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Pilot study assessing the ability of the dental spectrophotometer to measure the colour changes induced by natural pigments

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The dental spectrophotometers are good in identifying natural tooth colour in oral cavities but they have problems when encountering composite materials. In this pilot study we intend to evaluate the capability of the Easyshade Advance dental spectrophotometer to measure colour variations of the dental composite materials which have been introduced in solutions with food dyes. The values obtained by the Easyshade spectrophotometer revealed significant statistical results for the CIE L*a*b parameters measured within our study. The capacity of the instrument to measure the colour of dental materials in *in vitro* experiments *and* the various groups of food pigments represent further subjects of study.

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1. Introduction

Nowadays the aesthetics of teeth is very important for the patients. The dental solution for direct reconstruction is composite. Composite bonding procedures can enhance colour, form, and function [1]. Dental composite materials will change their original colour after coming into contact with fluids from the oral cavity (including water). One of the main group of natural colorants are anthocyanins. Anthocyanins are secondary metabolites of plants. They are water soluble pigments responsible for the colours red, violet, blue in flowers and fruits. Due to the biological abundance and the positive biological effects of these pigments on the human body there was observed an increase in the consumption of foods rich in these compounds [2].

The colour of the teeth can be determined optically or instrumentally. The instrumental technologies are required due to the fact that they are not influenced by the exterior conditions; they are precise, rapid and easy to practice [3].

The instrumental methods exceed the capacity of the human eye in detecting colour changes, thus helping us to choose the dental materials with a colour that comes closest to natural. The spectrophotometric shade analysis is more accurate and more reproducible compared with human shade assessment. [4] An important aspect is that these instruments are capable to measure tooth colour reliably and accurately. Reliability refers to the consistency of the device in matching the same specimen. Accuracy refers to the ability of the device to provide a correct match for a given specimen [5].

Our study is based on the capacity of the Easyshade spectrophotometer to determine shades according to the CIE standards (Commission Internationale de l'Eclairage) CIE LCh and CIE L*a*b. The CIE L*a*b* is used frequently in colour research and is based on the colour standardization of light sources and observers. A specific shade is defined by its location within the CIE L*a*b* system by 3 coordinates. The coordinate L* defines the lightness of the colour and can range between 0 (dark) and 100 (light). The coordinates a* and b* define the chromatic characteristics of the colour; a* refers to the red-green axis, and b* refers to the yellow-blue axis [5, 6]. The Vita Easyshade Advance spectrophotometer, has been specially designed for dental medicine, to determine the colours of the dental structure, but there are authors which determine the colours of the dental materials by means of it [5, 7]. Some authors claim that the Easyshade can be used in dental practice and dental research with some limitations [8].

2. Aim of the study

Dental spectrophotometers are good in identifying natural tooth colours in oral cavity but they have problems at encountering other materials. In our pilot study, we aimed to observe whether the Vita Easyshade Advance spectrophotometer, can determine *in vitro* the modifications of the L*a*b parameters of samples of the dental composite material after they have been introduced in solutions with food dyes.

The null statistical hypothesis is that Easyshade cannot determine the significant modifications of the parameters CIE L*a*b at the level of composite materials subjected to an in vitro experiment of extrinsic colour.

3. Material and method

We will measure the values of the L*a*b parameters of dental composite samples before and after their immersion in different solutions using the Vita Easyshade Advance spectrophotometer.

We created samples of the composite material Filtek Ultimate, with a height of 4mm, and a diameter of 8mm, using plastic mould with the above mentioned dimensions. Because the thickness of the layers of composite material cannot be larger than 2,5 mm regardless of the applied method and time of photo polymerization, the polymerization was performed on both faces with the photo polymerization lamp LED Elipar Freelight 2, 3M ESPE, guide Ø 8 mm, 1000mW/cm2, 20 sec [9]. The top surfaces of all specimens were then polished with fine and superfine polishing disks (Sof-lex, 3M, ESPE) with a low-speed hand piece [10].

Then, all prepared specimens were stored in distilled water at 37°C for 24 hours for rehydration and completion of the polymerization, similar to other recent studies from the specialist literature [6, 11, 12]. After 24 hours the L*a*b colour base values were recorded for all samples using a digital spectrophotometer (Vita Easyshade, Advance, Vita, Zahnfabrik Germany). Colour measurements were performed by positioning the specimens on a white background to prevent potential absorption effects [6, 11, 13].

There were created three groups of composite material of 5 samples each, each group corresponding to another solution. (11,12) The distilled water group was used as a control group, (group I), resembling to other studies from the specialist literature, group II was represented by red wine (ph 3, alcohol 14%) and group III was an acidified blueberry extract food colour (HCl acidified 70% Ethanol pH 4) [6, 11, 12]. The samples were immersed in the three solutions for 24 hours at 37°C, in concordance with other studies (6). Five measurements were taken with the active point of the spectrophotometer in the centre of each specimen. After 24 hours, the specimens were rinsed with distilled water and blotted dry with a tissue paper before re-measurements. The methodology used in the present study was in accordance with previous studies that used spectrophotometry and the CIE L*a*b* coordinate system, which is a recommended method for dental purposes [14].

4. Results

We have evaluated the parameters L*a*b by measurements done with the digital spectrophotometer (Vita Easyshade, Advance, Vita, Zahnfabrik Germany).

The independent variable (nominal) is that of a solution in which there have been immersed samples and the dependent variables (continuous, measurable, "scale") are the values for the parameters L*a*b. We performed measurements on the three parameters of the standard CIE L*a*b by means of the Easy Shade tool (Vita Zahnfabrick).

The statistical tests were performed by means of the statistic program SPSS 21. The normal distribution of the measured values has been confirmed by means of the Kolmogorov-Smirnov test.

The statistical primary evaluation on groups of two was performed by means of One-Way ANOVA. As the Levene test for the measurement of the equality of variances had significant results statistically, we completed the ANOVA test with a test such as Welch which is able to evaluate groups with unequal variances. In order to study the power of the applied statistical tests we used the program G*Power 3.1.6 with the settings F/equality of variance/posthoc. The values of the variance used in the calculations with the G*Power program were calculated by the program SPSS 21.

Table 1. Statistical result for parameter L (brightness of the colour).

Parameter	Immersing solution	Immersing solution	Sig. (α)	Power (1-β)
L	water 24 h (Base value)	water 48 h (Group I)	0,818	0,39
	water 24 h (Base value)	Wine (Group II)	0,000	1
	water 24 h (Base value)	Anthocyanin extract (Group III)	0,000	1
	water 48 h (Group I)	Wine (Group II)	0,000	1
	water 48 h (Group I)	Anthocyanin extract (Group III)	0,000	1
	Wine (Group II)	Anthocyanin extract (Group III)	0,777	0,09

The statistical tests revealed that both solutions with natural pigments, group II and group III, determined significant modifications in brightness (Table 1) not only compared to the measured base values (water 24h) but also to the measured values from the group I (water 48h). The statistical power for those comparative tests suggests that even with a small number of samples the results are relevant.

The results of the comparison tests between the L base values (water 24h) and the values L of the control group (water 48h, group I) as well as the values L of the group II and the group III, revealed no significant differences between the mentioned groups. Unfortunately the "statistical power" for these tests is reduced and this can lead to the occurring of errors of type II (false negative).

*Table 2. Statistical results for parameter *a (red-green axis).*

Parameter	Immersing solution	Immersing solution	Sig. (α)	Power (1-β)
***a**	water 24 h (Base value)	water 48 h (Group I)	0,588	0,97
	water 24 h (Base value)	Wine (Group II)	0,033	0,9
	water 24 h (Base value)	Anthocyanin extract (Group III)	0,000	0,92
	water 48 h (Group I)	Wine (Group II)	0,013	1
	water 48 h (Group I)	Anthocyanin extract (Group III)	0,000	1
	Wine (Group II)	Anthocyanin extract (Group III)	0,000	0,05

Keeping the samples in distilled water does not seem to be significant for the parameter values *a. The negative result (validation of the null hypothesis) in this case is supported by the "statistical power" at 97% (Table 2).

The statistical test results referring to the measured values of parameter *a comparing the group I (water 48h), and the solutions from natural pigments, group II, group III, revealed significant changes in the values. The "Statistical power" for these tests presented values between 90% and 100%.

The comparison of data for group II with group III, revealed in the statistical analysis of the data that the studied tool (Easyshade) identify significant differences between the colouring capacities of the natural pigments.

*Table 3. Statistical results for parameter *b (yellow-blue axis).*

Parameter	Immersing solution	Immersing solution	Sig. (α)	Power (1-β)
***b**	water 24 h (Base value)	water 48 h (Group I)	0,000	0,46
	water 24 h (Base value)	Wine (Group II)	0,857	1
	water 24 h (Base value)	Anthocyanin extract (Group III)	0,000	1
	water 48 h (Group I)	Wine (Group II)	0,519	1
	water 48 h (Group I)	Anthocyanin extract (Group III)	0,000	1
	Wine (Group II)	Anthocyanin extract (Group III)	0,000	0,98

There were identified significant changes between the base value (water 24h) and the group I (water 48h), for colouring parameter *b. The value for the "statistical power" of the test is reduced to 46% however it would have been more important only if NO significant differences were revealed (Table 3).

The statistical tests identified no significant differences in the values of*b between group II and group I, at a "statistical power of 100%.

The statistical tests applied on the values of *b of the group III (anthocyanin extract) revealed significant results statistically ($\alpha<0,05$) regardless of the set of comparison values. "Statistical power" for these tests lies between 98% and 100%.

5. Discussions

The determination of the tooth colour has a major importance in dental aesthetics [15]. Choosing the right colour of the tooth is a key point in the realization of direct or indirect dental restorations [3]. The coordinate L * is of great importance in the analysis of the results, as it represents the luminosity of the samples. The human eye is capable of perceiving the variations in this axis more clearly than in the *a and *b axes [16].

Tooth colour and stain measurement are currently assessed using a wide range of measurement methods divided into subjective (visual shade matching) and objective instrumental assessment such as by colourimetry, spectrophotometry and digital image analysis [17] These instruments are useful tools in colour analysis for direct or indirect restorations, communication

for indirect restorations, reproduction and verification of shade [15, 18].

Easyshade is a dental spectrophotometer. The performance of Easyshade was comparable or better than that of dentists, whilst the agreement between visual and instrumental findings was qualified as good to very good. [19] A study reveals that the VITA Easyshade reliability was 96.4% and the accuracy of VITA Easyshade device 92.6%., indicating predictable shade values from repeated measurements.[5] The quality of the VITA Easyshade tool for measuring the parameters CIE L*a*b has been often demonstrated by using it in numerous studies *in vitro* and *in vivo* [5, 7, 11, 20, 21].

Our study shows that the spectrophotometer Vita Easyshade, Advance, seems to be capable of detecting CIE L*a*b parameter modifications of some dental materials subjected to an *in vitro* test. In both solutions of our study, of natural pigments (anthocyanins from wild blueberries and red wine), the instrument identified significant modifications in the measured parameters compared to the initial values (base values, water 24h) or to the values of the control group (group I, water 48h). Significant differences were also identified in the effects generated by the two types of solution used, which contain natural pigments. More than that, there were identified significant statistical differences between the base values (water 24h) and the ones of the control samples (group I, water 48h) on axis *b. The modifications of colour were significant, even if the in vitro immersion time has been reduced, (6), compared to other in vitro studies which keep the samples immersed for 15 days (12), or up to 21 days (22).

The most interesting result is the one obtained from the measurements of parameter b*. At the end of the experiment the values measured for the red wine samples (group II) on axis*b were intermediary, situated between the base values (water 24 hours) and the control group (group I, water 48 hours). This result suggests that in case of the red wine, the modifications on this axis caused by the presence of water in the composition of wine could be influenced by the modifications caused by the natural pigments of wine.

The use of composite resins has become an important reality in restorative dentistry [20, 22]. Our study brings in discussion the group of nanocomposite materials considered the most stable at modifications of colour, i.e. with the smallest variation of the CIE L*a*b parameters [6, 23]. The modifications in colour of the composite materials are caused by extrinsic and intrinsic factors. The intrinsic factors are represented by the degree of conversion of the double residual bounds, resin filler type, type of resin matrix, composition and size of filler particles, the roughness of the fillings surface [10, 14, 21, 24, 25, 26, 27, 28]. The extrinsic factors are represented by food and colouring drinks which can modify the colour of the composites [26, 14, 23].

6. Conclusions

1. The values obtained by Easyshade revealed significant statistical results for all three parameters measured within our study. According to these results, the Easyshade dental spectrophotometer was able to reliable measure the three parameters L*a*b of the dental composite materials
2. Considering the results obtained in this experiment, we will continue to make future investigations on the capacity of the instrument to measure the colour of dental materials in *in vitro* experiments but also the colouring capacity of various groups of food pigments.

References

[1] B. P. LeSage, Dent Clin North Am., **51**(2), 359, 378 (2007).
[2] N. Motohashi, H. Sakagami, Top Heterocycl Chem. **15**, 1, 48 (2008).
[3] M. Ionaş, M. Sabău, A. Frăţilă, A. Bucă, B. Culic, T. Ionaş, A. Smarandache, A. Sas, Ed. ULB, Sibiu, 22-34 (2010).
[4] S. Paul, A. Peter, N. Pietrobon, C. H. Hammerle, J Dent Res, **81**, 578, 582 (2002).
[5] S. Kim-Pusateri, J. D. Brewer, El. Davis, A. G. Wee, The Journal of Prosthetic Dentistry, **101**(3), 193, 199 (2009).
[6] F. T. Topcu, G. Sahinkesen, K. Yamanel, U. Erdemir, E. A. Otkay, S. Ersahan, Eur J Dent. **3**(1), 50, 56 (2009),
[7] E. U. Çelık, A. Aladaq, L. S. Turkun, G. Yilmaz, J. Esthet. Restor. Dent., **23**(3), 179, 188 (2011).
[8] N. AlGhazali, G. Burnside, R. W. Smith, A. J. Preston, F. D. Jarad, Eur J Prosthodont Restor Dent. **19**(4), 168, 174 (2011).
[9] L. Ceballos, M. V. Fuentes, H. Tafalla, et al. Med Oral Patol Oral Cir Bucal. **14**(1), e51, e56 (2009).
[10] A. U. Güler, I. Duran, A. Ç. Yücel, P. Ozkan, J Appl Oral Sci., **19**(5), 505, 510 (2011).
[11] E. Sarkis, The Saudi Dental Journal **24**, 85, 89 (2012).
[12] F. A. Amin, S. I. Moosa, M. Abbas, Pakistan Journal of Medical Research, **51**(4), 123 (2012).
[13] C. C. Alandia-Roman, D. R. Cruvinel, A. B. Sousa, F. C. Pires-de-Souza, H. Panzeri, J.Dent. PMID: 23270748 DOI: 10.1016/j.jdent.2012.12.004 PII: S0300-5712(12)00327-2 (2012).
[14] I. Nasim, P. Neelakantan, R. Sujeer, C. V. Subbarao, J. Dent., **38** (Suppl. 2), e137, e142, (2010),
[15] S. J. Chu, R. D. Trushkowsky, R. D. Paravina, Review of clinical and research aspects. J. Dent., **38**(Suppl.2), e2, e16 , (2010).
[16] A. P. Samra, S. K. Pereira, L. C. Delgado, C. P. Borges, Braz. Oral Res., **22**(3), 205, 210 (2008).
[17] A. H. Brook, R. N. Smith, D. J. Lath, Int. DenT. J., **57** (5), 324, 330 (2007).

[18] S. R. Okubo, A. Kanawati, M. W. Richards, S. Childress, J Prosthet Dent, **80**(6), 642, 648 (1998).
[19] W. D. Browning, D. C. Chan, J. S. Blalock, M. G. Brackett, Oper. Dent., **34**(3), 337, 343 (2009).
[20] S. T. Fontes, M. R. Fernandez, C. M. De Mura, S. S. Meireles, J. Appl. Oral Sci. **17**(5), 388, 391 (2009).
[21] Z. Ergücü, L. S. Türkün, A. Aladag, Oper. Dent., **33**(4), 413, 420 (2008).
[22] H. Lu, l. Roeder, l. Lei, J. Power, J. Esthet. Restor. Dent.,**17**(2), 102, 108 (2005).
[23] N. M. Azard, Eur. J. Esthet. Dent., **2**(2), 236, 234 (2007).
[24] Q. Ye, Y. Wang, K. Williams, P. Spencer, J Biomed Mater Res B Appl Biomater. **80**(2), 440, 446 (2007).
[25] R. Janda, J-F. Roulet, M. Kaminsky, G. Steffin ,M. Latta, Eur. J.of Oral Sci., **112**(3), 280, 285 (2004).
[26] R. Bagheri, M. F. Burrow, M. Tyas, J Dent; **33** (5), 389, 398 (2005).
[27] A. F. Reis, M. Giannini, J. R. Lovadino, G. M. Ambrosano, Dent. Mater., **19** (1), 12, 18 (2003).
[28] A. U. Güler, E. Güler, A. C. Yücel, E. Ertaş, J Appl Oral Sci., **17**(2), 108, 112(2009).

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