Received: 21 July 2022 | Revised: 13 November 2022 | Accepted: 16 January 2023

DOI: 10.1111/cote.12674

ORIGINAL ARTICLE

Coloration Technology

Colour stability during production of printed ocular prostheses

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Abstract

Ocular prostheses have been used for centuries to restore patient confidence, psychosocial relationships and to improve quality of life. Methodology for producing accurate prostheses has improved with technological discoveries. Recently, hand painting ocular prostheses has been the go-to method for creating life like prostheses. However, digital printing a print to envelope around an acrylic prosthesis has been shown to decrease treatment and rehabilitation times, whilst still producing high-definition ocular prostheses. Despite these improvements, little is known about the colour stability of digitally printed ocular prostheses. To better understand the colour stability of digital prostheses 30 samples simulating ocular prostheses were created, containing 10 with blue iris, 10 combination/green iris and 10 with sepia (brown) irises. Colour measurements were taken using a data spectrophotometer, from two defined points, the iris and sclera for both pre-polymerisation and post-polymerisation to assess colour variance. Colour coordinate data was gathered and was analysed using a one-way analysis of variance test and a paired *t*-test, both with alpha = 0.05. Significant colour variations were found for each iris colour and for the sclera. The sclera showed the largest colour variation with a ΔE of 4.75, followed by the brown irises, the green irises and then blue irises with ΔE values of 3.29, 2.47 and 1.82, respectively. This is a significant decrease compared to current hand painting methods which have an average colour variance of $\Delta E = 20$. This shows a large increase in colour stability which can drastically improve patient satisfaction and quality of life.

1 | INTRODUCTION

Losing any part of the body can have a profound effect on both the function and aesthetic aspects of the part of the body, accompanied by an enormous psychological impact on the patient. This is particularly true when the moiety lost or damaged is facial in origin, as emotions are primarily communicated through facial expressions.[1] The facial region contains three of the six major sensing organs: the nose, the mouth, and the eyes. Each are essential for human relationships and communication. Removal of ocular organs may be necessary if ocular defects are present, or trauma occurs. Ocular defects can originate from many sources such as congenial deformities[2] or enucleation[3] due

to; blind painful eye, microphathalmos, endophthalmitis, cancer or trauma.[4] Quality of life has been found to be severely affected for anophthalmic patients, due to their perceptions of their social relationships.[5] A study by Wang et al revealed that prior to insertion of an ocular prosthesis, 49% of patients displayed clinical levels of depression and anxiety, with reduction to 10% with treatment.[6] Furthermore, McBain et al discovered that poor psychological well-being was directly linked to how the patient felt about their appearance and how accepted by society they felt.[1] Therefore, greater patient satisfaction has been found in patients who feel that their prosthesis is imperceivable to society.[7] Consequently, ocular prostheses must be as imperceivable and lifelike as possible, to best improve patient satisfaction and quality of life. Currently ocular prostheses cannot restore visual sight; however, they can restore facial aesthetics, prevent accumulation of lacrimal flux (excretions from the eye) and prevent further eyelid deformation. As well as restoring facial aesthetics, ocular prostheses also drastically improve the quality of life of patients, with psychosocial improvement observed alongside positive ocular prosthetic rehabilitation.[8]

1.1 | History of ocular prostheses

Ocular prostheses were first produced by the Romans and Egyptians in the fifth century BC. These prosthetics were created using painted clay attached to cloth and worn outside of the socket. Artificial eyes have been created using a wide variety of materials through-out history from gold, shells, coloured stones, coloured enamel, porcelain, clay, cloth and glass.[9,10] They have also been created in a multitude of varying forms from ekblephara and hypoblephara ocular prostheses, created using gold and silver, which were designed to be worn in front of or under the eye lids respectively,[11] to Venetian and German glass eyes[12] and the first acrylic eyes of the second world war.[13] The later technologies however, come in the form of hand-painted acrylics and inket printed acrylic prostheses. Recent studies have also been able to reduce the weight of the prostheses by introducing macroporosity to the back of the prosthesis, thus increasing patient comfort and satisfaction.[14] This technique can be applied to hand-painted and digitally-produced ocular prostheses.

1.2 | Hand-painted acrylic ocular prostheses (HPAOP)

Painted acrylic ocular prostheses have many benefits compared to earlier options. These include resistance against scratches, breakage, and attack from lacrimal secretions. They also have better customisability compared to stock painted acrylic ocular prostheses, where a generic eye of the same colour is given to the patient. Despite these advantages, painted acrylic prostheses are plagued by colour stability issues. In particular, changes in the iris and sclera colour during polymerisation of the colourless acrylic resin precursor remain a predominant issue.[12,13] Studies have also shown that attaining a perfect colour match between the resin precursor and the desired eye is difficult, as the commonly used paints are instable after acrylic resin polymerisation.[13] Most colour changes are clinically discernible resulting in a new prosthesis being required, regardless of the fit and physical state of the prosthesis.[15] This further slows down the process of prosthesis creation and the speed and quality of patient rehabilitation. The process of hand painting acrylic ocular prostheses can be long and complicated, taking a substantial amount of time to create. This is because professionals are required to be highly skilled and specialised, manually applying each stroke and layer of paint to recreate the iris. These requirements effect the price of the prostheses, the treatment time and hence, the speed of patient rehabilitation and subsequent psychosocial improvement times.[1]

Murphey et al has previously shown that pigments are free from colour alteration, if they are pure and of optimum quality.[16] Therefore, colour variance within paint types is due to reactions and interactions of additives, between themselves and the acrylic resin, when exposed to different curing techniques and ultraviolet (UV) degradation. These curing techniques include thermal curing, UV curing and microwave curing.[17] There have been several studies aimed at determining which paint type presents the least colour change upon polymerisation of the prosthesis. Research conducted by Sarjono et al showed that oil paints have better colour stability compared to gouache, water colour, acrylic and automotive paints.[18] The increased colour stability is due to the presence of an opacifier (zinc oxide) which increases temperature resistance and thus colour stability.[17] In comparison, automotive paints have been shown to have the highest colour variation when compared to other paint types. The colour instability observed is due to the presence and interactions of an acrylic resin binder, causing instability when cured,[17] especially with regards to thermal curing.[19] However, all paints that are used still present a colour change.[15,20] Not only does the type of paint chosen affect the colour stability, but the amount of paint used can cause issues such as discolouration and staining, both pre- and post-polymerisation. This is as a result of the UV degradation of the paints and becomes increasingly prevalent as the prostatic ages.[21,22]

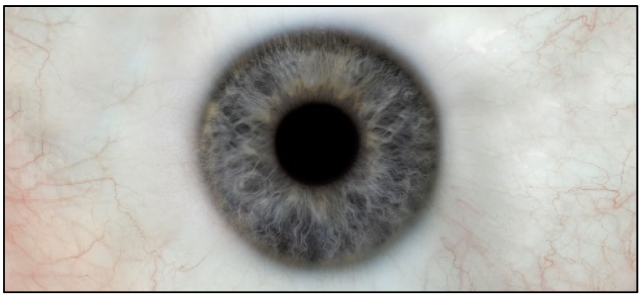

FIGURE 1 Digital image of the iris and sclera, showing the high-quality potential of digital prostheses.

1.3 | Digitally-printed acrylic ocular prostheses (DPAOP)

The fit and shape of the acrylic prostheses are found to be superior to those of alternative methods due to high patient comfort and satisfaction.[23] Unfortunately, the hand-painted method of colouring acrylic prostheses is flawed and requires improvement as colour alteration for both pre- and post-polymerisation is still present. This is a consequence of paint composition, as opposed to the pigments themselves presenting colour variance, as previously discussed in Section 1.2.

The use of digital printing in the production of ocular prostheses offers several advantages over conventional hand-painted ocular prostheses, compared to all paint types. Primarily, the ability to take a digital image of a patient's remaining eye allows for greater accuracy when replicating the patients eye with minimal colour alterations and modifications (Figure 1).

Furthermore, digital printing decreases treatment time and does not require the same skilled techniques which hand-painted prostheses require, increasing simplicity. Digital images can also be stored and easily accessed if a replacement prosthetic is required, allowing for faster replacement of an ocular prosthetic compared to hand-painted acrylic ocular prostheses (HPAOP). In general, the synthetic method for digitally-printed acrylic ocular prostheses (DPAOP) only differs in the iris being printed as opposed to hand painted, with some studies still choosing to paint/stain the sclera even when digitally printing the iris.[24]

A benefit of digital printing is that colour stability issues related to the amount of paint applied are no longer an issue, as methods of digital printing such as inkjet printing are both accurate and precise in image generation. Furthermore, the total treatment time where patients are required to be physically present for digitally-printed ocular prostheses is 5 h over two appointments. This is a significant reduction when compared to the 30 h required if the prostheses are made by conventional methods, and is likely to be more suitable and comfortable for the patient.[25] However, it is important to note that digitally-printed ocular prostheses require special digital photography equipment and computer software for image modification. Nevertheless, this could still be considered an improvement on previous techniques, as the training to use such software is faster than the training needed for hand painting the prostheses, which takes years of practice to master.

Another reoccurring problem is a potential colour change when the prosthesis undergoes polymerisation. If this problem is of significance, then the same issues as hand-painted prostheses would reoccur with mismatched colours, therefore lowering patient satisfaction and any possible psychosocial benefits. However, there is no literature of note which describes the quantification of colour variance in DPAOP.

In summary, DPAOP have been shown to have greater colour matching capabilities compared to conventional methods, which is paired with a substantial decrease in treatment time, increasing quality of life of the patient.[25] However, current literature is very limited regarding how ink composition affects colour stability and ageing techniques. Therefore, more research is required to gain a greater understanding of how ink composition and materials can affect colour stability, as well as the long-term colour stability of DPAOP, due to UV degradation.

1.4 | Production of acrylic ocular prostheses

When producing an acrylic ocular prosthesis there are a multitude of various techniques and compounds used for each stage. Figure 2 gives an overview of how a general acrylic ocular prosthesis is synthesised, detailing various additional methods used in the literature and which methods are the current best practice. Whilst Figure 3 details schematic representation of both HPAOP and DPAOP.

Both HPAOP and DPAOP share the same initial stages as an impression of the anophthalmic socket is needed for both techniques. This is typically achieved by using alginate impression creams[26] or irreversible hydrocolloid material[27] in tandem with different impression techniques. There are two main techniques utilised in the literature, external tray impression[26] and moulded shell impression.[28] External tray impression consists of an impression of the anophthalmic socket in tandem with the supporting structure of an external impression tray. Whereas the moulded shell technique utilises an impression tray shaped like a stock ocular prosthesis.

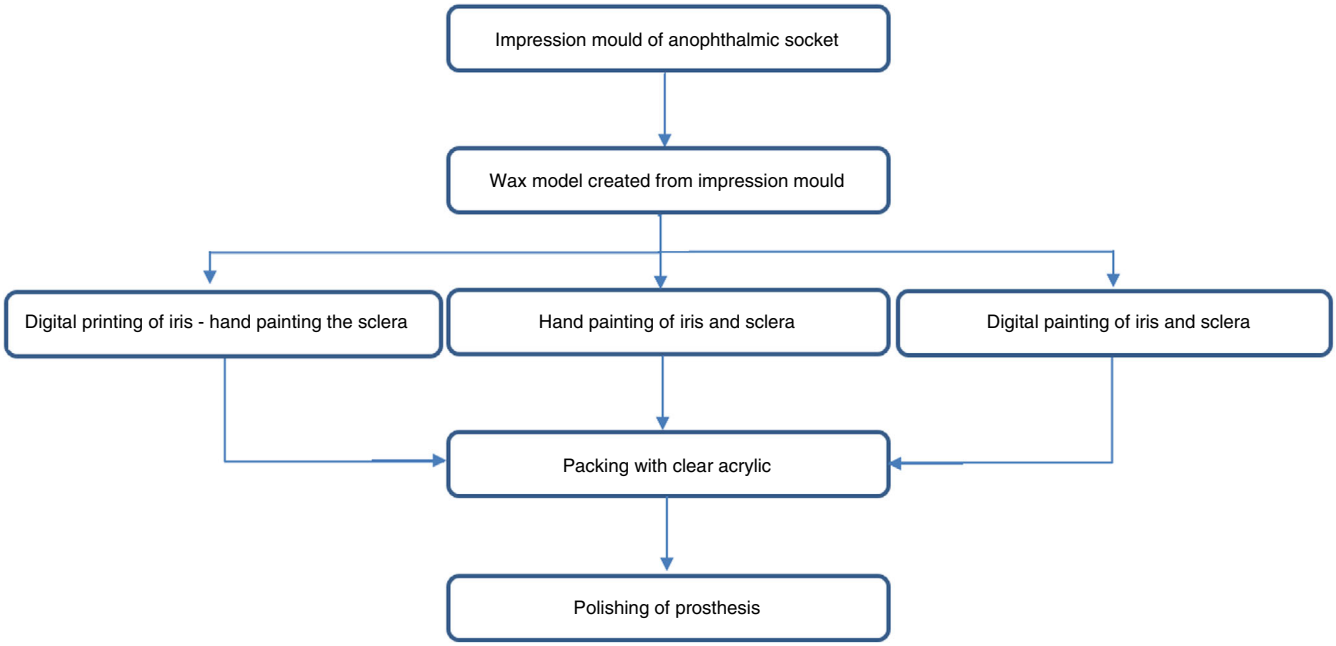

FIGURE 2 Flow diagram showing the key steps used to create an ocular prosthesis.

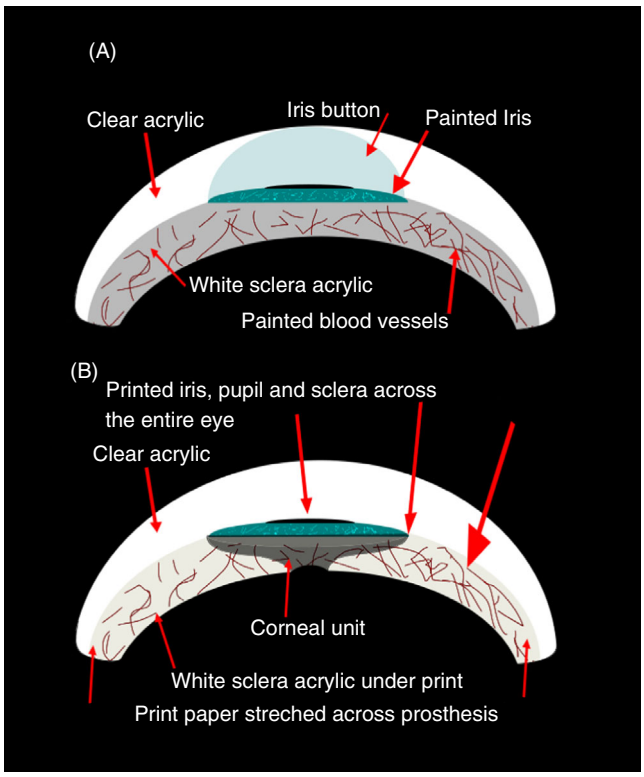

FIGURE 3 Schematic of ocular prostheses. (A) HPAOP schematic showing the clear acrylic, iris button, painted iris and white scleral acrylic with painted blood vessels. (B) DPAOP schematic showing the standard clear acrylic with white acrylic. However, a corneal unit is contained within the prosthesis to ensure the print is correctly aligned when fitted. The amalgamated print is aligned on top of the white acrylic and is overlayed over the top of the corneal unit.

The impression trays contain perforations which allows in the flow and retention of the impression material. During HPAOP production, various materials are used as a base for the iris painting of which autopolymerising acrylic resin discs[13] and paper discs[29] are frequently used. Once these bases have been painted, predominately in oil paint as this has previously shown the lowest colour variance, they are fitted with an artificial iris button. The iris button is a colourless acrylic cap which gives the iris appropriate contour and volume, this gives the iris the illusion of depth, appearing more natural.[21] Once the painted iris is fitted with the artificial iris button, the wax impression will be fitted into the patients anophthalmic cavity, marking where the pupil should reside. A concave dome will be carved out of the wax model to allow for the iris button to be affixed in the correct position. The wax impression will then be boiled out, leaving the iris button in the correct position, prior to packing with super clear acrylic along with a final polish.

In comparison the DPAOP will have the wax impression flasked, boiling out the wax, leaving the corneal unit in the correct orientation in the impression. The flask will then be packed with a white sclera acrylic and upon polymerisation, the white acrylic will then be trimmed back to reveal the corneal unit. The photo-quality paper containing the image of the iris and pupil will then be aligned on the prosthesis using the corneal unit as a guide. Once aligned the prosthesis will be sprayed with a waterproof fixative spray[13] to prevent smudging of the image. The prosthesis is then

re-flasked and packed with super clear acrylic prior to a final trim and polish. In summary, DPAOP and HPAOP are very similar in method, only differing in how the image of the eye is derived and affixed into the prosthesis.

2 | RESEARCH OBJECTIVES

The aim of this project is to assess and quantify colour variation between pre-polymerisation and post-polymerisation samples of inkjet-printed ocular prostheses.

The research objectives are as follows:

1. Primary research aim:
   1.1 To investigate whether there is a colour change following the acrylic resin polymerisation process when producing inkjet-printed ocular prostheses.
2. Secondary research aims:
   2.1 To explore which iris colour group gives the most significant change in stability during the polymerisation process.
   2.2 To examine if there is an identifiable constant variable in colour change for each colour group.

3 | SCOPE OF STUDY

Resolving these research issues could impact on the future synthetic methods for the creation of ocular prostheses. It has been suggested that each year globally over 1000 per one million people will require an ocular prosthesis, this figure is globally increasing yearly by 8%.[30] That is approximately eight million people who will need ocular prosthetics next year. If a better understanding of the colour changing variables can be attained, then this could have a profound effect on patient outcomes, helping to improve patient quality of life and easing trauma caused by the loss of an eye.

4 | PROCEDURES

4.1 | Participants

Ethical approval was attained from The University of Leeds Ethics' Committee (reference: LTCHEM-001). A total of 30 samples of simulated ocular prostheses were required for this research project, examples of which can be seen in Figure 4.

These 30 samples were required to contain 10 with blue iris, 10 with combination/green iris and 10 with sepia iris (brown iris). Therefore, 30 volunteers were gathered containing 10 of each colour group. A station, including a banner provided by the clinical photography laboratory to attract attention to the project, was set up in a corner of the canteen located in the Worsely building of The University of Leeds. This station included a Nikon D7500 digital single lens reflex (DLSR) camera fitted with a Nikon AF-S 105 mm f2.8G Macro lens using a Nikon SB-800 flash, which was set up with a custom white balance using a grey card. Staff and student volunteers were gathered using participant information sheets and consent forms. However, no patients were used as volunteers for this project. Participants were informed that the data given by them would be fully anonymised and they could opt-out of the project at any point prior to full anonymisation. Once a consent form was signed and dated, a digital photographic image was taken of an eye, with a measurement scale in a raw format. This ensured that the full iris was in view with no overlapping of the eye lid. A further photograph was also taken using an 18% grey scale with the same camera settings. Once digital photographic images were taken of a participant's eye, they were not required further for the project. A total of 33 volunteers were gathered with digital photographic images taken. The images from 30 of these participants were used to create simulated ocular prostheses, with

FIGURE 4 Examples of samples simulating ocular prostheses.

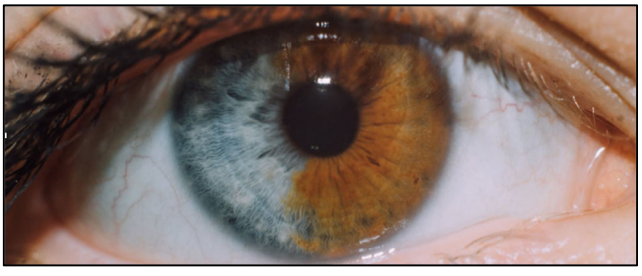

FIGURE 5 An example of an eye with heterochromia, a rare condition where more than one distinct colour is presented in the iris. The condition is only present in less than 1% of the global population. Adapted from the literature.[31]

10 samples of each colour group. Two of the omissions were due to a reclassification of eye colour. These eyes were originally thought to be green in colour, but upon further investigation, they were discovered to be hazel containing a combination of green, gold, and brown colouration. The final omission was due to a rare case of heterochromia being present within both individual eyes of the volunteer, an example of this condition can be seen in Figure 5.

4.2 | Simulated ocular prosthesis synthesis

The method selected for the synthesis of the optical prostheses is the standard technique described by Walshaw et al.[32] However, the full synthetic route was not required for synthesising the simulated ocular prostheses, as impressions of the anophthalmic cavity using wax patterns were not required. The technique was therefore adapted to fit the project, removing the unnecessary stages. A looped profiling system was utilised across the camera, monitor and printer to ensure the print and therefore pre-polymerised sample, accurately matched the patient's eye. This allowed for the true significance of any colour variation to be clearly visualised.

4.3 | Colour measurement procedure

Colour measurement of the prostheses was taken using a Datacolour Spectraflash SP600 plus spectrophotometer, gathering data for the CIELab colour space system, L^*, a^* and two distinct points for each prosthesis, the iris and the sclera, these measurements were taken for pre-polymerisation and post-polymerisation to assess potential colour variation.

Measurements were made using the following conditions:

- Specular—excluded
- UV%—0.0
- Aperture (dependent on location measured, iris = SAV, sclera = XUSAV)
- Flashes—2
- Cut-off—NONE

Measurements of the sclera were taken using a 3 mm^2 (XUSAV) aperture and measurement of the iris was taken using a 9 mm^2 (SAV) aperture. Each time a new aperture was used the data spectrophotometer was recalibrated to suit the aperture selected. Pre-polymerisation measurements were taken after the print was adapted to fit the prosthesis. The post-polymerisation measurements were taken at the end of the synthetic method when the simulated ocular prostheses were finalised and polished. These measurements were taken from the same points previously measured for each simulated ocular prosthesis. Data was recorded for a wide range of measurements, of these measurements; CIE $L^*a^*b^*C^*h^*$ colour coordinates, CIE XYZ colour space, grey scale measurements and reflectance spectral curve data were used to assess potential colour change. However, CIECh and CIE XYZ measurements were ignored as they provide similar data compared to CIELab values. Therefore, the data used was CIELab, Grayscale as a preliminary test and reflectance values for the visible spectrum, 400–700 nm.

4.4 | Statistical analysis

A paired *t*-test was used to compare pre-polymerisation and post-polymerisation colour variance significance. The paired *t*-test was used to analyse each attribute of colour; L^*, a^* and b^* to investigate if there is a variance in a singular attribute or if a constant colour variance is present in all attributes. A one-way analysis of variance (ANOVA) was then used to verify if any colour variance was significant between colour groups. All results gained were analysed at an alpha level of 0.05. A critical two-tail value was selected for the paired *t*-test to allow for colour variance to be measured from the mean, both increasing and decreasing in colour variance for the standard point set from the pre-polymerisation sample.

5 | RESULTS AND DISCUSSION

5.1 | Reflectance data

A reflectance spectrum is the fingerprint of a colour and contains key information such as the hue of the colour and the level of brightness. Reflectance spectral curves were taken of each prosthetic both pre- and post-polymerisation, for both the iris and the sclera. The reflectance data was measured within the visible region of the electromagnetic spectrum, 400–700 nm.

For ocular prostheses to become truly imperceivable, they must best match every attribute of the remaining healthy eye. This includes how ocular prostheses reflect light, as this can play a large part in how they are perceived. If the reflectance of an ocular prosthesis is significantly larger than that of a natural eye, then regardless of the perceived colour, the ocular prosthesis would be easily observed. For instance, within natural eyes, highlights

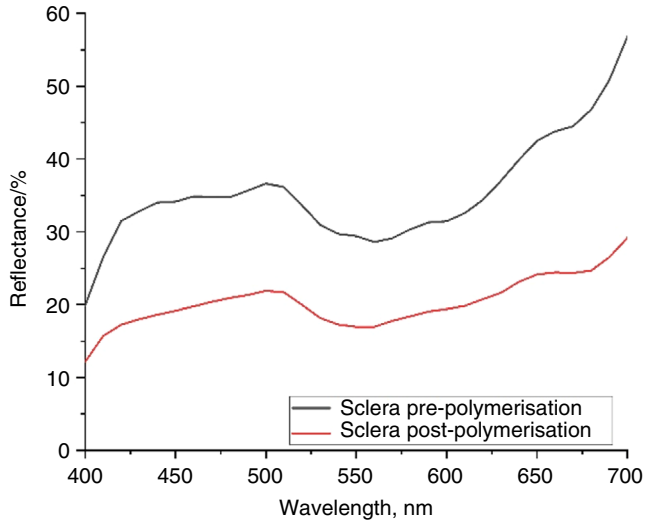

FIGURE 6 Reflectance spectral curves for the sclera averaged across all prostheses for both pre-polymerisation and post-polymerisation.

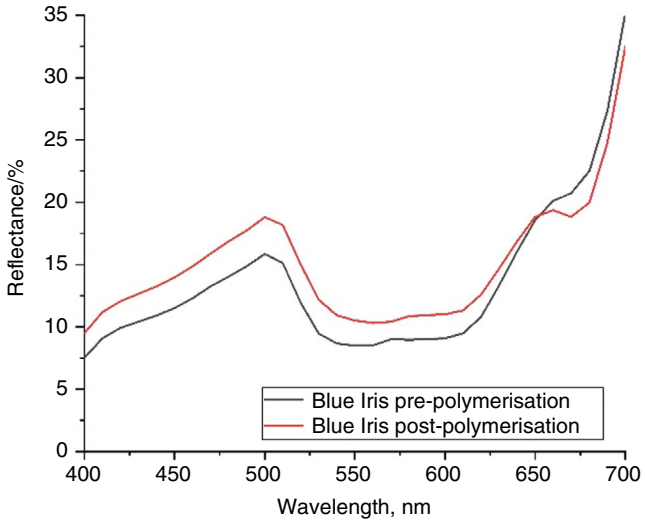

FIGURE 8 Averaged reflectance spectral curves for prostheses with blue irises for both pre-polymerisation and post-polymerisation.

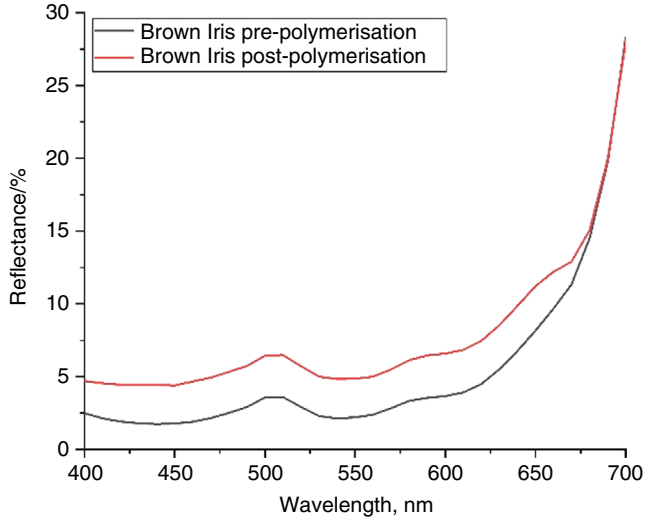

FIGURE 7 Averaged reflectance spectral curves for prostheses with brown irises for both pre-polymerisation and post-polymerisation.

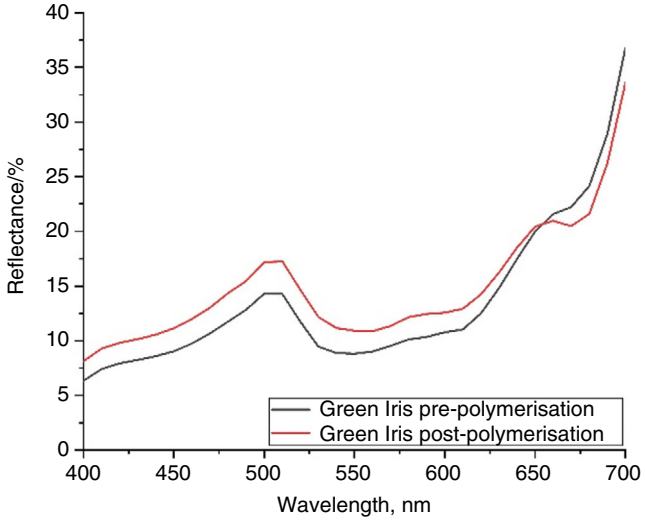

FIGURE 9 Averaged reflectance spectral curves for prostheses with green irises for both pre-polymerisation and post-polymerisation.

on the cornea show which direction an eye is looking. These highlights are the same on both eyes, this in turn tells the observer that the eyes are looking in the same direction. If this is not observed then eyes appear to be divergent or convergent, resulting in a crossed eyed appearance.[33] This undoubtedly shows how important reflectance is when creating an ocular prosthesis, as significant variance can result in the prosthesis looking unnatural.

This reflectance data gives insight into major attributes of colour and can be used in future work to assess the accuracy of digitally printed ocular prostheses, with regards to matching the average human eye reflectance. Perhaps in the future the reflectance from the individual's healthy eye can be analysed and a prosthesis fabricated to not only match colour and appearance but reflectance as well.

Examination of the reflectance spectral curves show that for each of the iris colours, Figures 6–9, the reflectance percentage increases throughout, only decreasing between the range of 650 and 700 nm for prostheses with blue and green irises. However, prostheses with brown irises showed a greater reflectance percentage difference across the entire spectrum measured, only showing

TABLE 1 Grayscale mean ratings given for the sclera, brown iris, green iris and blue iris. These ratings are means across all prostheses.

Sclera	Brown Iris	Green Iris	Blue Iris
1	1.5	2	2.5

similar values for reflectance between 675 and 700 nm. This shows, comparing pre-polymerisation and post-polymerisation between iris colours, that the reflectance and therefore brightness increases once the prosthetic has been polymerised. However, the average sclera does not exhibit the same trend, Figure 6, showing a significant decrease in reflectance in the post-polymerisation measurement compared to the pre-polymerisation measurement, with an average difference of a 10% decrease in overall reflectance. It is hypothesised that when printing the sclera, a reduced amount of ink is used to achieve the desired colour, this is due to the sclera being predominantly white with yellow/brown staining. This lack of ink results in the vinyl glossy photo-quality paper being more perceivable, resulting in a larger value of reflectance when compared to the reflectance values of each coloured iris. The inherent difference in materials, vinyl glossy photo-quality paper against the polymerised acrylic resin, results in a change in reflectance data as anticipated. However, areas where ink coverage is sufficient there is a minor increase in reflectance with brown coloured irises having a greater increase in reflectance when compared to blue and green eyes which have similar reflectance values. Whereas, areas where ink coverage is decreased, such as the sclera, the printing paper used vastly increases the initial reflectance values as it is perceivable, resulting in a significant decrease in reflectance post-polymerisation. Therefore, the sclera is significantly darker in the post-polymerisation stage that the intended print colour. The significance of a much darker sclera would result in the failure of the prosthetic, resulting in a replacement prosthetic being required as it would not match the patient's contralateral eye. This would increase the rehabilitation time for the patient reducing patient satisfaction and potentially decreasing the quality of life of the patient.

5.2 | Grayscale

Grayscale measurements were taken to access colour fastness in the pre-polymerisation and post-polymerisation stages of the prosthetic synthesis. These measurements were taken using a data spectrophotometer, using the same settings previously mentioned in Section 4.3. This data allowed for the visual assessment of the prostheses, giving them a rating from 1 to 5 (with four half steps) with 5 being no colour change and 1 being of significant variation in colour. The Grayscale ratings were used as a preliminary test to check for patterns and to see if there was any significant colour change (Table 1).

Grayscale ratings were calculated using an average across each relevant prosthesis iris colour. The sclera rating was derived from an average across all 30 prostheses with a range of ratings from 1 to 2 and a mean of one rounded to the nearest half step. This indicates that each sclera appears to present a significant colour variance.

The brown coloured iris prostheses had a range of 1 to 2 which is the same as the sclera. However, more prostheses were given a rating of 2 compared to the sclera and so the average is a value of 1.5. This also indicates that there is potential significant colour variance in the brown irises for pre-polymerisation and post-polymerisation. The green coloured iris prostheses had a range of 1 to 3 with an average of 2, showing that compared to brown irises and the sclera that there was a less significant colour change, however, there was still a potential colour variance present. The blue coloured iris prostheses had a range of 2 to 4 with an average of 2.5. This preliminarily shows that the blue iris prostheses present the lowest colour variance when comparing pre-polymerisation and post-polymerisation between iris colours. This data gives a valuable insight into the fundamental patterns presented and how each colour is affected by the polymerisation stage of prosthetic manufacture. CIELab has been utilised to further examine the trends observed in the Grayscale rating system and to quantify their colour variance.

5.3 | Colour analysis—CIELab

CIELab colour coordinates were measured for each prosthesis using a Datacolour Spectraflash SP600 plus spectrophotometer. Prostheses were analysed at two distinct points, the sclera and the iris, measuring the same points for both pre-polymerisation and post polymerisation. Four repeat measurements were taken for each distinct point, with the average value given. Variance within measurements was ± 0.02.

Future experiments will be undertaken with VeriVide's DigiEye system where high-definition digital images are used to measure each pixel of a sample. This will improve the colour measurement for spatially non-uniform samples, such as ocular prostheses. The averaged CIELab data for each iris colour group and the sclera is given above in Table 2.

The CIELab values are defined as:

TABLE 2 Table showing mean CIELab colour coordinates for each iris colour and the mean sclera. Difference in pre-polymerisation and post-polymerisation is given as ΔL^*, Δa^* and Δb^*. These values numerically quantify the colour difference between pre-polymerisation and post-polymerisation. The data is expressed as the mean (standard deviation).

Location	Polymerisation stage	L^*	a^*	b^*	ΔL^*	Δa^*	Δb^*
Brown iris	Pre	19.67	6.06	7.26			
	Post	28.80	4.47	5.09	9.13 (2.54)	−1.59 (1.60)	−2.17 (3.17)
Blue iris	Pre	38.55	−0.83	−3.29			
	Post	42.14	−3.11	−3.67	3.59 (1.40)	−2.27 (1.04)	−0.38 (1.42)
Green iris	Pre	35.18	0.38	6.73			
	Post	41.38	−1.70	4.44	6.20 (2.87)	−2.08 (1.68)	−2.29 (1.93)
Sclera	Pre	63.13	2.00	−2.77			
	Post	50.56	0.99	−0.25	−12.57 (4.15)	−1.01 (2.60)	2.52 (2.24)

TABLE 3 The *t* stat values for the paired *t*-test used to determine statistical significance pre-polymerisation and post-polymerisation. For irises, any value varying more than a value of 2.26 from 0.00 is classified as a significant colour change. For the sclera, any value varying more than 2.05 from 0.00 is classified as a significant colour change. Negative values simply indicate the direction of variance, for example a negative shift in L^* indicates that the post-polymerisation sample is darker and a positive shift indicates that the post-polymerisation sample is lighter in comparison to the pre-polymerisation sample.

Location	L^*	a^*	b^*
Brown iris	11.36	−3.12	−2.17
Blue iris	8.07	−6.87	−0.84
Green iris	6.82	−4.02	−3.75
Sclera	−16.55	−2.12	6.16

- L^* defines lightness ranging from 0 to 100, with 0 being black and 100 being perfect white.
- a^* denotes the red/green value ranging from −100 to 100, with −100 denoting green and 100 denoting red.
- b^* denotes the yellow/blue value ranging from −100 to 100, with −100 denoting blue and 100 denoting yellow.

5.4 | Paired *t*-test analysis

If any attributes were outside of the *t* critical two-tail value of 2.26 from 0 then this indicated that the colour variance was of significance. The paired *t*-test revealed that virtually all attributes of colour suffered significant variance, with each being larger than the critical two-tail value of 2.26 (Table 3).

Each iris colour showed varying mean values for each attribute, a brief description of each is provided later.

The sole omissions were for the b^* values for prostheses with brown or blue irises. The values given for these prostheses were −2.17 and −0.84, respectively. This shows that for prostheses with brown or blue irises that they do not show any significant colour variation in respect to b^* values. However, there was only this sole omission for colour variance.

- Blue irises showed a significant variance in lightness with a mean value of 8.07 showing that the colour was shifting towards perfect white, a a^* value of −6.87 indicating a significant variation and a b^* value of −0.84 which shows that there was no statistically significant change in b^*.
- Brown irises showed a significant variance in lightness with a mean value of 11.36 showing the largest shift of the three iris colour types towards perfect white. The a^* value was 11.36 showing a significant variation in a^* and a value of −2.17 for b^*, this was within the *t* critical value given and therefore brown eyes do not show any significant colour change in b^*.
- Green irises also showed a significant variance in lightness with a value of 6.82, this is however the lowest variance compared to brown and blue irises. The a^* value was −4.02 indicating a statistically significant change in a^* and the b^* value was −3.75, also indicating a significant variation in b^*.
- The *t* critical value for the sclera was given as 2.05. The mean sclera across all 30 prostheses showed the greatest variance of lightness with a value of −16.55, this indicates that the sclera is significantly darker in each of the prostheses. The a^* value was −2.12 showing a statistical significance in a^* with the b^* value 6.16 indicating a statistically significant variation.

It is important to note that variances in pupil size and iris stroma are more perceivable in blue and green eyes.

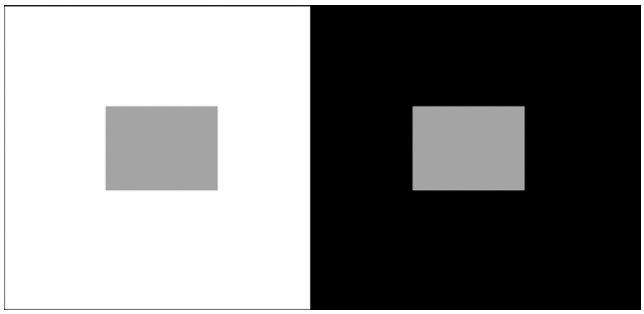

FIGURE 10 Demonstrating simultaneous contrast.[34]

This is especially true in green eyes, as humans perceive the colour green better than any other colour. This is due to its average wavelength residing at around 555 nm, which is near the centre of the visible spectrum. This means that whilst brown irises might show greater colour variation compared to green eyes, the variance in the green iris may be more noticeable to an observer.

Overall, each iris colour increases in lightness with values of 6.82, 8.07 and 11.36 for green, blue and brown irises, respectively. All iris colour groups showed statistically significant decrease in a^* values, whilst only green irises showed statistically significant variations in b^*, in which the b^* value decreased significantly. Both brown and blue irises showed no significant variation in b^* values. Whilst a significant decrease in lightness might not be as perceivable in the sclera when compared to the iris, it may still contribute to an overall decrease in aesthetics of a prosthetic. For instance, an inaccurate sclera, which is much darker in colour, could affect how a colour matched iris and overall prosthesis is perceived, thus visually accentuating the inaccuracy. This could be due to simultaneous contrast, where an adjacent colour can influence how the initial colour is perceived (Figure 10).

An effect such as this could result in irises appearing darker, meaning that whilst the iris could be a perfect colour match to the desired eye it may not be perceived as such, resulting in lower patient satisfaction and a slower rehabilitation due to a replacement prosthesis being required.

5.5 | Literature comparison

Unfortunately, current literature is limited in relevant data to compare the colour variance from this study to other digitally printed ocular prostheses. Ko et al have synthesised digital ocular prostheses using three-dimensional (3D) printing and dye sublimation techniques, however, the techniques are vastly different and they have not yet tested the colour variance of the prostheses and so it cannot currently be compared.[35] Most studies have simply tested digital techniques as a viable method of creating ocular prostheses, as opposed to testing the colour variance of the prostheses pre-polymerisation and post-polymerisation.[14,28,36,37] However, colour variance of digitally printed ocular prostheses has been recorded in two previous studies. Goiato and coworkers showed results of $\Delta E = 13.63$ and $\Delta E = 16.68$ for brown irises, with values of $\Delta E = 16.68$ and $\Delta E = 13.63$ for blue irises for digitally-printed prostheses, using microwave polymerisation.[13,21]

TABLE 4 The total colour difference for each iris colour and the sclera. The data is expressed as the mean (standard deviation).

Location	Total colour difference (ΔE)
Brown iris	3.29 (1.53)
Blue iris	1.82 (0.52)
Green iris	2.47 (0.68)
Sclera	4.75 (1.05)

The total colour difference between samples, shown in Table 4, were calculated using the formula:

$$\triangle E* = \left[(\triangle L*)^2 + (\triangle a*)^2 + (\triangle b*)^2\right]_{1/2}$$

The calculated values for total colour difference showed $\Delta E = 3.29$ for brown irises and $\Delta E = 1.82$ for blue irises. Comparing this to the literature shows that, although there is significant colour variance present, there is a major improvement in colour stability for both colours. Such a decrease in colour variation can significantly improve patient rehabilitation and quality of life. There are a myriad of factors affecting colour stability, so whilst this research project has been shown to have improved colour stability, there is uncertainty about where the methodology has truly improved. Factors include curing type, acrylic type, colourant type, paper type, printer type and quality (DPI) and a vast range of other materials used. The method used is currently superior with regards to colour stability, however, it still presents statistically significant colour variance. Future research is required to highlight which materials or techniques present optimum colour stability when combined to produce an ocular prosthesis. Furthermore, there are no studies within the current literature which analyse digitally-printed green irises or the sclera. These are important factors in how ocular prostheses are perceived, with the sclera showing the most significant colour variation of $\Delta E = 4.75$. This study suggests that sclera colour variation could have a large negative impact on the quality of digital prostheses. Future research is therefore

TABLE 5 The ANOVA test results.

Source of variation	SS	df	MS	*F*	*p*-Value	*F* Crit
Between groups	10.89	2.00	5.45	5.29	0.01	3.35
Within groups	27.81	27.00	1.03			
Total	38.71	29.00				

Note: SS, the sum of squares, this quantifies the variability between or within groups. df, degrees of freedom. MS, mean square, the average variation either between groups or within groups. *F*, the test statistic. *p*-value, the probability of obtaining test results at least as extreme as the results observed, assuming that the null hypothesis is correct. *F* crit, the value at which *F* is compared against to determine significance, if *F* is greater than the *F* crit value then the null hypothesis is rejected and hence there is a total colour difference between groups.

required to decipher the significance and true importance on the impact scleral colour variation has on how an iris or ocular prosthesis is perceived.

Comparing against different paint types of HPAOP, research papers very rarely include green coloured irises in pre-polymerisation, post-polymerisation analysis and tend to only focus on blue and brown irises. Unfortunately, this means that the values given for green irises cannot be compared to literature. However, the values calculated for brown irises and blue irises can be compared.

Some studies have measured total colour difference, however, they tested different black paint types,[18] meaning a clear comparison cannot be made. Whilst others focus on the total colour difference as the prosthesis ages, but not pre-polymerisation and post-polymerisation stage.[15,38]

Fortunately, Goiato et al have previously tested the total colour difference of prostheses when using varying paint types.[13] They tested gouache, acrylic and oil paint, showing results of $\Delta E = 12.75$, $\Delta E = 6.64$ and $\Delta E = 20.23$ for brown irises and values of $\Delta E = 14.50$, $\Delta E = 21.39$ and $\Delta E = 21.10$ for blue irises, respectively. Compared to values of $\Delta E = 3.29$ and $\Delta E = 1.82$ for brown irises and blue irises, respectively, this indicates that the DPAOP produced show significantly lower total colour difference compared to conventional HPAOP. However, the results still show that the calculated colour difference is significant for each iris colour group and the sclera.

In summary, the DPAOP produced have been shown to have much greater colour stability when compared to other digital printing methods and HPAOP methods. Meaning that patients can now receive a more accurate, less perceivable prosthesis which will increase patient satisfaction and quality of life. However, there is still significant colour variation present during the polymerisation of the acrylic resin. Future research is required to better understand what the cause of colour variation is and how to improve the current method of producing DPAOP, as there is still room for improvement.

5.6 | ANOVA

ANOVA revealed a statistically significant difference between colour groups ($p < 0.05$, Table 5). This indicates that not only was colour change significant across all colours but not all colour groups were affected by the same constant colour shift or variation.

All locations measured showed significant colour variation, with the sclera showing the greatest colour variation. The iris colour groups showed total colour variances of 1.82, 2.47 and 3.29 for blue, green and brown irises, respectively. Furthermore, when comparing iris colours, the greater the total colour difference the larger the standard deviation. Brown irises have the largest total colour difference and have the largest standard deviation, showing further instability in colour variation with less predictability for a constant in colour variation as variation increases.

Although total colour difference can be used to compare between groups, more research is needed into a suitable scale for what is a perceivable colour variance and a clinically acceptable level of variance. Studies by Khashayar et al have shown a clinical acceptable variance of $\Delta E = 1$ and a perceivable colour variance of $\Delta E = 3$.[39]

However, this study was for dental research and as such is not applicable for this research area as ocular prostheses are required to be abundantly more accurate with regards to colour. No research into ocular prosthetic colour variation of this kind has been undertaken. Research of this manner could result in the development of a means of testing prostheses following polymerisation, to test their quality prior to issuing out an ocular prosthetic. This would ensure high quality and confidence in the accuracy of the prostheses. Furthermore, this research has not analysed the effect of ageing on colour stability. Therefore, additional research will be required to test the colour stability of the digitally printed ocular prostheses with ageing techniques to test the longevity of a prosthesis. If the prostheses are found to have low colour stability as they age, then this will have to be compared to the cost effectiveness and effect on the

patient. Interestingly, as a patient ages, their anophthalmic cavity will naturally vary in size and shape for the first few years, resulting in a new prosthesis being required. This means that they will require a new prosthesis frequently, a necessity for patient comfort, satisfaction and rehabilitation. Without this necessity, if frequently replacing an ocular prosthesis is beneficial and low cost, then a failure of long-term colour stability might not be of significance as prostheses could simply be remade. However, if replacing an ocular prosthesis is detrimental to patient satisfaction, rehabilitation and overall quality of life then there is a much greater importance for research regarding colour stability of digitally-printed ocular prostheses, compared to other conventional methods. Discovering the effect of ageing on colour stability could also assist in calculating the correct replacement timing of an ocular prosthesis, to rehabilitate a patient in the most effective manner, balancing colour stability and frequency of replacing a prosthesis to best suit the patients need.

6 | FUTURE WORK

Although all research objectives have been answered there are still a myriad of variables present which have been left unexplained, thus requiring further research. Testing each factor to determine the fundamental cause of the colour variance may prove insightful. Further research will highly contribute to the validity of existing research and build fundamental knowledge, as current literature appears to lack papers in these topic areas.

7 | CONCLUSION

The results of a paired *t*-test and an ANOVA test, both with alpha = 0.05, showed that significant colour variations were present for each iris colour group and the sclera. The sclera was shown to have the most significant colour variation with an ΔE value of 4.75, brown irises displayed an ΔE value of 3.29, green irises displayed an ΔE value of 2.47 and blue irises displayed an ΔE value of 1.82. The total colour difference values attained were of statistical significance, signifying that colour variation was statistically unacceptable. However, there are no values for visual acceptability within current literature, meaning that whilst statistically significant some colour variation may be imperceivable or insignificant numerical data could be visually significant. Future work has been detailed for this area looking to ascertain values for visual acceptability, as this would allow for greater accuracy when producing DPAOPs. Furthermore, regardless of the statistical significance of the colour variation, the values for each colour group were significantly lower than the average total colour difference for HPAOP methods which stands at a mean ΔE value of 20.[15,17,20] This is a significant improvement compared to the conventional methods of creating ocular prostheses. This significant improvement in colour stability will decrease the perceivability of the prostheses, meaning that patients will have increased comfort whilst wearing the prosthesis, once again improving patient psychosocial rehabilitation and quality of life.

As well as a significant improvement in colour stability, constants were identified in each colour group. This will allow for preliminary alterations to be made to a colour profile when a DPAOP is being produced, increasing accuracy of the colour match and overall aesthetic of the eye. All these factors contribute to a better rehabilitation process and quality of life for a patient which is invaluable.

ACKNOWLEDGEMENTS

The authors would like to take this opportunity to express their sincere thanks to Dr Peter Broadbent for his assistance and guidance in the laboratory throughout the project.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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How to cite this article: Bunker E, Kazlauciunas A, Zoltie T, et al. Colour stability during production of printed ocular prostheses. *Coloration Technology*. 2023;139(5):596-609. doi:10.1111/cote.12674