MEDICAL UNIVERSITY
„Prof. Dr. Paraskev Stoyanov"
Faculty of Dental Medicine
Department of Conservative Dentistry
and Oral Pathology"

D-r Silviya Evtimova Stankova

STUDY OF THE INFLUENCE OF SOME FACTORS ON TOOTH BLEACHING

ABSTRACT

of a dissertation for acquiring of an

educational and scientific degree "Doctor"

SCIENTIFIC SPECIALTY:

"Therapeuphtic Dental Medicine"

SCIENTIFIC SUPERVISOR
Prof. Vladimir Emanuilov Panov, DMD, PhD, DSc

Varna, 2024

The PhD thesis was approved at a Department meeting of the Department of "Conservative Dentistry and Oral Pathology", Faculty of Dental medicine, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.

The dissertation contains 190 pages and is illustrated with 30 tables, 94 figures and 4 applications. The Bibliography consists of 424 sources, 23 in Cyrillic and 401 in Latin.

The official defense of the PhD thesis will take place on 30.09.2024 from …… in …………., Faculty of Dental medicine – Varna, and Webex system at an open session of the Scientific Jury.

Scientific Jury:
Chair*:* Assoc. Prof. Tsvetelina Iliyanova Borisova – Papancheva, DMD, PhD – internal member
Members:
Prof. Dr. Emilia Goshova Karova, DMD, PhD– external member
Assoc. Prof. Dr. Janet Kirilova Nikolova, DMD, PhD – external member
Assoc. Prof. Maria Stoyanova Dencheva, DMD, PhD, DSc – external member
Assoc. Prof. Maya Dimitrova Doychinova, DMD, PhD – internal member

The deffence materials are available at the Scientific Department of Medical University–Varna and are published on the website of MU-Varna.

Note: In the thesis summary, the numbers of the tables and figures do not correspond to the numbers in the PhD thesis.

Contents

ABBREVIATIONS

BDT - bleaching of devitalized teeth

BLC - bovin liver catalase

BP - bleaching products

CP - carbamide peroxide

DDM - dentists

FS (Fe2SO4.7H2O) - ferrous sulphate heptahydrate

HDT - hard dental tissues

HP - hydrogen peroxide

HRP - horse radish peroxidase

HVTB - at-home vital tooth bleaching

LED - light-emitting diode

OB - Opalescence Boost 40%, Ultardent

OH - Oral health

OHRQoL - "Oral Health and Quality of Life"

OVTB - in-office vital tooth bleaching

PO - Pola Office Bleaching Kit 35%, SDI

THS - tooth hypersensitivity

VBG - VITA Bleachedguide 3D-Master

VES - VITA Easyshade

TB - tooth bleaching

VTB - vital tooth bleaching

INTRODUCTION

Modern dentistry is associated not only with healing procedures for providing ensure full dental health, but also for restoring or improving the aesthetics of the face. Tooth bleaching is the most commonly performed and desired aesthetic procedure in many dental offices.

Tooth bleaching is the simplest, cheapest and non-invasive method of removing dental stains and lightening the color of vital and devitalized teeth, very successful in properly selected patients and appropriate means and protocol of work. Tooth bleaching improves the smile, appearance and quality of life of the patient.

The bleaching effect is most often achieved with peroxide-based chemical products as it generates free radicals that break down the organic structure of the pigment molecules in dentin and its color lightens.

The application of highly concentrated bleaching agents in the dental office is a subject of scientific researches for many years not only in terms of bleaching effect, but also in terms of potential side effects on hard tooth tissues (HDT), dental pulp and soft tissues in order to search for a relationship with the type and concentration of the bleaching agents, their pH, frequency and duration of administration, application of additional means for activation of the bleaching agent. Various physical and chemical factors are also object of scientific researches because of their potential to increase the effectiveness of bleaching agents for professional bleaching and at the same time to reduce the risk of adverse side effects when applied.

AIM

To study the experience of dentists and patients on tooth bleaching methods and to investigate the influence of certain physical and chemical factors on the bleaching mechanism and the effects of the application of tooth bleaching products.

TASKS

1. Survey of attitudes and experiences of dentists (DDM) and patients on tooth bleaching methods.
   1.1 Survey of DDM's opinion and experience on tooth bleaching methods (questionnaire method)
   1.2 Survey of patients' knowledge, attitudes and experiences about tooth bleaching (questionnaire method)

2. Study of the influence of some physical activators (LED light, electric current) on the rate of HP decomposition in tooth bleaching products
   2.1. To investigate by chemical method the influence of two physical factors – blue light (LED, 450 nm) and electric current (2mA) on the concentration and decomposition rate of HP incorporated in two tooth bleaching products.
   2.2. To compare by UV-VIS spectroscopy the influence of blue light (LED, 450 nm) and electric current (2mA) on the bleaching effect of HP incorporated in a tooth bleaching product on black tea extract.

3. Study of the potential of metal salts and enzymes to activate tooth bleaching products with different HP concentrations.
   3.1. To compare the potential for achieving bleaching effect of bleaching products with different HP concentrations with/without chemical activation in vitro.

3.2. To compare the possibilities of assessing the bleaching effect of methods for determining tooth colour by standard colour shade guide and by spectrophotometer.

4. Measurement of surface microroughness parameters of the enamel ot teeth subjected to in vitro bleaching in task 3 with bleaching products with different HP concentrations with/without chemical activation.

MATERIALS AND METHODS

Materials and methods for task 1

Materials and methods for task 1.1

Object of study: a total of 150 DDM (125 participants – on paper and 25 – through an online form of the survey) for the period April 2022 – April 2023

Criteria for selection of the participants:

Criteria for inclusion in the survey: DDM, familiar with the nature, purpose and voluntary nature of the study; clinically healthy persons aged 25-64, after signing informed consent (for paper surveys) or marking a button for consent in online surveys.

Criteria for exclusion from the study: DDM who did not give informed consent to participate in the study.

Materials and methods for task 1.2

Object of study: 122 patients, some of the patients who have passed through the clinical halls of the Department of Conservative Dentistry and Oral Pathology of the Faculty of Dental Medicine, MU-Varna for the period April 2022 – April 2023.

Criteria for selection of participants:

Criteria for inclusion in the study: ambulatory patients, clinically healthy persons, men and women aged 18-64, included in the survey voluntarily and after signing a "Declaration of Informed Consent" upon provided written information about the objectives and methods of the study.

Criteria for exclusion from the study: persons who have not signed a declaration of informed consent; persons out of the age group

18 - 64 years; persons with mental illness; persons with decompensated systemic diseases.

Methodology for task 1:

1. Survey method
2. Statistical methods from descriptive statistics (frequency distributions, relative shares and hypothesis estimation) using the software products MS Excel 2019 and SPSS 20.0:
3. Graphical methods (tables and diagrams)

Materials and methods for task 2

Materials and methods for task 2. 1

Materials:

1. Opalescence Boost 40% (Ultradent) (OB) (Figure 1): an in-office tooth bleaching product, containing HP and a photosensitizer specific to blue light absorption:
2. pH – Indicator (Figure 2)

Figure 1

Figure 2

Appliances:

1. Blue light source: light curing LED lamp (Masterdent, 450 nm, 750 mW/cm2; (Figure 3)
2. Source of electric current: Ionophorator, 2 mA (Figure 4)

Methodology: iodometric determination of the actual HP concentration in the bleaching agent by trituration (titration) of sodium thiosulfate (*Matis B, 2013),* according to the recommendation of the American Pharmacopoeia and ISO-Standards *(ISO 28399:2021)*

Algorithm of the study:

Group A: not activated tooth bleaching product: OB

Group B: OB activated by LED (Figure 3*)*

Group C: OB, activated by electric current (Ionophorator) with both electrodes immersed in the sample (Figure 4)

Figure 3

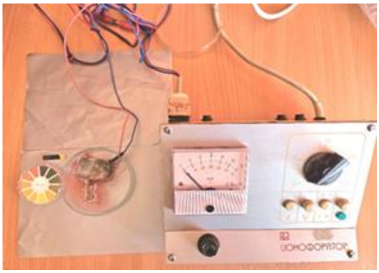


Figure 4

Materials and methods for task 2:

Materials:

1. Tooth bleaching product: OB (Figure 1)
2. Diluted liquid black tea extract /Camellia sinensis/, prepared immediately before the examination and cooled to 25°C (Figure 5)

Appliances:

1. Blue light source: LED lamp from task 2.2 (Figure 3)
2. Boeco UV/VIS Spectrophotometer model S-26 (Figure 6)

Figure 5

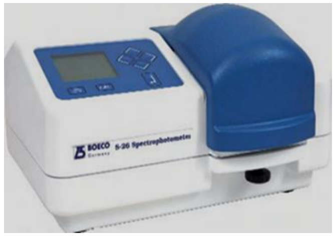


Figure 6

Methodology: When applying the method of UV-VIS spectroscopy to compare the bleaching effect of HP on black tea extract, the change of light absorption and optical density of the analyzed samples was taken into account.

Algorithm of the study:

Five groups of 10 samples were formed:

Group A: control group samples with filtrated black tea extract without tooth bleaching product

Group B: 10 samples with filtrated black tea extract and not activated tooth bleaching product OB

Group C: 10 samples of filtrated black tea extract and tooth bleaching product OB, activated by LED.

Group D: 10 samples of filtrated black tea extract and electric current-activated tooth bleaching product OB (Ionophorator)

Group E: For the validation of the analytical method, the absorption of light from a liquid extract of black tea mixed with HP (30%, 25%, 20%, 15%) in the same volume ratios was measured.

The optical density in all test samples was measured by Boeco UV-VIS Spectrophotometer S-26 at 1minute intervals over a period of 0 to 10 minutes.

Material and methods for task 3

1. Extracted teeth: 43 extracted upper and lower teeth from the groups of incisors, canines and premolars.
2. 10% neutral buffered formalin solution (Formaldehydi solution 10%; Himax Pharma, Bulgaria)
3. 0.9% sodium chloride (NaCl) - solution for infusion (B. Braun)
4. C-silicone impression material for making silicone matrix: ZetaPlus Putty, Zhermack
5. pH indicator band
6. Chemical activators:
   - 6.1 iron sulphate heptahydrate (FS; $Fe_2SO_4.7H_2O$)
   - 6.2 peroxidase from horse radish (HRP; Horseradish peroxidase: Peroxidase Type VI-A from Horseradish, essentially salt-free, lyophilized powder, ~1000 units/mg solid (using ABTS), 250-330 units/mg solid (using pyrogallol), Sigma-Aldrich): red-brown powdery substance
   - 6.3 catalase (CBL; Catalase from bovin liver, lyophilized powder, 2,000-5,000 units/mg protein, Sigma-Aldrich)
   - 6.4 alkaline buffer
7. Standard shade guide for visual determination of the color of the teeth VITA Bleachedguide 3D-Master (VBG) (Figure 7)
8. Spectrophotometer VITA Easyshade V (VES) (Figure 8)

Figure 7

Figure 8

9. Bleaching agents used:

- Group 1 bleaching agent: Factory bleaching system with 35% HP: Pola Office Bleaching Kit 35%, SDI; PO; Figure 9). According to the manufacturer's instructions, 2.0 mL of the liquid component in the syringe (35% HP) is mixed with 0.3 gr of gel-forming agent (powder) until a homogeneous blue-green mixture is obtained.

Figure 9

The choice of PO as a main bleaching product was due to the fact that the bleaching agent 35% HP is in the form of a single factory syringe. This allows both dilution with distilled water to reduce its concentration to 20% and also to include various water-soluble chemical agents as additional process activators for the purpose of the task.

- Bleaching agent for group 2: 1.144 mL 35% HP and 0.856 mL alkaline buffer were used to prepare 2.0 mL 20% HP 20% HP respectively. The 20% HP buffer thus obtained was mixed with a 0.3 gr gel former.
- Bleaching agent for group 3: 20 mg FS were dissolved in 0.856 mL 20% HP alkaline buffer. 0.3 gr gel former was added to the resulting solution and mixed until the formation of a gel of green color.
- Bleaching agent for group 4: 20 mg HRP were suspended in 0.856 mL 20% HP alacle buffer using a vortex-mixer. The resulting suspension was mixed with 0.3gr gel former until a red hydrogel is formed.

- Group 5 bleaching agent: 20 mg CBL was dispersed in 0.856 mL 20% HP buffer by means of a vortex mixer. The prepared suspension was mixed with 0.3 gr gel former until white gel is formed.
- Group 6 was a control: no bleaching agent was applied.

The sample teeth are numbered and randomly distributed in 6 groups - 5 experimental and 1 control. For easy and repeatable positioning, the specimens of each group were included in a silicone matrix so that only the vestibular surface of the tooth crowns and roots remained accessible for processing and observation.

Group 1: with 35% HP
Group 2: with 20% HP
Група 3: с 20% HP + FS
Group 4: with 20% HP + HRP
Group 5: с 20% HP + CBL
Group 6: control group of not treated teeth

Shade registration was carried out immediately before and after the experiment by two methods: visual (by VBG) and instrumental (by spectrophotometer VES). By the visual method, the color determination was based on the most visual matching between the color shade in the middle third of each tooth-sample with the color of 15 shade guides of the VBG, as the color was registered in digital units Shade Guide Units (SGU) from 1 to 29, according to the recommendations of ADA (Figure 10). The difference in tooth color (ΔSGU) was calculated according to the number of units by which the color index changes after bleaching. This method is subjective because it depends mainly on the color vision of the researcher, as well as on the illumination of the room, which should be the same during the analysis.

Figure 10

By the instrumental method, the color of the sample teeth was registered by VITA Easyshade V spectrophotometer (VES). In the "Bleaching mode", the color was read in the same SGU units (Shade Guide Units) according to the VITA Bleachedguide 3D-MASTER color. This mode facilitates the reporting of bleaching efficacy (*Aydın N* et al., 2022). Thus, for the colour shade registration by both methods, identical for VBG and for VES indices SGU under ADA in digital units (1 to 29) were used. This allowed to correctly and directly compare the results obtained by the two methods.

Three consecutive applications of the respective bleaching agent were carried out on the teeth of each of the five experimental groups (Figure 11). Each application was for 10 minutes. Changes in the tooth shade were recorded with the indicated methods in numerical units and were registrated in a table.

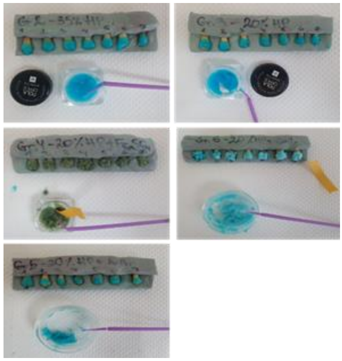


Figure 11

The colour registration data of task 3.1 were used for the performance of task 3.2 and comparison of the possibilities for evaluation of the bleaching effect by the visual method, using standard shade guide VBG and by VES spectrophotometer.

For the statistical processing of the results, the following statistical methods were applied to test hypotheses: statistical verification of a hypothesis about the difference between the average of two interdependent samples (Paired t-test), as well as the method for statistical verification of a hypothesis about the difference between the average of two independent samples (Independant t-test). Tabular and graphical methods were used to illustrate the results.

Materials and methods for task 4

The object of the study in task 4 were the teeth in task 3, distributed in the same experimental groups.

Apparatus:

1. Apparatus for microroughness measuring Surftest SJ-210 (Mitotoyo Corporation, Takatsuku, Japan) (Figure 12)
2. Olympus SZ51 microscope equipped with digital camera №TP6080000B) (Figure 13)

Figure 12

Figure 13

Study design:

The arm of the apparatus was sliding along the vestibular surface of each examined specimen, accounting the changes in the surface microroughness. For each specimen 3 measurements were made along the vesturbular surface in the gingivo-incisal direction.

The measurement of the microroughness was carried out twice after forming the experimental groups: before the application of the bleaching procedure, as well as 24h after its completion. The studied parameters were: Ra – mean arithmetic deviation of the profile, Rq – root mean square deviation of the profile (average amplitude) and Rz – maximum height of the profile; Average characteristics along the height direction: Rsk - the asymmetry (distortion) of the distribution above and below the average, Rku – kurtosis of the distribution; relative acuity parameter at the apex of the distribution curve. The BAC and ADC curves of the surface profile of the specimens before and after bleaching were also automatically registrated. The data reported on the display of the apparatus were registered in tables.

RESULTS AND DISCUSSIONS

Results and discussion on task 1:

Results and discussion on task 1.1:

The distribution of DDM – participants according to the characteristics "offering procedures for TB" and "specialty" is presented in Table. 1. DDMs who have not given an answer are 6 pcs. (4%). Given that 52% of the participants are without an acquired specialty, the highest percentage share is also for DDM without an acquired specialty (53.2%), followed by DDM with acquired specialty "Operative Dentistry and Endodontics" (24.2%).

Table 1

Specialty * Offering_TB Crosstabulation

			Offering_TB		
			No	Yes	Total
Specialty	No dental specialty	Count	9	66	75
		% within Offering_TB	45,0%	53,2%	52,1%
	Conservative Dentistry and Endodontics	Count	1	30	31
		% within Offering_TB	5,0%	24,2%	21,5%
	Prosthetic Dentistry	Count	1	6	7
		% within Offering_TB	5,0%	4,8%	4,9%
	Oral Surgery	Count	6	2	8
		% within Offering_TB	30,0%	1,6%	5,6%
	Periodontology	Count	0	2	2
		% within Offering_TB	,0%	1,6%	1,4%
	Pediatric Dentistry	Count	1	5	6
		% within Offering_TB	5,0%	4,0%	4,2%
	Orthodontics	Count	1	0	1
		% within Offering_TB	5,0%	,0%	,7%
	General Dentistry	Count	1	9	10
		% within Offering_TB	5,0%	7,3%	6,9%
	Other	Count	0	4	4
		% within Offering_TB	,0%	3,2%	2,8%
Total		Count	20	124	144
		% within Offering_TB	100,0%	100,0%	100,0%

The main part of the survey aims to investigate the clinical experience of the DDM respondents in terms of TB procedures by analyzing the answers to the 15 questions included.

Question No. 2 "Do you offer teeth bleaching to your patients in your practice?" was chosen as a control question. The DDMs who answered negatively to it are 22 pcs. (15%) and their answers to the remaining questions were not included in the analyses to exclude unreliable answers that were not based on personal clinical experience (Figure 14).

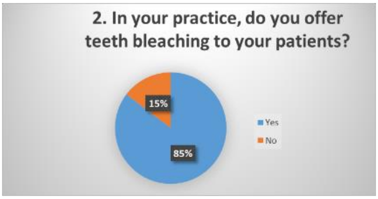


Figure 14

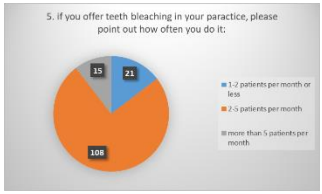


Figure 15

In terms of the frequency of VTB, 75% of participants indicated 2-5 patients per month as an answer to question 5 (Figure 15).

A comparison of the results with literature data indicates a significantly higher frequency in the current study, which can be associated with both greater demand from patients and greater accessibility of the procedure.

The main object of interest of this survey is the protocol for the OVTB applied by the respondents (questions No. 6, 7, 8, 9 and 10).

According to the answers to question No. 6 "What is the percentage of HP in the bleaching products you use for OVTB according to the product leaflet?", the most commonly used (by 49% of the surveyed DDMs) are BP with a percentage of HP from 25% to 40%, and the least (from 8%) – those with 6% HP.

The distribution of the answers on the OVTB protocol (questions 7 and 8) is presented on Figures 16 and 17:

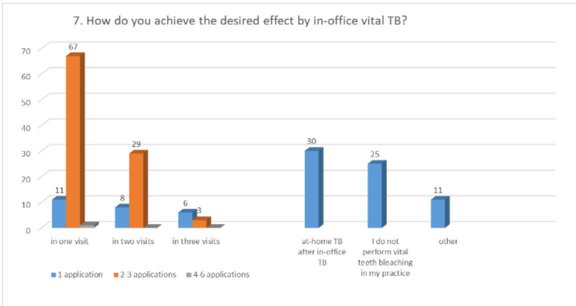


Figure 16

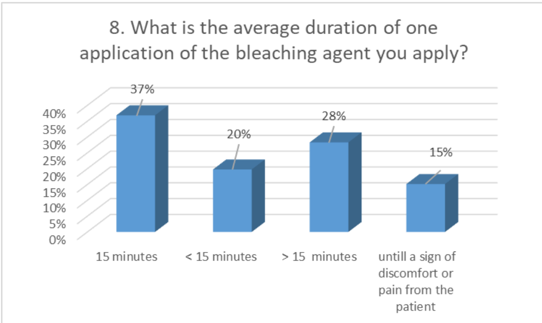


Figure 17

Peydro-Herrero et al. (2020) in their study applied different IP with a high concentration of HP for 2 or 3 applications of different duration (8-10 minutes) in one visit and found that the achieved bleaching effectiveness after the different clinical protocols applied was the same. *Meireles et al. (2021)* did not find a difference in the achieved bleaching effect after 2 or 3 applications of the same BP.

The role of light as an accelerator of in-office bleaching is included in questions No. 9 "Do you apply a light source as an accelerator to the bleaching of vital teeth?" and No. 10 "Do you think that light accelerates and increases the bleaching effect of the applied bleaching system?" . The results are presented in Figures 18 and 19.

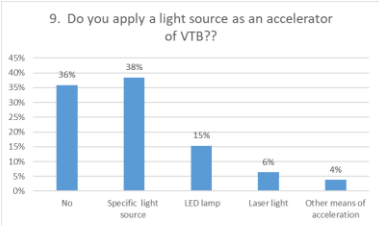


Figure 18

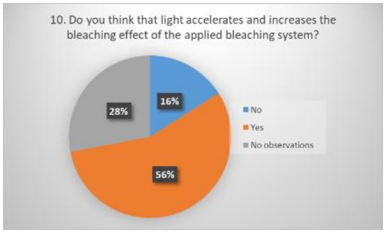


Figure 19

These results correspond to the results of other studies according to which the application of a light source increases the effect of the BP (*Féliz-Matos L et al., 2015; Ferrarazi M et al., 2003; Kury M et al., 2020).*

Light-activated OVTB is the most commonly preferred bleaching procedure both by the participants in the current survey and in a similar study conducted by *Mazilu Moldovan et al*. (2022) in Romania. As factors determining the choice of the most appropriate clinical protocol, these authors indicate the cost of the procedure, followed by the number of sessions required, the time in which the results were obtained, the effectiveness of bleaching and the degree of risk of side effects.

However, in the publications of recent years, the accelerating role of light in the process of TB has been increasingly rejected. *Alghonaimy HA and col. (2021)* do not distinguish between non-activated bleaching and light-activated bleaching (via BP-specific light source or laser). In their meta-analyses, Maran BM et al. (2018; 2019) conclude that, regardless of the different protocols for the OVTB and the concentration of HP, the activation of the bleaching agent with light does not have a significant impact on both the improvement of tooth color and sensitivity *(Maran BM, 2018; 2019).* Given the high proportion (68%) of the participants in the current study who apply BP in combination with a light source, 56% of them believe that this accelerates and increases the bleaching effect of the applied BP and only 16% are of the opposite opinion. 28% of the participants indicate that they have no observations.

Results and discussion on task 1.2:

For the purposes of statistical data processing (determination of frequency, percentage share and distribution - mean SD), the participants are divided into the following age groups: 18-25 years old, 26-35 years old, 36-45 years old, 46-55 years old, with the largest percentage share (47%) being the participants from the age group 18-25 years old, and the lowest percentage share (5%) being the oldest participants (56-65 years old). In terms of gender distribution, women (65%) have a higher percentage share.

Despite the high percentage of individuals (96%) who have heard of tooth bleaching (question No. 1), the percentage of individuals who have never visited a dentist for bleaching is significantly higher – 72% (Figure 20). Interestingly, the percentage of respondents who would perform a dentist-appointed TB is also very high - 79% and is even slightly higher than that of respondents who have never visited a dentist for bleaching purposes (Figure 21).

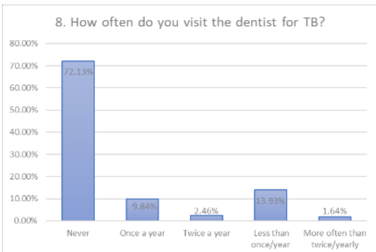


Figure 20

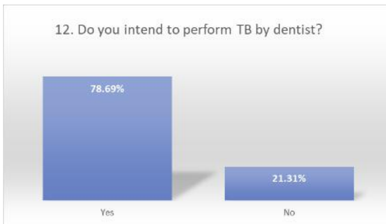


Figure 21

Based on the results of the present study, it can be concluded that a very large proportion of participants (68%) do not have any negative experience with THS after TB (Figure 22). Almost 1/3 of them (29%) define the onset of THS as "mild". For those participants who did not experience any THS or had only mild THS after TB (96% overall), TB procedures were not associated with worsening of OHRQoL due to psychosocial discomfort or pain. Literature data on the frequency and intensity of THS after TB are varied. Meireles SS et al. (2021) indicate a frequency of THS greater than 40% after the HTB (*Meireles SS et al., 2021)*. Given that the answers to the question do not require a link between the emerging THS and the type of bleaching method applied, the comparison of these results with the literature data is incompatible.

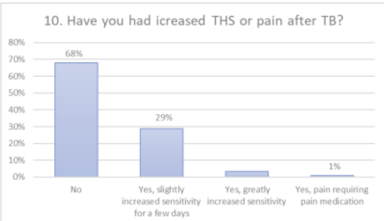


Figure 1

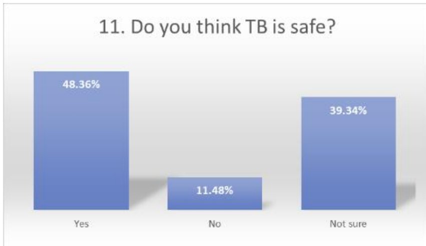


Figure 2

Regarding the safety of TB procedures, the opinion of participants is distributed as follows: almost half of them perceive TB as safe and only 11% as unsafe, but the percentage of participants (40%) who are not sure of safety is relatively high (Figure 23). This clearly outlines the authority of the DDM and the credibility in it, which will also be necessary for the wider information of patients about the safety of TB in compliance with all indications.

Results and discussion on task 2:

Results and discussion on task 2.1

The results of task 2.1 are presented graphically in Figure 24.

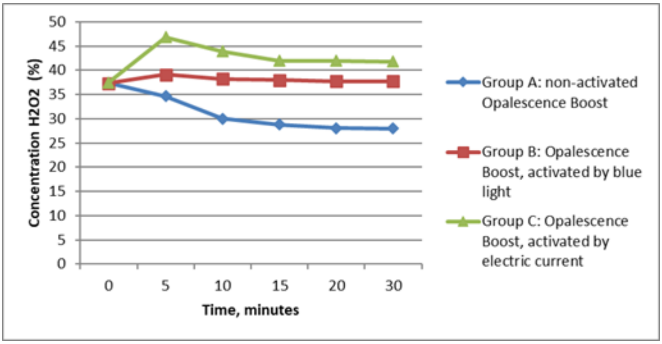


Figure 24

During the iodometric study, an increase in the oxidation potential as a peak at the 5th minute was visible in the groups with samples exposed to light (group B) and electric current (group C), which was not observed in the samples without activation (group A).

The outcome of the TB procedure depends mainly on the type and concentration of the bleaching agent, the agent's ability to reach the chromophore molecules, and the number and duration of the agent's contact with these molecules (*Dahl, J.E. et al., 2003*). By iodometric titration, differences between the reported actual concentration of HP (37.36%-37.49%) and the expected concentration (40%) according to the BP manufacturer's leaflet (Opalescence Boost, Ultradent) were detected in all three experimental groups of the current study. The reported differences were within the limits of the permissible ones, according to the ISO standard for BP for external TB (*ISO 28399:2021).* According to this standard, the difference between the expected and actual concentrations is permissible when it is in the range of +10 % and −30 % of the manufacturer's request. The results of this study confirm the data from the literature sources studied, *(Matis B et al., 2013; Majeed A et al., 2015)*, whose authors also found differences in concentration again within the permissible interval. As probable reasons for this, they point to distortions both in the production process and in the supply and storage, mixing and application of the BP. Storage of some BPs in a refrigerated environment may result in faster degradation of HP and hence lower actual concentration.

The increase in the concentration of HP in the light-activated samples is probably due to a photochemical reaction based on the specific photosensitizer included in the TB product. According to the manufacturer, the photosensitizing substance included in the chemical composition of Opalescence Boost is carotene, which, as part of the carotenoid group, has the potential to be an activator of HP degradation (*Buchalla W et al., 2007; Ontiveros JC, 2011).* Carotenoids, due to their orange-red color, mainly absorb light with a wavelength of 400-500 nm (blue light), which is a part of the visible spectrum. This is why the manufacturer's instructions do not include activation with an additional blue light source. In this task, the application of LED activation is included in order to clarify the role and activation potential of blue light.

When analyzing the data from the table and graph, it was found that in the group without physical activation (group A) there was a gradual decrease in the concentration of HP for 15 minutes, followed by maintaining almost the same concentration (about 28%) for up to 30 minutes. In the experimental groups with samples activated by physical factors (groups B and C), an increase in HP concentration was initially found at the fifth minute, most significant in samples subjected to electric current (group C – with a positive difference of 9.37%) compared to the concentration of HP in samples exposed to blue light (group B – with a difference of 1.75%). After the fifth minute, the concentration of HP in both groups gradually decreased without reaching the baseline values. An increase in HP concentration at the fifth minute is associated with an increased oxidative and bleaching potential of HP.

The reported large positive difference in group C samples confirms the literature data (*Nguyen BL et al., 2006)* for a probable process of regenerative ionophoresis in which electrochemical reactions of HP-resynthesis take place on the cathode additional amount of bleaching agent. By recycling free ions and radicals and increasing the initial concentration of HP, the bleaching process can become faster, may require a smaller amount of bleaching agent, and may not require renewal or reapplication of additional bleaching agent. The undeniable effect of electric current on HP concentration can be used to activate BPs with a lower output HP concentration.

The high concentration of HP recorded at the fifth minute (47%) and the HP electrophoresis process itself should be further investigated with regard to the risk of damage to the pulp cells in order to preserve pulp vitality. There is a lack of data in the scientific literature on clinical outcomes after OVTB with ionophoretic activation. In clinical settings, ionophoretic activation of BP could be applied by preparing a special individual tray for the placement of BP, in which either both electrodes should be included (as in the present experimental setting), or only the active electrode should be switched on, and the passive electrode should be in the patient's hand. The last setting with only an active electrode included was used for ionophoretic BDT *in vivo*, in which the authors achieved excellent aesthetic results in a short time *(Dimitrov Sl, Radeva E, 2009; Kouzmanova Y, 2022).* However, a in a publication from 2022(*Kouzmanova Y, 2022*) two cases of internal cervical resorption following similar BDT were reported.

Ionophoretic activation implies a deeper penetration of the released ions into the tooth structure (*Kouzmanova Y, 2022),* which in OVTB creates a risk of their entry into the dental pulp with subsequent complications from what requires serious scientific studies.

Results and discussion on task 2.2

Spectrophotometry as a method of spectroscopy is most widely used for quantitative determination, using the relationship between the concentration and intensity of the absorbed monochromatic light. In the conducted study, the method was used to track in dynamics the degradation of chromogenic compounds in a tea extract subjected to the action of HP, included in TB products. Boeco UV/VIS Spectrophotometer S-26 was used to measure the bleaching effect (Figure 6). The Spectrum Scanning mode allows scans to be made in any selected part of the range from 198 to 1000nm. The Auto-ranging Absorption axis allows scanning to be started without prior knowledge of the expected peak absorption level of the samples examined.

The results of the study are reflected in the graph of Figure 25:

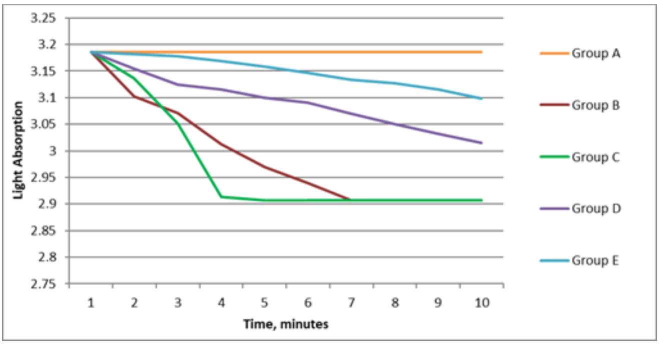


Figure 25

Group A: control group of samples with filtrated black tea extract without tooth bleaching product

Group B: 10 samples with filtrated black tea extract and not activated tooth bleaching product OB

Group C: 10 samples of filtrated black tea extract and LED-activated OB tooth bleaching product.

Group D: 10 samples of filtrated black tea extract and electro-activated OB tooth bleaching product (Ionophorator)

Group E: validation samples

The spectrophotometry performed showed a decrease in light absorption from the samples in all groups, except for those from the control group A. It was most pronounced in the samples subjected to activation with blue LED light (group C). In this group, light absorption and bleaching effect occured in a shorter time - 4 minutes compared to samples without activation (group B) - 7 minutes. There was no change in light absorption after the fourth and seventh minutes respectively in both groups, which was probably due to HP depletion. Under the influence of electric current (Group D), the absorption of light from the samples in the group decreased gradually until the tenth minute. The achieved final lightening effect was the same for groups B and C and weaker for group D. For validation of the analytical method, the absorption of light from liquid extract of black tea mixed with HP (30%, 25%, 20%, 15%) in the same volume ratios (Group E) was measured, as a function of time.

The application of UV-VIS spectroscopy on black tea extract subjected to HP allows to avoid the complexity of the tooth structure in terms of both the diffusion of HP in it and the penetration of light into it (*Young N et al., 2012)*.

The results of the study confirm the potential of the applied physical factors to increase the bleaching effect and efficacy of the BP used. The physical factors provide additional energy (increased temperature and blue light), thereby increasing the stimulated dissociation of HP and the formation of free radicals (*Buchalla W* et al., 2007). The most likely main mechanism of action of all light-activated BPs is the occurrence of a photochemical reaction through the absorption of light photons by the specific photosensitizers involved.

The mechanisms by which light can improve bleaching are different and complex:

- direct photobleaching of chromogenic molecules by breaking the bond and losing conjugation in their structure

- direct light-induced cleavage of HP into two radicals, which subsequently react with chromogenic molecules

- chromogens absorb photons and transfer their energy to the HP molecule, which leads to its cleavage (*Young N et al., 2012).*

As a result of the photochemical reactions in the black tea extract samples, the achieved final bleaching effect was equal, but occured almost twice as fast after activation with blue light compared to the effect without activation. The observed peaks in absorption were followed by a plateau with no change in light absorption, which is probably due to the depletion of HP. In relation to OVTB procedures, the application of the results obtained is related to the possibility of shortening the duration of the application of the specific BP in the cases of light activation. It should be taken into account that the faster depletion of HP after light activation, in addition to the occurrence of a faster bleaching effect, may also be associated with a higher risk of subsequent THS.

The mechanism by which electric current can improve bleaching is different from that of light and is described in the discussion on problem 2.1 as the so-called regenerative ionophoresis. It implies a shorter, more efficient and facilitated bleaching process, in which, according to the resynthesis of HP, it is likely that no new applications of BP are needed. According to the results of task 2.1, ionophoretic activation of BP leads to a significant increase in the initial concentration of HP by more than 9%, which also implies a faster and more pronounced bleaching effect. However, the results of task 2.2 reject this. The final lightening effect in the ionophoretic activation group is the weakest compared to that in the other groups and occurs gradually. As a supposed reason for this, the experimental setup itself with the immersion of both electrodes in the samples can be indicated. This allows the generation of more ions, resulting in an increased concentration of HP. At the same time, however, it is very likely that the movement of ions takes place only between the two immersed electrodes, which limits the interaction of the ions with the chromophores, respectively, and the bleaching potential of the ionophoretically activated HP.

Results and discussion on task 3

Results and discussion on task 3.1

The numerical indices determined (before and after the experiment) visually and spectrophotometrically are entered in a table.

The brightness index measured after the TB procedure is subtracted from the brightness index before it and the resulting difference is reflected as ΔSGU. It serves to evaluate the achieved bleaching effect. Depending on the ΔSGU values, bleaching is considered ineffective (≤0.8), moderately effective (>0.8, ≤1.8), with good effect (>1.8, ≤ 3.6), with very good effect (>3.6, ≤ 5.4) and with excellent effect (>5.4) (*Paravina RD et al., 2019).*

By the visual method, the color shade difference is indicated by ΔSGU – BG (Shade Guide Units by Bleaching Guide), and in the spectrophotometric method – by ΔSGU – ES (Shade Guide Units by Easy Shade). In the second method, the color difference is calculated for each of the three zones of the teeth from each group: cervical (VES-C), middle (VES-M) and incisal zone (VES-I), (Figure 26).

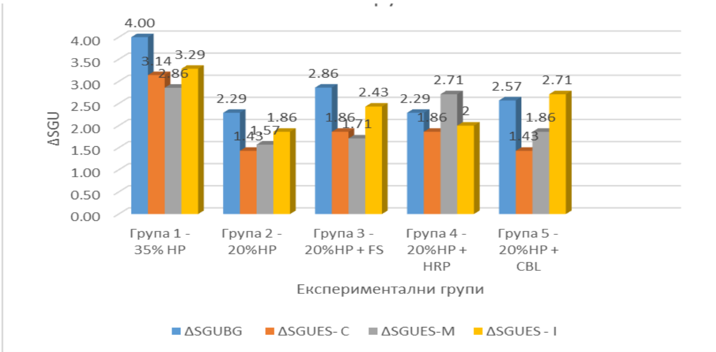


Figure 26

Reported by groups, the largest absolute difference in brightness ΔSGUES was found in all three zones of the samples of group 1, and the smallest in those of group 2. Reported by zones, the largest ΔSGUES was found in the incisal (VESG-I) zone in groups 1, 2, 3 and 5, and for group 4 - in the middle (VESG-M) zone. Although that the cervical (VESG-C) zone has the highest spectrophotometric color indices before the application of bleaching agents, the ΔSGUES found in it are at second place after those in the incisal zone for groups 1 and 3. For groups 2, 4 and 5, they take the last place. In all groups, the bleaching effect was observed to be above the accepted minimum threshold of PT = 1.4 for the evaluation of the bleaching effect by ΔSGUBG (*Aydın N* et al., 2022; *Paravina RD et al.,2019).*

The role of HP concentration in achieving a significant bleaching effect is confirmed by the fact that the smallest bleaching effect is observed in group 2, in which it is 20%. The addition of FS, HRP and CBL as activators to the same bleaching agent (20% HP) increases its effect. In no tooth zone, however, the effect of the applied product with the highest concentration - 35% HP was reached.

When analysing the identified intergroup differences in the degree of bleaching of different tooth zones, the inclusion of samples from different dental groups (incisors, canines and premolars) with expected different histomorphology and colour should be taken into account.

The activators for HP decomposition were selected according to literature data. The inclusion of metal salts and enzymes aims to establish whether this could reduce the concentration of HP applied while maintaining its bleaching potential. In addition, the expectations for these activators are, on the one hand, to "neutralize" the residual, not reacted HP, and on the other hand, to limit the diffusion of released aggressive radicals in pulp direction, which reduces the risk of its damage.

Fenton was the first scientist who has established the pronounced ability of some metals/metal ions to form highly reactive hydroxyl radicals (•OH) when they come into contact with HP. Subsequently, the reaction was named in his honor - the Fenton reaction. It is easy to implement and is the main method used to catalyze HP decomposition (*Ameta R et al., 2018*). The formed hydroxyl radicals have a high oxidation potential. They interact spontaneously with aromatic compounds, as most pigment molecules are. They also interact with existing intact HP molecules to form hydroperoxide radicals (NO2•). The latter interact with the resulting Fe^{3+} ions, regenerating them again to Fe^{2+}. This allows the reaction to be repeated many times - until the HP invested is completely exhausted.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{-}OH + \bullet OH$$
$$Fe^{2+} + \bullet OH \rightarrow Fe^{3+} + OH^-$$
$$Fe^{3+} + HO_2\bullet \rightarrow 2\ Fe^{2+} + 2\ OH^-$$
$$Fe^{3+} + HO_2\bullet \rightarrow Fe^{2+} + H^+ + O_2$$
$$H_2O_2 + OH\bullet \rightarrow HO_2\bullet + H_2O$$

The latter reaction represents the total reaction of HP decomposition to water and radicals *(Ameta R et al., 2018)*.

Catalase and peroxidase are oxidoreductase enzymes catalyzing electron transfer between the so-called molecule reducer (electron donor) and oxidizing molecule (electron acceptor) (*Whitaker JR et al., 2003).*

Catalase is an enzyme from the group of metalproteins - tetrameric chemoprotein, in the center of which is the iron trivalent ion. The biochemical role of catalase is to catalyze the decomposition of the formed HP into oxygen and water through a two-step process (*Cao C et* al., 2003; *Boon EM et al., 2007):*

$$H_2O_2 + Fe(III)\text{-}E \rightarrow H_2O + O{=}Fe(IV)\text{-}E(.+)$$
$$H_2O_2 + O{=}Fe(IV)\text{-}E(.+) \rightarrow H_2O + Fe(III)\text{-}E + O_2$$

Catalase itself does not have a bleaching effect (*Pauli MC et al., 2018*). It only accelerates the decomposition of HP, including CP *(Heck DE et al., 2010).* According to *Banerjee A and Friedman* J (2005), catalase increases both the O_2 release rate and the number of hydroxyl radicals, which also reduces the time it takes to achieve a rapid bleaching effect. According to Silva RB et al. (2014) the inclusion of 5mg catalase will lead to the breakdown of the entire amount of HP (35 to 37%), in just 3 min. without the need for light activation or pH changes.

Peroxidase enzymes have a certain self-bleaching effect. By analogy with catalases, HRP accelerate the destruction of HP by identically activating the action of peroxide-based BP. Thus, HRP reduce the negative effect of BP on odontoblasts (*Ortecho-Zuta U et al., 2019).* In a study by *Duque CCO et al. (2018),* it was found that after application, *in vitro,* of a combination of 10% HP with HRP, the resulting bleaching effect was similar to that obtained after the application of 35%

HP gel. Unlike the latter, the combination used did not cause a cytotoxic effect.

During the experiment, changes in the pH of the bleaching agents were also tracked. For this purpose, a pH indicator strip was used. The latter made it possible to determine *in situ* even the changes in the pH of the applied bleaching material; a measurement that could not be established with conventionally used pH meters.

For the factory BP used for group 1, the reported pH was acidic (~ 4.0). This is a prerequisite for demineralization of tooth enamel, which is a risk for deterioration of its physical, optical and aesthetic properties. Despite the inclusion of alkaline buffer in groups 2, 3, 4 and 5, the finding regarding pH was the same.

In the activator groups (groups 3, 4 and 5), a violent exothermic reaction was observed when HP was mixed with the activator, despite the lower concentration of HP used (20%). This interaction was accompanied by the release of multiple gas bubbles. They probably consisted of oxygen molecules released during the chemical reaction, which changed the consistency of the bleaching agent into a foam. The latter made it difficult to apply with a brush. Gradually, over time, the number of gas bubbles decreased, and the bleaching agent liquefied. A probable reason for this can be pointed out the rapid generation of oxygen radicals, as well as the release of water as a reaction product. Such a change in consistency in possible clinical application would require increased attention to the isolation of the surrounding soft tissues in order to prevent their damage.

Given the exothermic nature of the reaction, it is also necessary to determine the amount of heat released during the reaction in order not to threaten pulp vitality in case of an increase in the intrapulp temperature of more than 5,4 °C. Pulp vitality can also be threatened by the rapid release of oxygen radicals and the oxidation-reduction nature of the bleaching process.

Despite the lower concentration of HP and the alleged reduced side effects, the effects of a combination of HP and activators should be thoroughly investigated, by means of chemical analysis and thermometry, with a view to preserving pulp and gingival health in possible clinical application.

In the statistical processing of the results for all experimental groups, a research hypothesis, H1, was put forward, according to which the brightness of the teeth increased after the application of a bleaching agent or respectively the numerical value of the determined color decreases. For the null hypothesis, H0, it was assumed that this value was equal to or greater in value, due to the absence of a bleaching effect. Due to the small sample size, normality of the distribution was checked by the Shapiro Shapiro-Wilk and the hypotheses were tested only if there was a normal distribution in the samples. A normal distribution was found for all experimental groups. Control group 6 was excluded from statistical processing, given the zero differences in SGU. For statistical verification of the hypothesis of the difference between the means of two interdependent samples, the Paired t-test was used**,** with a significance level $\alpha = 0.05$**.** For each group, t - Student's coefficient, SD - the same standard deviation of the two samples, p-value - statistical significance and ES - effect size (Cohen's D) were calculated. While *p-value* is an indicator that only confirms or denies the existence of a difference between groups, *ES* is an indicator for the quantitative expression of this difference (Table 2).

Table 2 Effect Size (ES) – interpretation (Todorova S, 2022)

Effect Size (ES)	**Interpretation**
0,00≤ES<0,20	Negligible
0,20≤ES<0,50	Small
0,50≤ES<0,80	Medium
0,80≤ES<1,30	Large
1,30≤ES	Very large

The results are presented and summarized in Table 3. In all experimental groups, statistically significant differences were found with a very large effect size (ES). The largest numerical value (Cohen's D) was reached in group 1, followed by group 2. In groups 3, 4 and 5, the effect was approximately equal. The size of the effect also determined the practical significance of the results of the scientific study (Table 2).

For all groups H0 was rejected in favour of H1. This means that the mean colour shade value for the representative sample of treated teeth is lower than the mean colour shade value of the same sample before treatment with bleaching agents; or there is statistically significant bleaching at a p-value of $< \alpha = 0.05$.

Table 3

Statistical hypotheses and effect size in dependent samples		**Value**	**Color Difference (ΔSGUES)**
Group 1: 35% HP	Statistical significance	p-value (0.000) < α	**Significant**
	Effect Size	Cohen`s D = 5.79	**Very large**
Group 2: 20% HP	Statistical significance	p-value (0.000) < α	**significant**
	Effect Size	Cohen`s D = 2.61	**Very large**
Group 3: 20% HP + FS	Statistical significance	p-value (0.001) < α	**Significant**
	Effect Size	Cohen`s D = 2	**Very large**
Group 4: 20% HP + HRP	Statistical significance	p-value (0.001) < α	**Significant**
	Effect Size	Cohen`s D = 1.87	**Very large**
Group 5: 20% HP + CBL	Statistical significance	p-value (0.001) < α	**Significant**
	Effect Size	Cohen`s D = 2.04	**Very large**

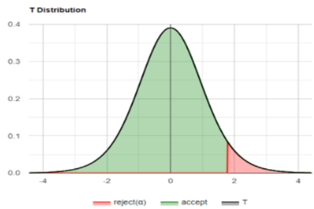
To compare the difference in the bleaching effect of HP with or without activators of the process, the control method is 35% HP bleaching in group 1 and therefore it is accepted as a control group. The effect of 35% HP is compared with the effect of the application of experimental bleaching agents in the other experimental groups (Table 4).

Table 2

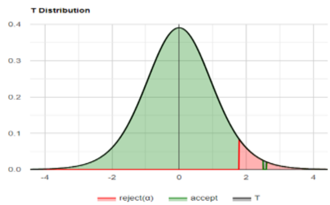
Color Difference (ΔE*)	N	Mean	Std. Deviation	Std. Error Mean
Control group	7	3.0952	,53452	,20203
Group 2:20% HP	7	1.6190	,62148	,23490
Control group	7	3.0952	,53452	,20203
Group 3: 20% HP + FS	7	2.0000	1,000	,37796
Control group	7	3.0952	,53452	,20203
Group 4: 20% HP + HRP	7	2.1905	1, 16837	,44160
Control group	7	3.0952	,53452	,20203
Group 5: 20% HP + CBL	7	2.0000	,98131	,37090

For hypothesis zero (H0), the bleaching effect in group 1 it was assumed to be equal to or less than the bleaching effect in the other groups. The test was carried out by *the Independant t-test* (statistical verification of the hypothesis of the difference between the mean of two independent samples) at a selected significance level $\alpha = 0.05$ (Figure 27).

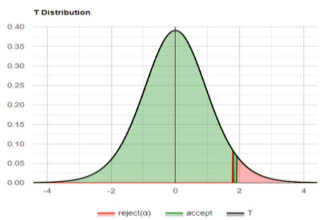
Group 2, 20% HP



Group 3, 20% HP+FS



Group 4, 20% HP+HRP



Group 5, 20% HP+CBL

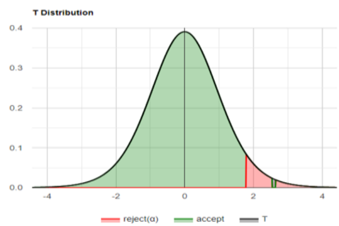


Figure 27 Student's t-distribution

The aggregated results are presented in Table 5. For all experimental groups in the sample, the difference in bleaching of the control group was found to be greater than that of the compared group and statistically significant at significance level $\alpha = 0.05$ and effect size (ES) very large (for groups 2, 3 and 5) and large (for group 4). The greater the ES, the greater the statistical difference between the bleaching effect in the experimental group and the same in the control group. It follows that the bleaching effect in group 4, in which horseradish peroxidase (HRP) was added as an activator, is closest to the bleaching effect of 35% HP in the control group (Cohen's D=1).

Comparing the values of the ES - Cohen's D indicator, it can be concluded that the addition of ferrous sulphate (FS) and catalase (CBL) has a similar effect on the bleaching effect. Of all the groups, the bleaching effect is weakest in group 2 (20% HP), given that for her Cohen's D = 2.55,

Table 5

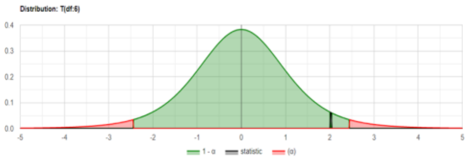
Statistical hypotheses and effect size in independent samples		**Value**	**Control group: Group 1: 35% HP**
Group 2: 20% HP	Statistical significance	p-value (0.000) < α	**Significant**
	Effect Size	Cohen`s D = 2.55	**Very large**
Group 3: 20% HP + FS	Statistical significance	p-value (0.013) < α	**Significant**
	Effect Size	Cohen`s D = 1.37	**Very large**
Group 4: 20% HP HRP	Statistical significance	p-value (0.044) < α	**Significant**
	Effect Size	Cohen`s D = 1	**Large**
Group 5: 20% HP CBL	Statistical significance	p-value (0.012) < α	**Significant**
	Effect Size	Cohen`s D = 1.39	**Very large**

Results and discussion on task 3.2

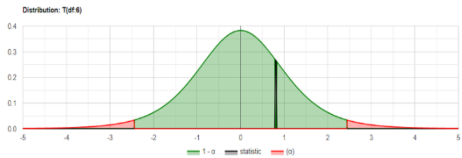
For the implementation of task 3.2, the purpose of which is to compare the possibilities for assessing the bleaching effect of the visual method using the standard color shade guide VITA Bleachedguide 3D-Master (VBG) and the instrumental method using the VITA Easyshade (VES) spectrophotometer, the data from the color registration of task 3.1, carried out immediately before and after the experiment under the same lighting conditions, were used.

The results were processed using the method for statistical verification of the hypothesis of the difference between the means of two interdependent samples - *Paired t-test*. The aim was to establish whether there were statistically significant differences between the recorded data using the visual (ΔSGUBG) and instrumental (ΔSGUES) methods**.** The null hypothesis (H0) excluded the presence of statistically significant differences. The alternative hypothesis (H1) included the presence of such.

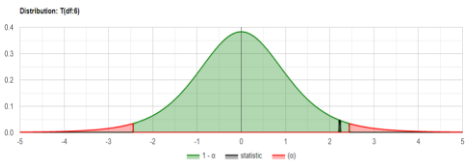
For each group, the *mean color differences of the two methods* (ΔSGUES and ΔSGUBG), *the differences between them* (d = ΔSGUES - ΔSGUBG), *Student's t-coefficient*, SD - the same *standard deviation of the two samples*, *p-value* - statistical significance and *ES-size of the effect* (Cohen's D) were calculated. The results are presented on Figure 28 and Table 6. Due to the small sample size, the Shapiro-Wilk test was applied to check for normality and the hypotheses were tested only in the presence of a normal distribution in the samples.



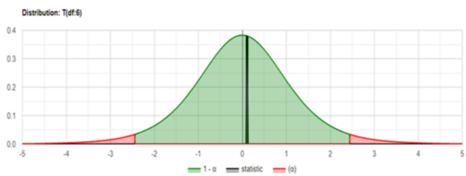
Group 1, 35% HP



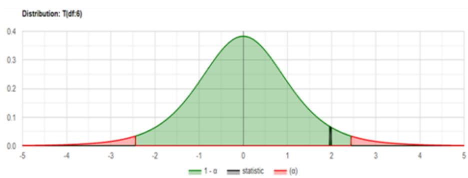
Group 2, 20% HP



Group 3, 20%HP+FS



Group 4, 20%HP+HRP



Group 5, 20%HP+CBL

Figure 28 Student's t-distribution

Table 6

Statistical hypotheses and effect size in dependen samples		Value	ΔSGUES -ΔSGUB
Group 1: 35% HP	Statistical significance	p-value (0.0892) >α	**Inignificant**
	Effect Size	Cohen`s D = 0.77	**Medium**
Group 2: 20% HP	Statistical significance	p-value (0.4471) >α	**Insignificant**
	Effect Size	Cohen`s D = 0.31	**Small**
Group 3: 20% HP + FS	Statistical significance	p-value (0.06702) >α	**Insignificant**
	Effect Size	Cohen`s D = 0.84	**Large**
Group 4: 20% HP + HRP	Statistical significance	p-value (0.9172) > α	**Insignificant**
	Effect Size	Cohen`s D = 0.04	**Very small**
Group 5: 20% HP + CBL	Statistical significance	p-value (0.0948) > α	**Insignificant**
	Effect Size	Cohen`s D = 0.75	**Medium**

For none of the experimental groups, H0 could be discarded in favor of H1, due to the lack of data for a statistically significant difference between ΔSGUES and ΔSGUBG at significance level α=0.05 and p-value<α. This was confirmed by the calculated effect size (ES), which was large only in group 3, and in the rest groups it was very small to medium. In group 3, the occurrence of a statistically significant difference could be expected in case of an increase in the sample volume. With the acceptance of the null hypothesis, the correctness of the visual method by the VBG- Shade guide is confirmed in the assessment of the bleaching effect. According to literature data, instrumental methods are characterized by more accurate analytical parameters (accuracy, precision, repeatability, etc.) than visual methods in color shade determination for prosthetic restorations (*Liberato WF et al., 2019*). In spectrophotometry, the CIE Lab system allows to distinguish minimal differences in tooth color.

Using the visual method, VBG Shade guide only quantifies changes in the degree of brightness of specimens by numerical units (SGUs) by ADA color indices from 1 to 29. This makes the two values ΔE and ΔSGU, respectively, both methods incomparable for direct comparison (*Basson RA et al., 2013).* To perform the current task, VES was used in the "Bleaching Mode”. In this mode, the color of the teeth is registered by the ADA color indices from 1 to 29. The latter are identical to those of VBG coloration, which allows to correctly compare and compare the results obtained by both methods. One of the main advantages of spectrophotometric color determination is that it does not depend on the conditions of the room, as well as on the fatigue of the human eye („eye fatigue“).

However, it should be taken into account that spectrophotometry data, especially from CIE Lab, exceed the capabilities of the naked human eye to capture subtle differences in colour before and after bleaching. A spectrophotometer measures one wavelength at a time from the reflectivity or transmittance of an object, rather than using color filters that approximate the spectral function of the eye *(Kielbassa AM et al., 2009)*. In comparative studies, it has been found that the results of spectrophotometric color determination are more accurate than the results of visual color determination as there is a match in approximately 45-47% of cases *(Kielbassa AM et al., 2009).*

Although objective and repeatable, the obtained spectrophotometric assessment of color shade cannot always overcome the subjective opinion/perception of patient’s color shade. Therefore, VBG Shade Guide should remain the main method in the tootth bleaching clinical protocol (*Igiel C et al., 2017).* The ideal color determination is the combination of visual and digital methods under the right conditions (*Yilmaz B, 2020).*

Results and discussion on task 4

Based on the literature data, the measurement of the surface roughness of the teeth treated in experimental task No. 3 is necessary given the oxidation-reduction nature of the TB process, as well as in view of the established acidic pH of all bleaching agents applied in the task, which is associated with expected demineralization processes in HDT and subsequent increased enamel roughness.

A statistical check was also carried out for a correlation between the achieved bleaching effect and the roughness of the tooth surfaces.

In the course of the task, three measurements were made for each sample tooth in the gingivo-incisal direction along the axial middle third of the vestibular surface using the microroughness measuring device (Mitotoyo SJ). The measurement of the microroughness parameters was carried out twice after the formation of the experimental groups: before the bleaching procedure, as well as 24 hours. after its completion.

For the analysis of the results of the tables for each indicator, 2 graphs have been prepared: by the absolute average values of the indicator and by percentage differences.

Of the parameters studied, the most commonly used parameter to describe roughness is Ra in μm: the arithmetic mean of the roughness profile (Average roughness) *(Goel A et al., 2017; Gadelmawla ES et al., 2002).* Ra provides stable results because the reading of average roughness is not significantly affected by single irregularities, contamination or noise when measuring.

When comparing the size of changes in the mean values of Ra (Ra Average; Figure 29), as well as in the percentage differences in the Ra (Figure 30), it was found that in the individual groups after the implementation of the different BP the most significant changes were in group 5 (20% HP+CBL) – 22%, followed by group 3 (20%HP+FS) – 16%, group 2 (20% HP) -10% and group 4 (20%HP+HRP) – 5%. In the control group, the change is expected to be not significant (3%), but its presence indicates that both the environment and the storage time probably have a minimal impact on the values of Ra. For group 1 (35% HP), however, the change is negative. Of all the experimental groups, only for group 1 was applied a completely factory product – PO 35%. In the same group, the most pronounced brightening effect (ΔSGU) was also reported. In the groups with BP with additional activators, the increase in Ra most likely corresponded to the nature of the chemical mixing reaction described in Task 3.1 – a violent exothermic reaction with an uncontrolled release of multiple gas bubbles. The chemical reaction after applying the factory BP to the teeth of group 1 was also accompanied by release of gas bubbles, but their amount was significantly less and the reaction was not exothermic. Probably because of this, despite the established acidic pH of the BP, reduced values of Ra were estabisged. It can be concluded that with the single addition of activators to the BP, the violent exothermic reaction is associated with a risk not only for the vitality of the tooth pulp, but also for the tooth enamel. As an alternative, a precise reduction in the amount of the respective activators can be offered.

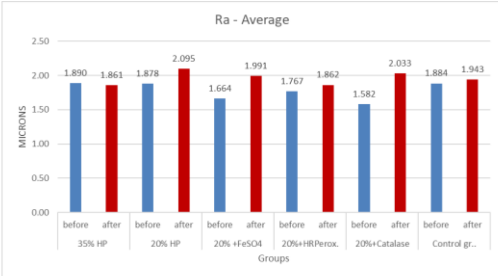


Figure 29

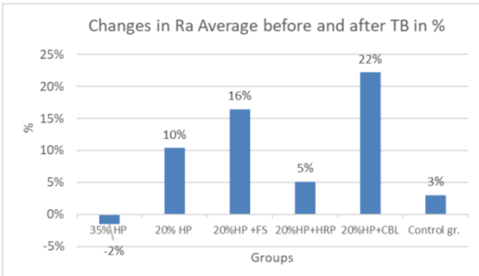


Figure 30

To establish whether or not there is a significant relationship between the Ra and ΔSGU indicators, a correlogram was prepared for each experimental group using the ΔSGUES values reported in task 3.1. In Task 3.1, the highest absolute ΔSGUES was reported in Group 1 and the lowest in Group 2. Reported by zone, the highest ΔSGUES was found in the incisal (VESG-I) zone in groups 1, 2, 3 and 5, and for group 4 - in the middle (VESG-M) zone. Although the cervical (VESG-C) zone has the highest spectrophotometric color indices before the application of bleaching agents, the ΔSGUES found in it are second only to those in the incisal zone for groups 1 and 3. For groups 2, 4 and 5, they take the last place. The higher the value of

ΔSGU, the greater the color shade difference between two objects, and accordingly, this difference is more noticeable to the human eye.

For all groups, the correlation coefficients between the variables Ra (dRa) and d_Color (ΔSGU) according to Kendall's and Spearman's, as well as the statistical significance coefficient Sig.2-tailed (Table 7 and |Table 8) were calculated. A positive correlation coefficient was found in group 1, but it was not statistically significant (Sig. (2-tailed) =0.878, Sig.2-tailed >α). In the other groups, negative correlation coefficients were found, and only in group 2 and group 5 they were statistically significant (Sig.2-tailed >α=0.05), which means that the surface roughness Ra decreases with increasing tooth brightness due to bleaching. In Group 2, Kendall's correlation coefficient was negative (-0.720) and statistically significant (Sig. (2-tailed) = 0.029, Sig.2-tailed <α), which means that as brightness increases after bleaching, roughness decreases.

However, on the correlograms prepared for each experimental group (Figure 31) there is not a pronounced linear dependence between the variables Ra (dRa) and ΔSGU (d_Color), there is even a scattering of the values. This is most likely due to the small sample size (n = 7) and it is recommended that the sample size to be increased in a future study. In their study Vieira-Junior WF et al. (2018) also did not establish any statistically significant relationship between Ra and the indicator for the total color change ΔSGU, but a deeper analysis of the cited team found that the change in Ra is associated with changes in specific indicators from CIELAB: in tooth brightness (lightness) and along the green-red axis of tooth color. (a* and b*).

Table 7 Correlation coefficients between the variables Ra and ΔSGU in groups 1 and 2

Correlations

			dRa_35%HP	d_Color_35% HP
Kendall's tau_b	dRa_35%HP	Correlation Coefficient	1,000	,050
		Sig. (2-tailed)	.	,878
		N	7	7
	d_Color_35%HP	Correlation Coefficient	,050	1,000
		Sig. (2-tailed)	,878	.
		N	7	7
Spearman's rho	dRa_35%HP	Correlation Coefficient	1,000	,164
		Sig. (2-tailed)	.	,726
		N	7	7
	d_Color_35%HP	Correlation Coefficient	,164	1,000
		Sig. (2-tailed)	,726	.
		N	7	7

Correlations

			dRa_20%HP	d_Color_20% HP
Kendall's tau_b	dRa_20%HP	Correlation Coefficient	1,000	-,720*
		Sig. (2-tailed)	.	,029
		N	7	7
	d_Color_20%HP	Correlation Coefficient	-,720*	1,000
		Sig. (2-tailed)	,029	.
		N	7	7
Spearman's rho	dRa_20%HP	Correlation Coefficient	1,000	-,863*
		Sig. (2-tailed)	.	,012
		N	7	7
	d_Color_20%HP	Correlation Coefficient	-,863*	1,000
		Sig. (2-tailed)	,012	.
		N	7	7

*. Correlation is significant at the 0.05 level (2-tailed).

Table 8 Correlation coefficients between the variables Ra and ΔSGU in groups 3,4 and 5

Correlations

			dRa_20% HP_FeSO4	d_Color_20% HP_FeSO4
Kendall's tau_b	dRa_20%HP_FeSO4	Correlation Coefficient	1,000	-,293
		Sig. (2-tailed)	.	,362
		N	7	7
	d_Color_20%HP_FeSO4	Correlation Coefficient	-,293	1,000
		Sig. (2-tailed)	,362	.
		N	7	7
Spearman's rho	dRa_20%HP_FeSO4	Correlation Coefficient	1,000	-,324
		Sig. (2-tailed)	.	,478
		N	7	7
	d_Color_20%HP_FeSO4	Correlation Coefficient	-,324	1,000
		Sig. (2-tailed)	,478	.
		N	7	7

Correlations

			dRa_20% HP_ horseradish_ peroxidase	d_Color_20% HP_ horseradish_ peroxidase
Kendall's tau_b	dRa_20%HP_ horseradish_peroxidase	Correlation Coefficient	1,000	-,250
		Sig. (2-tailed)	.	,442
		N	7	7
	d_Color_20%HP_ horseradish_peroxidase	Correlation Coefficient	-,250	1,000
		Sig. (2-tailed)	,442	.
		N	7	7
Spearman's rho	dRa_20%HP_ horseradish_peroxidase	Correlation Coefficient	1,000	-,364
		Sig. (2-tailed)	.	,423
		N	7	7
	d_Color_20%HP_ horseradish_peroxidase	Correlation Coefficient	-,364	1,000
		Sig. (2-tailed)	,423	.
		N	7	7

Correlations

			dRa_20% HP_catalase	d_Color_20% HP_catalase
Kendall's tau_b	dRa_20%HP_catalase	Correlation Coefficient	1,000	-,683*
		Sig. (2-tailed)	.	,033
		N	7	7
	d_Color_20% HP_catalase	Correlation Coefficient	-,683*	1,000
		Sig. (2-tailed)	,033	.
		N	7	7
Spearman's rho	dRa_20%HP_catalase	Correlation Coefficient	1,000	-,811*
		Sig. (2-tailed)	.	,027
		N	7	7
	d_Color_20% HP_catalase	Correlation Coefficient	-,811*	1,000
		Sig. (2-tailed)	,027	.
		N	7	7

*. Correlation is significant at the 0.05 level (2-tailed).

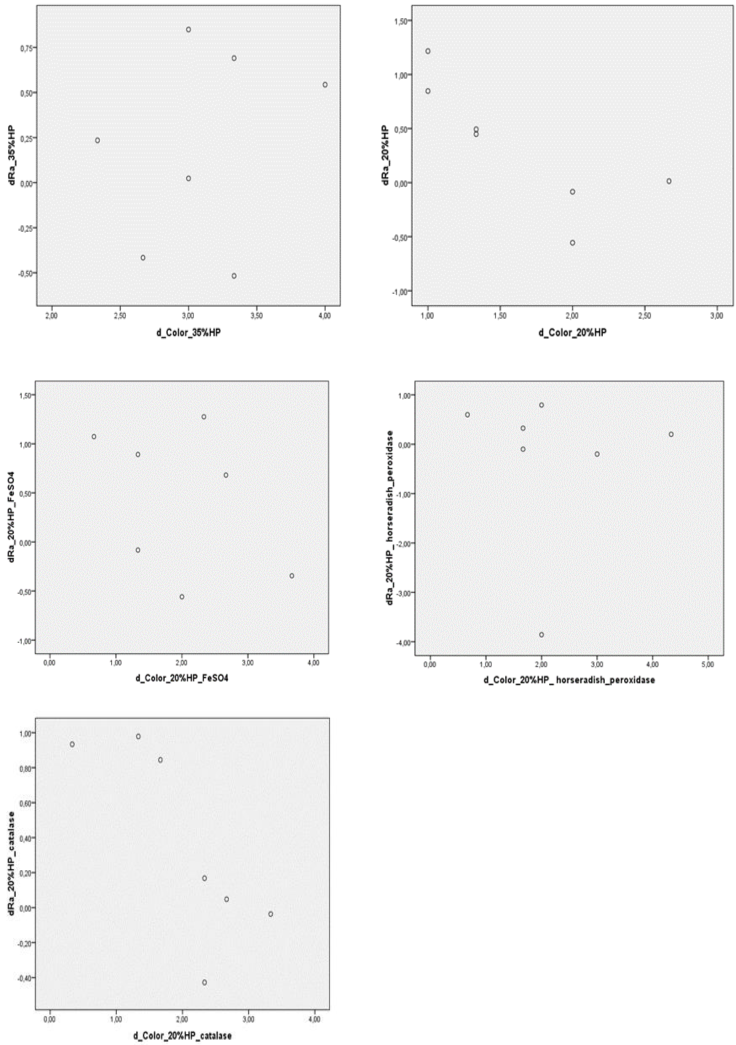


Figure 31 Correlograms by groups

Another indicator of surface roughness is Rz - the average roughness depth, which is formed from the arithmetic mean of the single maximum roughness depths (Rzi), i.e. the maximum height of the peak to the valley of the profile within one reading length, according to *ISO 21920-2:2021*.

In the studied sources from the scientific literature, Ra is the only parameter studied in most of them. Rz is a less frequently studied parameter, and for the other parameters (Rku, Rq, Rsk) there is no literature data on measurements carried out for the purposes of dental medicine.

When analyzing the Rz values reported in this study, the largest percentage increase was reported in group 3 (by 15%), group 1 (14%) and group 5 (14%), while in group 2 it was only 4%. In groups 4 and 6, a decrease in the average roughness depth Rz was reported (Figure 32 Figure 33).

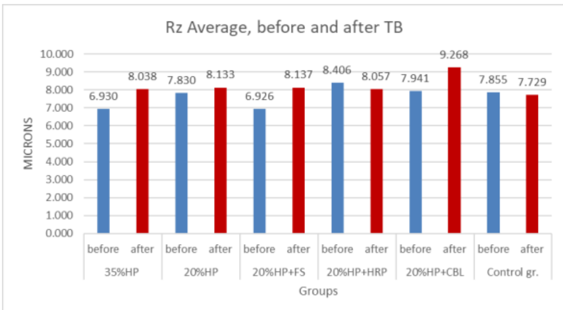


Figure 32

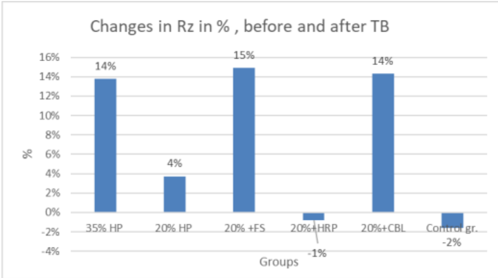


Figure 33

The value of Ra will always be less than or equal to half of Rz, since Ra is a measure of changes on both sides of the average, and not like Rz, from the peak to the valley. Typically, Ra values are much less than half of Rz *(Whitehouse D, 2002).* Rz is much more sensitive than Ra for detecting surface irregularities (peaks and valleys). This was also observed in the analysis of the results of measurements carried out in all groups of the present study, in which the average ratio between the values of Ra and Rz was about 1:3.5. In addition, it was found that in group 1, although the reported change in Ra was negative, i.e. the average roughness decreases after bleaching, then the change in Rz is significant (14%). This means that peaks and valleys are likely to be reported and present, and the enamel surface is not perfectly smooth. Therefore, it is necessary that the measurement of Ra is accompanied by a measurement of Rz, in order to make the surface characteristic more objective.

Rq is a parameter for the square mean. It is not particularly used in standard roughness measurements, but its values are associated with the optical properties of surfaces. When reporting the parameter Rq by groups (Figure 34) the highest percentage differences before and after IC were reported in Group 5 (20% HP+CBL) – 20%, Group 3 (20% HP+FS) – 13% and Group 2 (20% HP) – 8%. The percentage differences in group 1 (35% HP) and in the control group are low and the same – 2%, and in group 4 (20% HP+CBL) they are minimal, but with a negative sign: -1% (Figure 35).

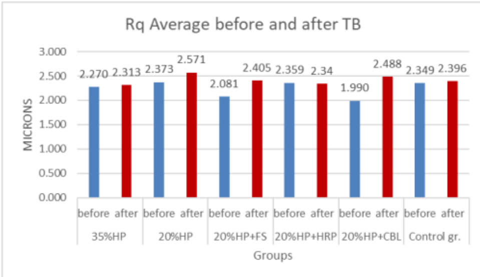


Figure 34

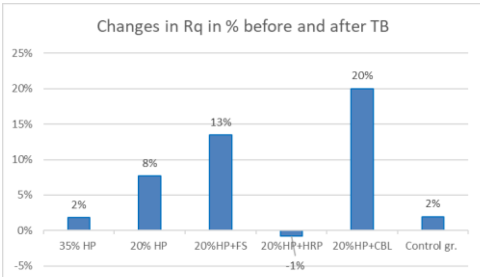


Figure 35

In the course of measuring the roughness indicators, the software associated with the device automatically generates a profile assessment for each sample examined (Figure 36), as well as BAC - curve and ADC - curve (Figure 37) . The main elements of the profile are "mountains", "peaks" and "valleys". BAC (bearing area curve): the curve shows the tooth-profile ratio in the evaluated profile length. ADC (amplitude distribution curve) is the amplitude distribution curve of the profile - the amplitude density, which is the percentage ratio of the sum of the horizontal lengths of the sections of the evaluated profile, which fall between the levels of 2 segments and the length of the evaluated profile. The ADC curve is constructed using the first level as the ordinate and the amplitude density for each segment as the abscissa *(Dikova C et al., 2022)*

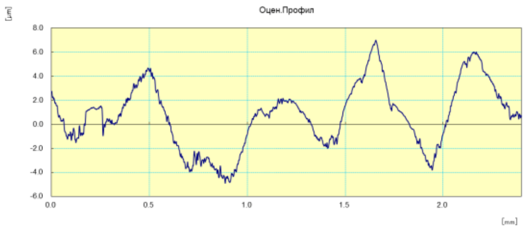


Figure 36 Profile evaluation

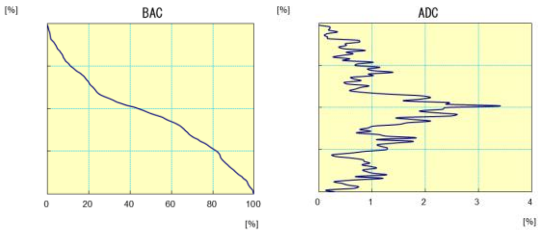


Figure 37 BAC and ADC profile curves

Rsk takes into account the degree of deviation in the upward or downward direction of the amplitude distribution curve (ADC), i.e. Rsk is an indicator of the asymmetry of this above-average and below-mean distribution. An ideal symmetric distribution has Rsk=0. A large positive distortion means thin peaks sticking upwards. A large negative distortion means narrow indentations on the surface. Rsk with values below and equal to 0 is associated with high abrasion resistance, and values above 0 – with low abrasion.

In terms of the tooth surface, the ideal values are as close to 0 as possible. In the study, the mean values of Rsk were close to 0, with the highest in group 4 (20% HP+HRP) before TB (0.818μm) (Figure 38). However, in the same group, the percentage difference in Rsk before and after TB was most pronounced (80%), followed by the percentage difference in group 1 (35%HP) - 49% and -36% for the control group, and in group 2 (20%HP), group 3 (20%HP+FS) and group 5 (20%HP+CBL) the percentage differences were approximately the same -15/-16% (Figure 39). In all groups, the Rsk values decreased significantly after bleaching and come even closer to 0, i.e. the distribution remained almost perfectly symmetrical at Rsk=0.

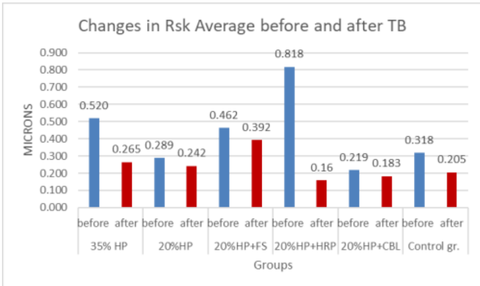


Figure 38

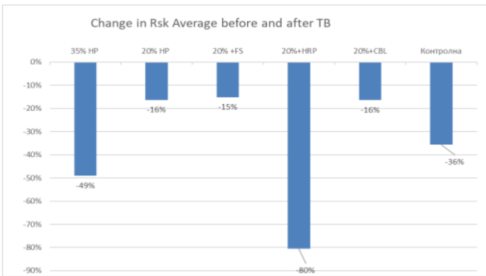


Figure 39

Rku is a parameter for the sharpness of the roughness profile and the excess of the distribution (curtosis). The interpretation of its values from literature data (*Gadelmawlaa ES et al., 2002)* is as follows:

Rku <3: The elevation distribution of the profile has relatively few peaks and valleys above the middle plain

Rku=3: The height distribution is normal (sharp parts and concave parts coexist)

Rku>3: The elevation distribution has relatively many peaks and valleys

The data from this study are graphically presented on the Figure 40 and Figure 41.

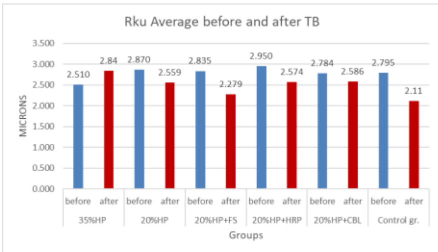


Figure 40

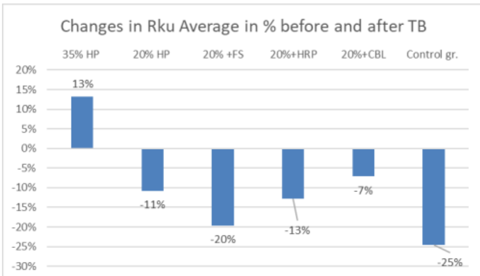


Figure 41

Despite the reported significant percentage differences (Figure 41), the Rku values in all groups were below 3 or close to 3, which means that the height distribution is normal or with single peaks and valleys.

CONCLUSIONS

I. About the surveys in task 1:

A) among DDMs

1. The most common clinical protocol for OVTB among DDM participants involves the administration of BP with a high concentration of HP in one visit with 2-3 applications of the bleaching agent with an average duration of 15 minutes.

2. Light activation of the BP is used by 80% of the participants, and according to a little more than half of them, it accelerates and increases the bleaching effect of the applied BP.

B) among patients

1. TB procedures were not associated with worsening of OHRQoL due to psychosocial discomfort or pain by more than 95% of participants.

2.The percentage of persons who have never visited DDM for bleaching is significantly high, but the proportion of those who would perform TB prescribed by a dentist is higher than it, which outlines the authority of the DDM and its credibility in the wider information of patients about the safety of TB methods.

II. About laboratory studies:

A) task 2

1. The differences between the actually reported concentration of HP in the studied BP and the expected concentration established by iodometric titration are within the acceptable limits compared to the ISO standard for BP for externalTB ISO 28399:2021.

2. By iodometric titration, a gradual decrease in the concentration of HP in the samples with BP without additional activation was detected over a period of 20 minutes. The application of physical factors (blue light - 450 nm and electric current) as BP activators leads to an initial increase in the HP concentration with a peak at the fifth minute, followed by a gradual decrease without reaching its initial values.

3. The achieved final bleaching effect on black tea extract after application of BP without or with blue light activation is equal, but occurs almost twice as fast after activation. Ionophoretic activation leads to a weaker, but smoothly occurring bleaching effect.

B) task 3

1. The concentration of HP in the BP has a fundamental role in achieving the best bleaching effect.

2. Horseradish peroxidase (HRP) increases the whitening effect to 20% HP to the greatest extent. Ferrous sulfate (FS) and catalase (CBL) have a similar effect on the bleaching effect of HP 20%, but weaker than that of horseradish peroxidase.

3. The addition of metal salts and oxido-reductase enzymes to the BP with 20% HP causes a rapid and violent exothermic reaction, which in clinical conditions would pose a risk to the surrounding soft tissues and pulp vitality.

4. Under the correct conditions for the execution of the visual method by VITA 3D Bleaching Guide and the spectrophotometric method by VITA EasyShade in Bleaching Mode no statistically significant difference was found between them in terms of the assessment of the bleaching effect.

C) task 4

1. The average surface roughness of the teeth (Ra) increases after bleaching. Only in the group in which an entirely factory product with 35% HP was used, Ra decreased after bleaching and the percentage difference was negative. The percentage differences in Ra after administration of the different BPs were most significant in the catalase activator group and the smallest in the horseradish peroxidase activator group.

2. A negative correlation was found between Ra and the achieved bleaching effect in the 20% HP group and the 20% HP group and catalase activator. However, the prepared correlograms lacked a pronounced linear relationship between the two variables.

3. For a more objective characterization of each surface examined, it is necessary that the measurement of the average surface roughness Ra is accompanied by a measurement of the average depth of roughness Rz.

4.In all groups, the Rsk values decrease significantly after bleaching and come even closer to 0, i.e. the distribution remains almost perfectly symmetrical at Rsk=0, and the Rku values in all groups are below 3 or close to 3, which corresponds to a normal distribution of profile height.

CONCLUSION

Tooth bleaching is a procedure that utilizes great popularity. The radiant smile is an important part of our vision, it is a symbol of health and youth. This procedure, although generally considered as cosmetic, can have a significant psychological effect on a person's self-esteem and social life by improving his appearance and increasing confidence.

Like any medical procedure, tooh bleaching has its benefits and risks that must be carefully considered. It is important to choose the right method to ensure a balance between efficiency and safety. The most important is to not damage the hard tooth tissues, pulp, periodontium. Knowledge of the awareness, actions, experience of patients and dentists could improve the qualities and control of this manipulation, as well as its predictability. Achieving a faster and more effective procedure is a desirable outcome for both patients and dentists, and the balance between the desire for aesthetics and health is a key to long-term success and satisfaction.

A healthy and beautiful smile is not only a matter of aesthetics, but also of overall health and well-being

SELF-ASSESSMENT OF CONTRIBUTIONS IN RELATION TO THE DISSERTATION

1. **Scientific and applied contributions**

1.1. With original character

1.1.1. An in-depth analysis has been carried out regarding the awareness, experience and various aspects of bleaching procedures among both DDM and patients.

1.1.2.The potential of the physical factors blue LED light and electric current to activate BP with HP is compared.

1.1.3.For the first time, the microroughness parameters Rq, Rsk and Rku, as well as the BAC and ADC curves of the surface of teeth subjected to in vitro bleaching were determined.

1.2. With confirmatory nature

1.2.1. The possibilities of iodometric titration as a chemical method for detecting changes in the concentration and rate of degradation of HP have been confirmed.

1.2.2. The possibilities of UV-VIS spectroscopy for comparison of the bleaching effect achieved by the dental BP on black tea extract have been confirmed.

1.2.3.The concentration of HP in the BP is confirmed to play a key role in achieving the best bleaching effect.

1.2.4. The administration of BP has been confirmed to result in changes in the mean surface roughness Ra of teeth whitened in vitro.

2. Applied contributions

2.1.Of original character: A comparison of the possibilities for estimating the bleaching effect of the visual method and of the spectrophotometric method using VITA EasyShade in Bleaching Mode was carried out.

2.1.2. With confirmatory nature: The established activation potential of the studied physical and chemical factors provides an opportunity for changes in the clinical protocols for OVTB.

PUBLICATIONS ON THE TOPIC OF THE DISSERTATION:

1. Stankova S. Changes in tooth enamel due to bleaching Varna Medical Forum. 2023;12(1):188-195.

2. Stankova S. Methods for evaluating the bleaching effect on vital teethVarna Medical Forum. 2023;12(2):189-194

3. Stankova S. Patients' awareness, attitudes, and experiences regarding tooth bleaching methods: a survey study. Varna Medical Forum. 2023;12(2):195-199.