between the groups. *Post hoc* analysis was performed using Tukey's honestly significant difference test. Pre- and post-results were analyzed by paired sample t test. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software program, version 24.0 (IBM, Chicago, Illinois).

Results

The comparison of mean ΔL^* values of scleral heat-cured scleral resin disc immersed in eye lubricant, ophthalmic irrigation solution, and neutral soap solution was carried out. A statistically significant difference ($P < 0.001$) was observed among the groups. The ophthalmic irrigation solution group at 8 weeks (0.28 ± 0.17) showed the least changes than the original ΔL^* values of scleral heat-cured scleral resin disc at baseline (0.45 ± 0.10) [Tables 1–3].

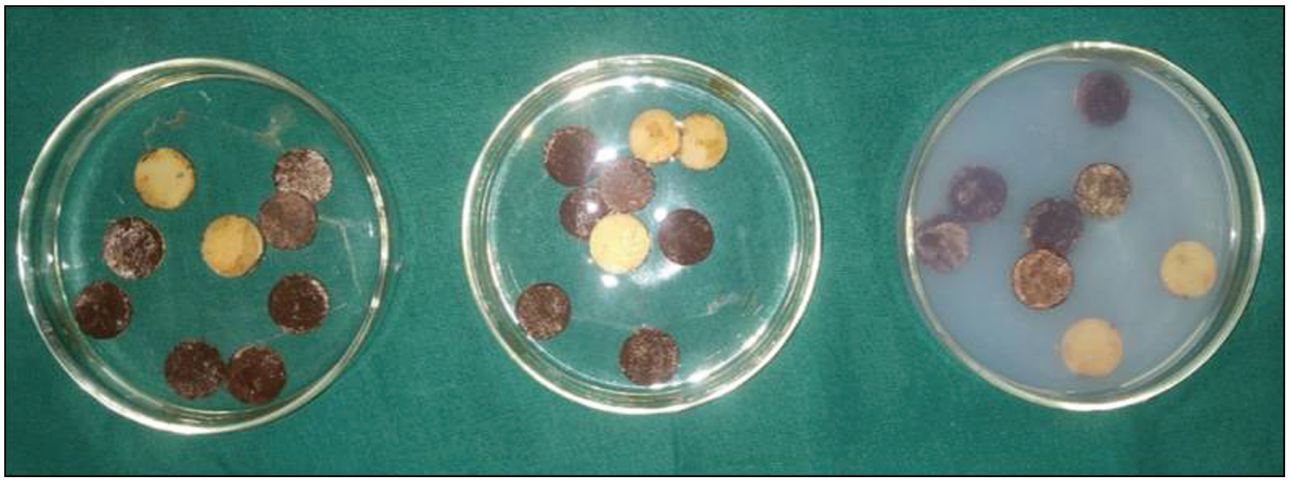

Figure 5: Samples immersed in three different solutions

The comparison of mean Δ*a** values of scleral heat-cured scleral resin disc immersed in eye lubricant, ophthalmic irrigation solution, and neutral soap solution was made. A statistically significant difference ($P < 0.001$) was observed among the groups. The ophthalmic irrigation solution group at 8 weeks (–2.09 ± 0.51) showed the least changes than the original Δ*a** values of scleral heat-cured scleral resin disc at baseline (–3.01 ± 0.53).

The comparison of mean Δ*b** values of scleral heat-cured resin disc immersed in eye lubricant, ophthalmic irrigation solution, and neutral soap solution was made. A statistically significant difference ($P = 0.006$) was observed among the groups. The ophthalmic irrigation solution group at 8 weeks (–1.55 ± 0.49) showed the least changes than the original Δ*b** values of scleral heat-cured scleral resin disc at baseline (–1.49 ± 0.30).

The comparison of mean Δ*E** values of scleral heat-cured scleral resin disc immersed in eye lubricant, ophthalmic irrigation solution, and neutral soap solution was made. A statistically significant difference ($P = 0.04$) was observed among the groups. The

Table 1: Comparison of mean Δ*E values of heat-cured acrylic resin disc immersed in eye lubricant, ophthalmic irrigation solution, and neutral soap solution**

Variables	*N*	Mean	SD	SE	MD	95% CI	*t* value	*P* value[#]
*L**	30	38.66	0.34	0.06	12.28	11.41–13.14	28.988	<0.001[†]
	30	26.38	2.21	0.40				
*a**	30	5.32	0.18	0.03	–0.02	–0.19–0.13	–0.364	0.719
	30	5.34	0.37	0.06				
*B**	30	6.07	0.51	0.09	1.67	1.38–1.96	11.669	<0.001[†]
	30	4.40	0.45	0.08				
Δ*L**	30	0.45	0.10	0.01	0.75	0.66–0.84	17.712	<0.001[†]
	30	–0.30	0.18	0.03				
Δ*a**	30	–3.01	0.53	0.09	–0.96	–1.34–0.57	–5.128	<0.001[†]
	30	–2.05	0.74	0.13				
Δ*B**	30	–1.49	0.30	0.05	–0.01	–0.36–0.34	–.057	0.955
	30	–1.48	0.83	0.15				
Δ*E**	30	3.14	0.28	0.05	0.53	0.14–0.93	2.781	0.009[†]
	30	2.61	0.97	0.17				

SD = standard deviation, SE = standard error, MD = mean difference, CI = confidence interval

[#]*P* value derived from paired *t* test

[†]Significant at $P < 0.05$

Table 2: Comparison of mean *L, *a**, *B**, Δ*L**, Δ*a**, Δ*b**, and Δ*E** values at baseline and after 8 weeks of heat-cured acrylic resin disc immersed in ophthalmic irrigation solution**

Variables	*N*	Mean	SD	SE	MD	95% CI	*t* value	*P* value[#]
*L**	30	38.66	0.34	0.06	4.13	3.88–4.37	34.232	<0.001[†]
	30	34.53	0.55	0.10				
*a**	30	5.32	0.18	0.03	0.70	0.52–0.88	8.186	<0.001[†]
	30	4.61	0.38	0.07				
*B**	30	6.07	0.51	0.09	0.88	0.61–1.14	6.907	<0.001[†]
	30	5.19	0.48	0.08				
Δ*L**	30	0.45	0.10	0.01	0.16	0.08–0.24	4.452	<0.001[†]
	30	0.28	0.17	0.03				
Δ*a**	30	–3.01	0.53	0.09	–0.92	–1.20–0.64	–6.769	<0.001[†]
	30	–2.09	0.51	0.09				
Δ*B**	30	–1.49	0.30	0.05	0.06	–0.13–0.25	0.641	0.527
	30	–1.55	0.49	0.08				
Δ*E**	30	3.14	0.28	0.05	0.50	0.22–0.79	3.647	0.001[†]
	30	2.64	0.66	0.12				

SD = standard deviation, SE = standard error, MD = mean difference, CI = confidence interval

**P* value derived from paired *t* test

[#]Denotes significance

[†]Also denotes significance

ophthalmic irrigation solution group at 8 weeks (2.64 ± 0.66) showed the least changes than the original Δ*E** values of scleral heat-cured scleral resin disc at baseline (3.14 ± 0.28). The eye lubricant (2.61 ± 0.97) and neutral soap solution (1.92 ± 1.05) values had shown statistically significant changes in Δ*E** values [Tables 4 and 5 and Figures 6 and 7].

Discussion

One frequent failure during eye prostheses processing is alteration in iris color. It is difficult to obtain the exact color of iris mainly when the color stability of the ink cannot be controlled following polymerization.

To maintain what has been achieved in terms of color stability, this study aimed to evaluate which of the immersion media such as ophthalmic irrigation solution, eye lubricant solution, and neutral soap solution will be the best to increase the longevity as well as color stability of the iris of ocular prostheses.

Fernandes *et al.*[3] stated that ocular prostheses have color stability and can be used up to 10 years, without

Table 3: Comparison of mean *L, *a**, *B**, Δ*L**, Δ*a**, Δ*b**, and Δ*E** values at baseline and after 8 weeks of heat-cured acrylic resin disc immersed in neutral soap solution**

Variables	*N*	Mean	SD	SE	MD	95% CI	*t* value	*P* value[#]
*L**	30	38.66	0.34	0.06	13.27	12.53–14.02	36.457	<0.001[†]
	30	25.39	1.84	0.33				
*a**	30	5.32	0.18	0.03	0.50	0.32–0.68	5.858	<0.001[†]
	30	4.81	0.35	0.06				
*B**	30	6.07	0.51	0.09	1.19	0.98–1.40	11.560	<0.001[†]
	30	4.87	0.36	0.06				
Δ*L**	30	0.45	0.10	0.01	0.61	0.51–0.71	12.679	<0.001[†]
	30	–0.16	0.25	0.04				
Δ*a**	30	–3.01	0.53	0.09	–1.44	–1.77–1.10	–8.819	<0.001[†]
	30	–1.57	0.62	0.11				
Δ*B**	30	–1.49	0.30	0.05	–0.55	–0.93–0.17	–2.998	0.006[†]
	30	–0.94	1.05	0.19				
Δ*E**	30	3.14	0.28	0.05	1.19	0.74–1.64	5.457	<0.001[†]
	30	1.95	1.05	0.19				

SD = standard deviation, SE = standard error, MD = mean difference, CI = confidence interval

L value is known as whiteness or brightness of the sample; *a* value represents the quantity of red color (+ve value) and green color (–ve value); *b* value represents yellow color (+ve value) and blue (–ve value); Δ*E* is for color change; and Δ*L*, Δ*a*, and Δ*b* stand for their mean

[#]Denotes significance

[†]Also denotes significance

Table 4: Comparison of mean Δ*E values of heat-cured acrylic resin disc immersed in eye lubricant, ophthalmic irrigation solution, and neutral soap solution**

Groups	*N*	Mean	SD	SE	95% CI	Min.	Max	*F* value	*P* value[#]
Eye lubricant	30	2.61	0.97	0.17	2.24–2.97	1.08	3.93	5.881	0.004[†]
Ophthalmic irrigation solution	30	2.64	0.66	0.12	2.39–2.88	0.49	3.56		
Neutral soap solution	30	1.92	1.05	0.19	1.53–2.31	0.29	4.12		

SD = standard deviation, SE = standard error, CI = confidence interval

[#]*P* value derived from one-way analysis of variance test

[†]Significant at $P < 0.05$

Table 5: Pairwise comparison of mean Δ*E values between the groups**

Groups	MD	95% CI	*P* value[#]
Eye lubricant vs. ophthalmic irrigation solution	–0.02	–0.59–0.53	0.991
Eye lubricant vs. neutral soap solution	0.68	0.12–1.25	0.013[†]
Ophthalmic irrigation solution vs. neutral soap solution	0.71	0.15–1.27	0.009[†]

MD = mean difference, CI = confidence interval

[#]*P* value derived from Tukey's honestly significant difference *post hoc* test

[†]Significant at $P < 0.05$

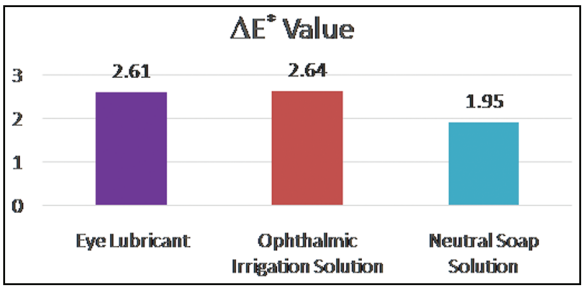

Figure 6: ΔE^* values

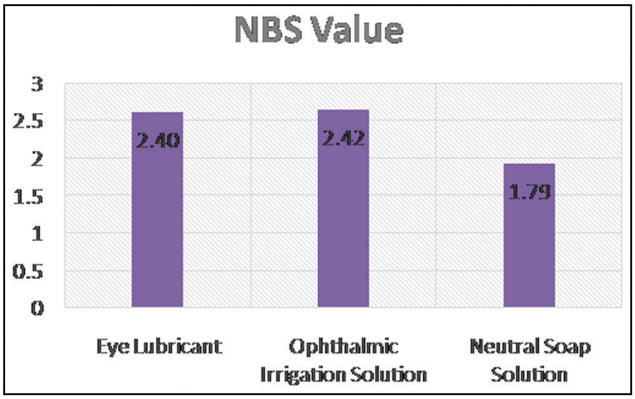

Figure 7: National Bureau of Standards (NBS) values

undergoing color differences, but the paints that are to be used should be pure pigments of good and optimum quality and should be biocompatible.

Some studies have used different coloring paints and materials. Oil paints that were used in the studies conducted by Fernandes *et al.*[3] and Goiato *et al.*[4] contained lead that was considered to be toxic causing irritation and itching.

In our study, the samples were painted using iris pigment NEAS. NEAS is a naturally occurring mineral containing metallic oxides that are organic dry earth pigments.[5] They are made by adding the monopoly syrup. The primary colors comprise ochre, sienna, and umber. The advantages of these colors are that they are fast setting, relatively inexpensive, and most importantly, they are nontoxic as they do not contain lead. So, it can be safely used in the fabrication of the ocular prosthesis. This is a unique study in which these stains are used in combination with different media to study the color stability of the scleral resin. Similar pigments were also used in the study conducted by Shreshtha and Thaworanunta.[5]

After painting and drying of samples, a total of 90 samples were divided into three groups for evaluating pre- and post-immersion color alteration.

They were grouped as follows [Figure 4]:

- Group A: Samples immersed in eye lubricant solution
- Group B: Samples immersed in an ophthalmic irrigation solution
- Group C: Samples immersed in a neutral soap solution

These were then immersed in respective media for 8 weeks after which a comparison was made using spectrophotometry. The solutions that are common solutions prescribed by the ophthalmologist were selected for the study.[6,7]

In a case of eye prosthesis, the patient is advised to clean the prosthesis with soap solution. Accordingly in our study, we have used neutral soap solution (immersion media). It consists of lye and excess amounts of oils.

The ophthalmic irrigating solution was the second immersion solution used for the study. As the ophthalmic irrigating solution is commonly used for clearance of debris through anophthalmic socket, this was the solution taken into consideration for this study. It contains sodium chloride in distilled water. Normal saline is the commonly used ophthalmic irrigating solution, having a pH of 4.5–7.2. Herr *et al.*[8] in their study compared the use of four different ophthalmic irrigating solutions, which include normal saline, lactated ringers, normal saline adjusted to pH 7.4, and sodium bicarbonate.

The patients with eye prosthesis are commonly prescribed the eye lubricant solution, which is the third immersion media used in the study. It contains the lubricant hydroxypropylmethylcellulose (HPMC), a cellulose derivative, widely used in artificial tears and comfort drops. The same solution was used in the study by Thai *et al.*[9]

Eye lubricant solution contains (Add Tears 10 mL, Cipla, Noida, UP, India) carboxymethylcellulose sodium, stabilized oxychloro complex as a preservative, and aqueous vehicle. It also contains magnesium chloride, calcium chloride, potassium chloride, and sodium chloride. It works as a protectant against burning, irritation, discomfort, and dryness of eyes. Roth *et al.*[6] in their study evaluated carboxymethylcellulose, sodium hyaluronate, and glycerin in patients with dry eye.

The samples were immersed in neutral soap solution, eye lubricant solution, and ophthalmic irrigation solution groups and were cleaned daily. Cleaning involved immersing them in a solution of distilled water

at an initial temperature of 37°C for 15 min, followed by rinsing in running water for 30 s using physical rubbing with gauze for 1 min. The samples in each group were cleaned after every 8 h.

After cleansing of samples, they were subjected to observation for color change. This was performed using an i7 reflective spectrophotometer. The baseline readings were measured using spectrophotometric analysis before immersion, and after iris painting. It was kept in for 8 weeks in three different immersion media, that is, neutral soap solution, ophthalmic irrigation solution, and eye lubricant solution. Again the scleral resin discs were analyzed through spectrophotometry. All the measurements were recorded in CIELAB coordinates (CIE L*a*b* (CIELAB) is a color space specified by the International Commission on Illumination (French Commission internationale de l'éclairage, hence its CIE initialism)) and transferred to the computer. The CIELAB measurements make it possible to measure the color. "CIELAB" coordinates are considered the gold standard for measurement analysis of the color change (ΔE) value. The "CIELAB" system quantifies color variations through three-dimensional coordinates and hence it is used for the color change (ΔE) analysis. The L^* parameter assesses the luminosity (where 100 represents white and 0 represents black), the a^* coordinate measures the amount of redness (positive values) and greenness (negative values), and the b^* coordinate measures the amount of yellowness (positive values) and blueness (negative values). This system was selected because it recognizes small color changes among specimens and has been extensively used.[10]

In this study, the color change (ΔE) value of custom-made scleral resin disc immersed in neutral soap solution was $\Delta E = 1.95$, in eye lubricant solution, it was $\Delta E = 2.61$, and in ophthalmic irrigation solution, it was $\Delta E = 2.64$). All the values were less than 3; thus, the color change was clinically acceptable.

The values of ΔE are also expressed as National Bureau of Standards (NBS) units by the following formula to quantify the color changes according to this system[11]:

$$NBS = \Delta E \times 0.92.$$

According to NBS, the color change from 0.0 to 0.5 is marked as trace, 0.5 to 1.5 is slight, 1.5 to 3 is noticeable, 3 to 6 is appreciable, 6 to 12 is much, and >12 is very much. By the aforementioned formula, NBS values for this study after immersion in neutral soap solution, eye lubricant solution, and ophthalmic irrigating solution are 1.79, 2.40, and 2.42, respectively, suggestive of noticeable, but clinically acceptable color change.

After a comparison of solutions using spectrophotometry, it was found that among the three solutions, neutral soap solution showed the least color change as compared to the other two groups, whereas eye lubricant solution showed maximum color change as compared to neutral soap solution and minimum color change as compared to ophthalmic irrigation solution; and ophthalmic irrigation solution showed the highest color change as compared with the other two groups.

Color changes, which occurred after immersion in the different solution, can be because of the following reasons:

According to the American Chemical Society, solvents can affect stability, solubility, and rate of reaction. Thus, choosing the appropriate solvent permits the thermodynamic and kinetic control over a chemical reaction.[12]

Although the best results among all the three groups were shown by neutral soap solution as compared to control readings, the samples of this group showed noticeable color change as per the NBS values (NBS = 1.79).

This can be attributed to the fact that neutral soap solution comprises water, cocamidopropyl betaine, PEG-80 Sorbitan Laurate, sodium trideceth sulfate, citric acid, and tetrasodium ethylenediamine tetraacetic acid (EDTA). Different solutions were used for carrying out a study by Taqa *et al.*[13] One of the solution in his study comprised of EDTA. EDTA was also one of the component in the solution of neutral soap which was used in our study. The reason for the slight color instability of the samples immersed in neutral soap solution could be because acrylic resin is hydrophilic and is subjected to water sorption, which acts as a plasticizer. Due to this, there is a formation of cracking zones resulting from adsorption and absorption cycles, leading to hydrolytic degradation and gradual deterioration of the acrylic resin, and the color pigments.

The results of this study do agree with the results of a study by Mehta and Nandeeshwar,[14] in which they used different solutions and other extraoral aging conditions in which neutral liquid soap solution was used as one of the media to evaluate the color variation. They concluded that immersion of samples in neutral soap solution produced the least color change irrespective of the material used.

Samples in Group A (eye lubricant solution) showed slight color change but more than Group C as per the NBS values (NBS = 2.40). The reason for this can be attributed to the fact that the eye lubricant contained

hydroxymethylcellulose, which leads to mass growth, as stated in a study by Boruvkova and Wiener.[15]

So, after immersion the same phenomena must have taken place, which led to the expansion of mass and fading of color.

Samples in Group B (ophthalmic irrigation solution) showed the highest color change in comparison with the other two groups, as per the NBS values (NBS = 2.42). Ophthalmic irrigation solution consists of a 0.9-g sodium chloride solution; sodium has a higher absorption rate. Although in more concentrated solutions, less water is taken up owing to greater osmotic pressure. Thus, the inherent capacity, column flow rate, level of regeneration, the composition of the solution, size of a particle, temperature, and distribution of resin, all these factors, affect the material as well as its properties, which may be the probable cause of color change in our study.[16]

This study concludes that neutral soap solution is the best immersion and storage media, followed by eye lubricant solution and ophthalmic irrigation solution, which showed the highest color change among all the three immersion media. The results in all the media were statistically significant and were clinically acceptable. The values of ΔE (color variation) showed the clinically acceptable result with all the three solutions.

1. As this was an *in vitro* study, there may be variation in the result when used *in vivo* because of various factors such as environment and secretions coming in contact with the prosthesis, which may affect the surface and color of iris of the prosthesis. So, further *in vivo* studies are required to confirm these results.
2. To assess the color change, the most commonly used artificial media were selected for this study. But there are innumerable brands with different compositions, so different brands may give different results.

Clinical significance: Neutral soap solution proved to be the best medium, in which ocular prosthesis should be stored as it may help in the longevity of the prosthesis as it is proved to be most color stable among all the three immersion media.

Conclusion

Within the limitations of the study and the results obtained, the following conclusions can be drawn:

1. The neutral soap solution proved to be the most color stable.
2. Color changes were seen in all the groups.
3. The values of ΔE (color variation) obtained in the study were clinically acceptable.

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Conflicts of interest

There are no conflicts of interest.

Author contribution

Seema Sathe Kambala, Deepika Rathi : study conception, data collection, CAB data collection manuscript writing. Anjali Borle , Rajanikanth K : data acquisition and analysis. Tanvi Jaiswal, Mithilesh Dhamande: Data analysis & Manuscript writing.

Ehical policy and institutional review board statement

This study was carried out after the ethical clearance from the Institutional Ethics Committee (DMIMS (DU)/ IEC/2016-17/5086).

Patient declaration of consent

As this is in vitro study patient's consent is not required.

Data availability statement

The data set used in the current study is available.

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