

Chemical Interactions among Some Antimicrobial Solutions and Chelating Agents Used in Endodontics for Irrigation of Infected Root Canals

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The root canal irrigants are of utmost importance in the soft tissue dissolving, the removal of microorganisms, hard-tissue debris, and smear layer. This study sets forth to investigate the stability and synergic interactions of different systems with various combinations among antimicrobial substances and chelating agents, frequently used in endodontics for the irrigation of the infected root canals through electrochemical evaluation, physicochemical and spectrophotometric analysis. The pharmaceutical products indicated as the antiseptic solutions (sodium hypochlorite - NaOCl, chlorhexidine - CHX) and chelating agents (EDTA, citric acid - CA) have large pH variations on the entire pH domain. The physicochemical parameters' (pH, conductivity) assessment at the CHX and CA solutions shown good stability in time and also electrochemical behaviour (OCP, CV). There is a dissociation of ions in aqueous medium and a competition of the chemical balances among the structures, as an intense electrochemical activity between the functional groups through the intensification of the anodic current currents, and a shift of the potential registered. This allows the formulation that chemical interactions are not always possible among diverse types of antimicrobial solutions and chelating agents for endodontic use.

Keywords: root canal irrigation, antimicrobial solutions, chelating agents, electrochemical evaluation, spectrochemical analysis

The elimination of bacteria and their by-products from the infected root canals is one of the main goals in endodontic treatment, which is achieved by simultaneous mechanical enlargement and tissue debris flushing out with antimicrobial liquids. Along with the disruption of endodontic biofilms, the root canal irrigants have an additional beneficent role in soft tissue dissolution, and in hard-tissue debris and smear layer removal [1-4].

A single solution cannot entirely fulfil all these actions, hence the need for substance association [5-9]. However, in conjunction with the considerable improvement of cleaning the root canal harmful effects were also noticed. Accordingly, numerous studies have investigated the antagonistic interactions that emerge when the sodium hypochlorite (NaOCl), chlorhexidine (CHX), EDTA and citric acid (CA) are mixed while using in the root canal treatment [10].

NaOCl is used for cleaning and washing the root canals due its dissolving effect in the interaction of the necrotic or inflamed pulp tissue and removal of the organic component of the smear layer. NaOCl also penetrates into the accessory canals and the dentinal tubules that are inaccessible for endodontic instruments. It is recommended in the removal of the dental pulp remnants during the mechanical enlargement of the root canal. It cleans the canal and it removes the organic fraction of smear layer to exposing the dentinal tubules openings before the root canal filling. It has a low stability and it decomposes into hypochlorous acid, which is a very weak acid. The hypochlorous acid penetrates the cytoplasmic membrane, hence the germicide action. To attack proteins requires a suitable

concentration and a prolonged contact ensured through the irrigation of the lesion. Sodium hypochlorite mostly attacks the necrotic pulp tissue [11,12].

Chlorhexidine (CHX) is an antiseptic that facilitates the treatment of the infected root canal and it is active towards the gram-positive germs (especially) and gram-negative; mycobacteria and spores are resistant [9,11,13]. It is recommended for the flushing of the root canals in persistent chronic apical periodontitis because it facilitates the biofilm removal and prevents its recurrence. It is efficient in the case of infection with *Enterococcus faecalis* and *Candida sp.*, which are high resistant to usual endodontic antiseptics such as NaOCl and calcium hydroxide. It is also recommended to be used in cases of endodontic retreatments.

Ethylendiaminetetraacetic acid (EDTA) is an irrigating agent used for the removal of smear layer and cleaning the root canal before the final obturation. EDTA forms a complex soluble in water, a chelate which decalcifies substances and dissolves the smear layers, increasing, thus, the adherence of the filling material. EDTA is also useful in the mechanical enlargement of calcified root canals. After the EDTA irrigation the root canal must be thoroughly rinsed to avoiding a further demineralization of root dentine and surrounding apical bone [11].

Currently calcium hydroxide ($\text{Ca}(\text{OH})_2$) is the most accepted endodontic dressing in two-visit treatment option due to its remarkable antimicrobial effect against the relevant pathogens harboured in infected root canals and its established biocompatibility as well [14-16].

Calcium hydroxide ($\text{Ca}(\text{OH})_2$) is a strong base with high pH (12.5 - 12.8) that develops antiseptic activity into the

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infected root canals against planktonic micro-organisms by ionic dissociation of Ca^{2+} and OH^- . Unfortunately, its efficacy against endodontic biofilms is still questioned [17]. However, calcium hydroxide confirmed to be an efficient local medication of the root canals usual endodontic practice because it is dedicated for inactivation of bacterial endotoxins and drying of the root canals with secretions [11,18].

The use of EDTA or CA as chelating agents is necessary in endodontic treatment to remove and prevent the forming of smear layer associated with the canal instrumentation. The antagonistic interactions include the loss of free available chlorine for NaOCl, when it contacts other chelating agents, which, as a result, would reduce the capacity of tissue dissolving and, to a lesser extent, the antimicrobial activity. When CHX and NaOCl are in a blend, a precipitate is formed, that can present less desired effects for the endodontic treatment, including tooth coloration and levigating risk with potential of unidentified chemical substances in the periradicular tissues [10].

The aim of this study was to investigate the possible interdependency processes and to emphasise the mutual, synergistic or antagonistic interactions that may happen between some antiseptic substances and chelating agents frequently used in root canal irrigation. The possible chemical interactions among some antiseptic substances and chelating agents with various combinations were evaluated through physicochemical parameters and spectrophotometric analysis (UV-Vis). It was also performed the evaluation of the electrochemical behaviour to highlight the electro-oxidative properties by cyclic voltammetry (CV) and open circuit potential (OCP), an approach which is only a preliminary exploration.

Experimental part

There were used the following pharmaceutical products used in endodontic practice: chloraxid (5.25 % NaOCl), gluco-chex (2 % chlorhexidine), calasept (17 % EDTA), calcium hydroxide plus (42 % $\text{Ca}(\text{OH})_2$ cones) and citric acid (CA). All solutions e.g. CA of 10 %, 15 %, 20 %) which were prepared in the laboratory following the recommendations of RFX [19]. Also, different combinations from all used reagents were performed.

The electrochemical measurements (pH, conductivity) were made with the Consort C862 equipped with two electrodes: mixt electrode (glass electrode for pH) and a special electrode (platinum electrodes for conductivity). Before measurement, the pH meter was calibrated with buffer solution of known pH (pH 4.0, 7.0 and 10.0) and the conductometric measurements using solution of KCl 1 n, 10^{-1} n and 10^{-2} n.

The electrochemical evaluation by open circuit potential (OCP) and cyclic voltammetry (CV) measurements were performed into classical electrochemical cell of 20 mL consisting of three electrodes: platinum electrode with a 1.6 mm^2 (as working electrode- WE), saturated calomel electrode (SCE, $E_{\text{SCE}} = + 0.242 \text{ V}$ vs ENH) as reference electrode (RE) and a platinum counter electrode (CE). The WE surface was periodically prepared before measurements through repeated washing and cleaning of, before and after each utilisation, with abrasive paper of numerous sizes (800 – 1000 μm). There was used Bio-Logic SP 150 Potentiostat Galvanostat equipment. The applied potential was from -1V to 1V vs SCE. CV measurements were performed at various scanning speeds ($100 \text{ mV}\cdot\text{s}^{-1}$, $50 \text{ mV}\cdot\text{s}^{-1}$, $20 \text{ mV}\cdot\text{s}^{-1}$).

The spectrophotometric analysis was performed with the T90+ UV-Vis spectrophotometer with double fascicle

using quartz optical prisms with 5 mL capacities, in the UV-Vis wavelength range from 190 to 900 nm.

Several types of solutions were made using blends of antiseptic substances (NaOCl, CHX), chelating agents (EDTA, CA) and $\text{Ca}(\text{OH})_2$. The volume was set at 100 mL, which was considered sufficient for all measurements (physicochemical, electrochemical and spectrochemical).

Type A solutions were prepared with 17 % EDTA (constant volume) and NaOCl in different concentrations (2.62 % - A1; 3.5 % - A2; 4.5 % -A3 and 5.25 % - A4) in 1:1 (v/v) ratio.

Type B solutions were prepared with 1 % NaOCl and 17 % EDTA in various ratios (v/v): 1:1 - B1; 1:5- B2; 5:1- B3.

Type C solutions were prepared with 1 % NaOCl, 10 % CA and 17% EDTA in 1:2:1 (v/v/v) ratio.

Type D solutions were prepared with 1 % CHX (D1) and CA at different concentrations: 10 % - D2; 15 % - D3; 20 % - D4, in 1:1 (v/v) ratio.

Results and discussions

In the experiment, there were used antiseptic solutions (NaOCl, CHX) and chelating agents (EDTA, CA and $\text{Ca}(\text{OH})_2$) in different combinations and the results were evaluated through physicochemical parameters (pH and conductivity) and it was performed the electrochemical evaluation of the capacity of the systems (open circuit potential-OCP; cyclic voltammetry -CV) and spectrophotochemical analysis (UV-Vis).

Physicochemical evaluation

In the case of the type A solutions with 17 % EDTA (constant volume) and NaOCl in various concentrations (from 2.62 to 5.25 %), there are no significant pH variations (from 8.57 to 8.34). The solutions of citric acid (CA), recommended in different concentrations, indicate a highly acidic pH, around of 1.5, which in concentrations between 10 – 20% remains constant.

By adding 10 % citric acid (CA), in the type A solutions with at least two pH units, with variations of the concentration of the hydrogen ions from solution to solution was observed (fig. 1). It is possible that the chemical balances established among the three compounds structures not to be stable throughout the pH measurements; there are intense acid-base exchanges among the functional groups. It is observed that for different concentrations of NaOCl the pH remains slightly acid for a lower CA added volume and increases with approximately one unit when the CA added volume is also increased. However, if in systems with NaOCl (higher than 4 %) and 10 % CA when 17 % EDTA (chelating agent) was added, the solution's pH remains slightly basic and a weak increase of 0.5 pH units are registered.

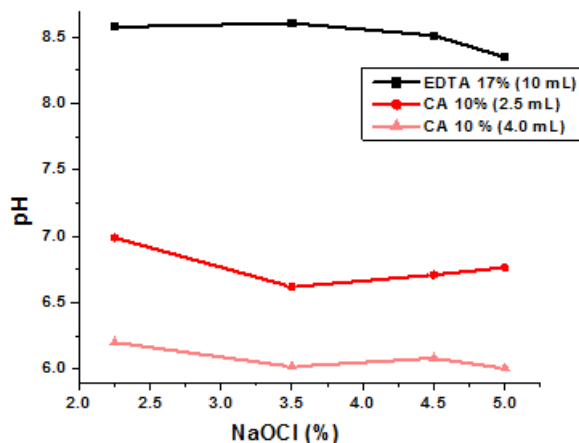


Fig. 1. pH's evolution in solutions with 17 % EDTA and 10 % CA at the addition of various concentrations of NaOCl

For the type B solutions, prepared from 1 % NaOCl and 17 % EDTA, in various ratios, the pH of the solutions registers a slightly alkaline pH around 9 (fig. 2) as opposed to the pH of the initial solution of 5.25 % NaOCl (pH of 11.5). The lowest pH value of 8.35 is registered for 1:1 (v/v) ratio and an increasing of the pH value of the blend (pH of 8.70) is registered when the ratio of NaOCl is ten times lower.

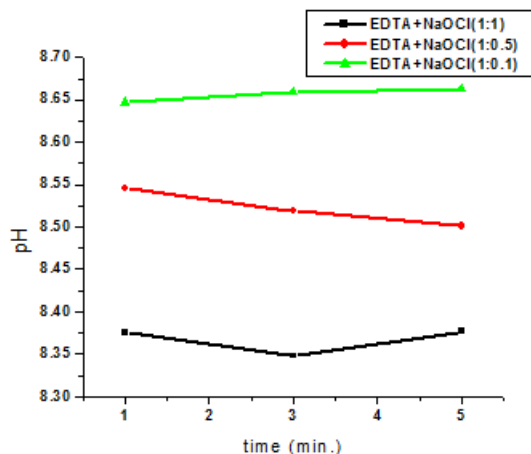


Fig. 2. pH's evolution in the blends prepared with 1 % NaOCl and 17 % EDTA in various ratios

In the case of the type C solution, the blend of 1 % NaOCl and 10 % CA, the pH's value is 2.26 in comparison with the individual solutions of 1 % NaOCl (pH of 11.74) and 10 % CA (pH of 1.43), respectively, changes significantly when 17 % EDTA is added (pH of 6.90). Thus, it can be concluded that the competitive balances among the antiseptic solutions are complex, with significant changes in the presence of two chelating agents, precisely, because of the competitive balances.

The results obtained make possible the assertion that the tested pharmaceutical products, indicated as antiseptic solutions (NaOCl, CHX) and chelating agents (EDTA, CA), have increased variations of the pH across the entire pH domain.

While the individual solutions of 5.25 % NaOCl indicate a highly alkaline pH (of 11.5), the CA solutions of different concentrations have a highly acid pH (around 1.50), the 17 % EDTA having a slightly alkaline pH (of 9.02). In the tested systems, with EDTA being considered a pH leveller in various blending ratios, there are registered pH modifications with up to three pH units in the more acidic range.

The individual solution of 1 % CHX indicates a pH of 4.28 and by the addition of a chelating agent CA (10 - 20 %) the pH drops somewhere around of 1.50 - 1.60. If the initial solution of 1 % CHX has registered a weak conductivity of the $\mu\text{S}/\text{cm}$ order, when CA is added it reaches the mS/cm order, which is the effect of an active dissociation. In terms of the stability evolution registered for a certain time, the pH values and conductivity parameters for the type D solutions, after four days, it is observed a time constancy of the registered values which confirms their stability. A slight decrease in pH and conductivity parameters is observed at the addition of $\text{Ca}(\text{OH})_2$ solution.

By applying, in time, the lavage on the root canal after the 5.25 % NaOCl treatment, the pH suffers modifications (fig. 3). If initially the pH is > 10.5 and maintains unchanged, after three minutes, there can be noticed a significant decrease of pH. For the irrigation with distilled water or physiological serum the pH drops with almost one unit, behaviour comparable to when a 50 % alcohol solution is used for the irrigation. But the dropping of the pH is more

obvious, approximately 3.5 pH units, four minutes after when a 70 % alcohol solution is used in procedure.

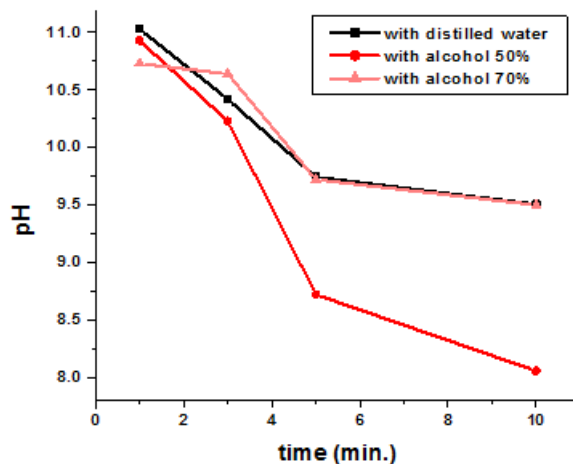


Fig. 3. pH's evolution in the lavage procedure after the utilisation of NaOCl solutions

The citric acid (CA) chelating agent and the tested antiseptic solutions in various combinations were subjected to conductometric measurements also. For CA, it is noticed a constancy of the conductivity in range of mS cm^{-1} and an increase of salinity with concentrations. The conductivity of the type A solutions, 1:1 (v/v) blend of 17 % EDTA and NaOCl in different concentrations are in mS cm^{-1} range (from 78.3 to 103.3) and for higher the concentration of NaOCl, the higher of the values is obtained. This can be explained by the fact that there are more dissociated ions generated in solutions than in the case of the blend which also contains 10 % CA. When 10 % CA is added, the conductivity drops with 3 - 6 mS. The lowest value of conductivity was registered for the type A1 solution with lower NaOCl concentration (2.52 %) both when adding 2 mL 10 % CA (75.3 mS cm^{-1}) and 4 mL 10 % CA (72.1 mS cm^{-1}).

For the systems formed from NaOCl and EDTA in a variable ratio (type B solutions), the conductivity decreases significantly to values around 60 mS cm^{-1} by comparison with the sterile ultrapure water (fig. 4). The lowest value is registered for the type B2 solution (48.20 mS cm^{-1}), where the ratio NaOCl : EDTA is 1:5.

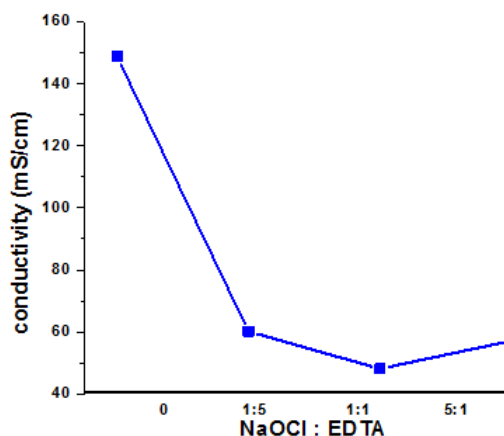


Fig. 4. The values of conductivity of the NaOCl and EDTA solutions in different ratio

When in the NaOCl solution in various concentrations are added the two chelating agents (17 % EDTA and 10 % CA, respectively), an increase of conductivity is registered for higher NaOCl concentrations, both in the EDTA and CA systems (fig. 5).

These results indicate a dissociation of the ions in the tested conditions and a competitiveness of the chemical

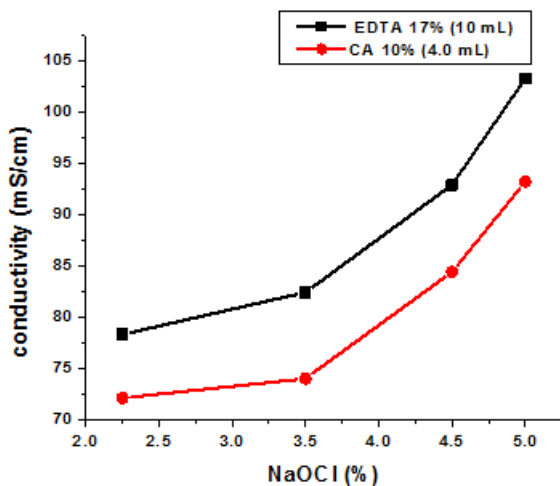


Fig. 5. Conductivity of the NaOCl solutions in various concentrations with 17 % EDTA and 10 % CA, respectively

balances among the components structures in the aqueous medium.

Electrochemical evaluation

To establish if the pharmaceutical products display electrochemical interactions when administered together, to test the electro-oxidative properties through cyclic voltammetry (CV) and open circuit potential (OCP), an approach which is only a preliminary exploration in this way was performed.

There were registered OCP values in two cases, which highlights an electrochemical activity between the component substances: the blend formed from the CHX antiseptic and CA chelating agents (fig. 6.a) and the blend formed from the CHX antiseptic and the CA chelating agent

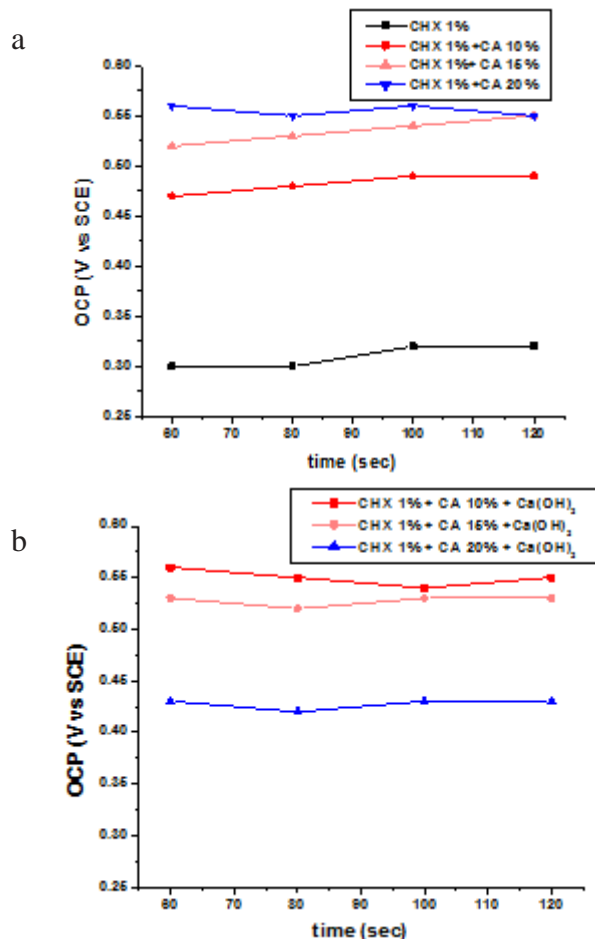


Fig. 6. The OCP's evolution of the system with CHX and CA (a) and respectively with CHX, CA and $\text{Ca}(\text{OH})_2$ addition (b)

to which $\text{Ca}(\text{OH})_2$ was added (fig. 6.b). The measurements were performed for reading times between 60 and 120 s, as an optimal time for chemical action in endodontic treatments [20].

The lowest OCP value is registered for 1 % CHX in individual solutions and the electrochemical activity intensifies when CA is added. The value doubles when 20 % CA is added and there are no significant changes when $\text{Ca}(\text{OH})_2$ is also added; however, in the case of a higher concentration a slight decrease of the values is possible, via the potential forming of some complexes of coordination with CA (fig. 6.a).

For the analysed antiseptics and the combinations among them, cyclical voltammograms were obtained only for one system, namely the one formed from CHX and CA (type D solutions) as it can be observed in Figure 7. If the NaOCl and EDTA, as well as NaOCl and CA systems did not indicate OCP values, the system formed from CHX and CA indicated an evolution of the electrochemical parameters and the registering of a current at the sweeping of the potential applied between the -1 V to 1V/vs SCE. CV measurements were performed at various scanning speeds ($100 \text{ mV}\cdot\text{s}^{-1}$, $50 \text{ mV}\cdot\text{s}^{-1}$, $20 \text{ mV}\cdot\text{s}^{-1}$), but the most representative voltammograms were noticed for the $100 \text{ mV}\cdot\text{s}^{-1}$ scanning speed, the electronic changes being more rapid and useful to this study (table 1).

In the domain of the negative potential around the -400 mV vs SCE, there is an anodic peak, which indicates a slight moving of the chemical potential registered at the changing of the scanning speed.

There is a reversible process owed to the protonation / deprotonation of the functional groups which contain hydrogen. When the scanning speed is changed for the

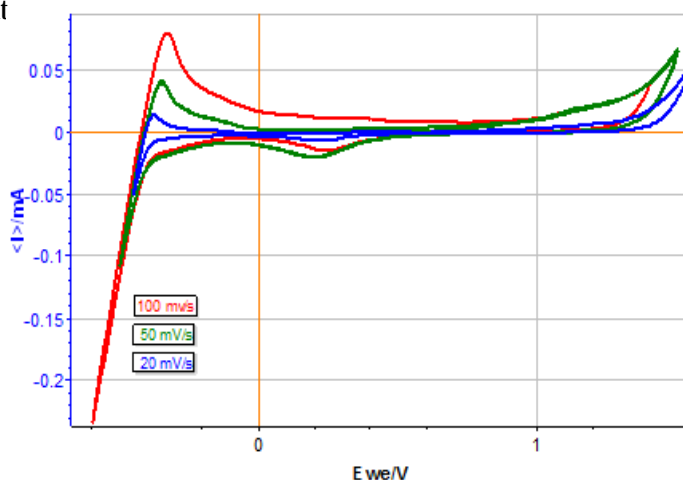


Fig. 7. The cyclic voltammograms for the system formed from CHX and CA

Table 1
ELECTROCHEMICAL PARAMETERS FOR THE SYSTEM FORMED WITH CHX AND CA

Scan rate ($\text{mV}\cdot\text{s}^{-1}$)	E_{p1} (mV)	E_{p2} (mV)	$\Delta E_p/56.5$ (mV)	I_p (μA)
100	-378	298	1.41	78.528
50	-402	320	1.45	43.809
20	-420	344	1.34	18.431

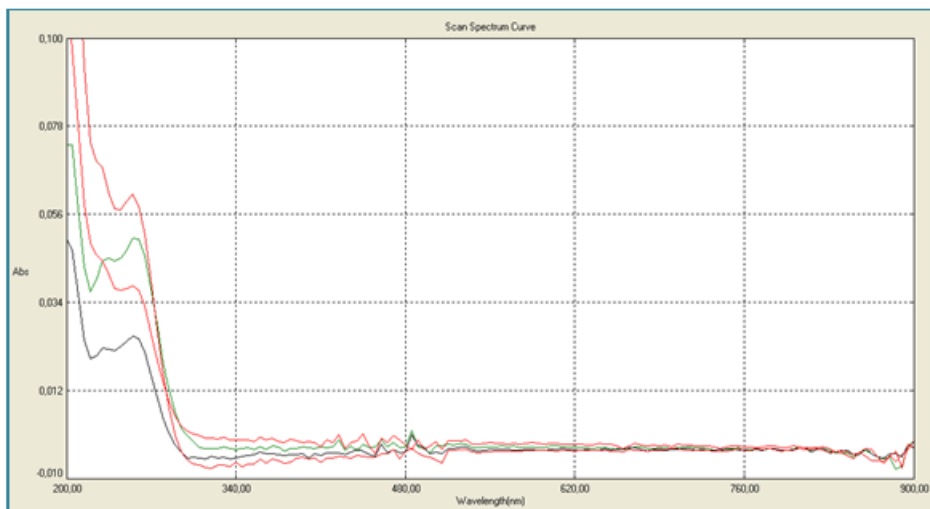


Fig. 8. UV-Vis spectra registered in solutions of 1 % CHX (orange), with various concentrations of CA (20 % CA - red, 15 % CA - green, 10 % CA - black)

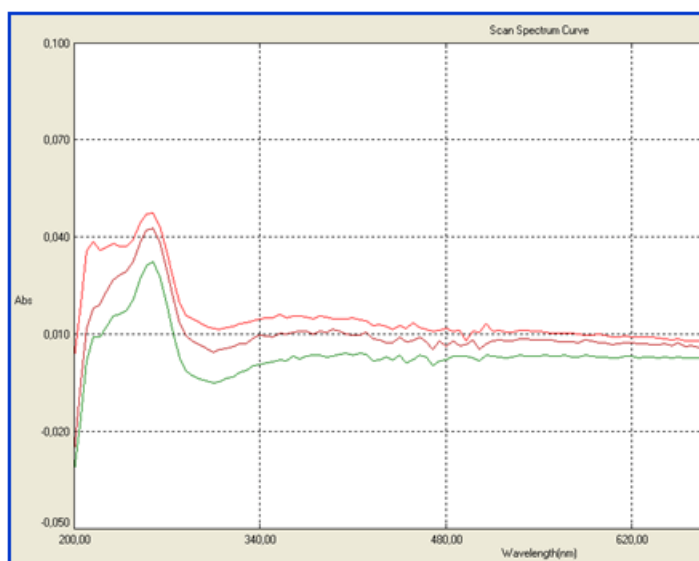


Fig. 9. UV-Vis spectra registered in solutions of 1 % CHX, Ca(OH)₂ with various concentrations of CA (20 % CA - orange, 15 % CA - red, 10 % CA - green)

cyclic voltammograms performed for the blends of CHX and CA, there is an intensification of the currents of anodic current but also a movement of the potential, which shows an intense electrochemical activity between the functional groups and a reversible process through the electronic change on the anodic branch. Table 1 shows the electrochemical parameters. The potential differences indicate a change of approximately 1.5 electrons in the studied systems, considering that 56.5 mV are required for the transfer of an electron between the functional groups [21-23].

Spectrofotocemical analysis

The UV-Vis measurements were performed for all solutions combinations but conclusive spectres were obtained for two solutions, which indicate the absence of optic activity in the other solutions. There were analysed solutions containing 1 % CHX with CA (10 - 20 %) through repeated dilutions (fig. 8) in ultraviolet and visible range. The UV spectra registered indicate an absorbance at 270 nm, which it intensifies with the increased added CA concentration. Thus, an intensification of the optical activity of the compounds formed from the dissociation of the antiseptic and of the chelating agent could be observed.

For the same system containing CHX (1%), various concentrations of CA to which Ca(OH)₂ was added UV-Vis spectra were registered also (fig. 9). The maximum of absorption is observed at the same wavelength which indicates that the presence of some compounds with

optical activity in solutions and dependence on the CA concentration.

Conclusions

There were investigated the chemical interactions between the usually antiseptic substances and chelating agents used in endodontic treatment such as: NaOCl, CHX, Ca(OH)₂, EDTA and CA. The pH changes only when the EDTA : NaOCl ratio is changed; there is a pH drop, each substance influences the system and the action period is always different. When CA is added into the NaOCl and EDTA system, the pH drops with approximately 1-2 units. The significant reduction of pH takes place and when the CHX system is associated with CA, and an increase in conductivity is noticed. At the same time, there is registered a drop in the UV range, which indicates a lower optical activity, which is dependent on the concentration of the CA solution. There are electrochemical interactions between the antiseptic solution CHX (1%), associated with the chelating agent CA, but also with Ca(OH)₂.

It can be stated that chemical interactions cannot always be made among antiseptic solutions for dental use, the blends being mostly electroactive, with chemical potential and with changed action, because of the possible antagonistic mutual interactions among them.

The mutual chemical interactions, resulting from the association of some antiseptic solutions (NaOCl, CHX) and chelating agents (EDTA, CA) require thorough investigations, even when they are used as irrigants of the root canal in endodontic treatments, as the competitive chemical processes are very complex.

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