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Examination of Effect of Electrochemically Activated Water Solutions on Candida Albicans after Different Periods of Storage

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Abstract

Studies to determine the sensitivity of *Candida albicans* to anolyte and catholyte of 0.5% NaCl and a combination of 0.5% NaCl and 0.5% Na_2CO_3 were performed. Both anolytes killed the tested strains in suspensions (10^6 cells/ml) within 5 min. The catholytes showed low antimicrobial activity. The inhibitory properties of the tested anolytes were fully preserved over a period of 2 months when stored in the dark at room temperature, but of the catholytes - for no more than 2 weeks. These results show that anolytes are promising antimicrobial agents with very high activity against *C. albicans*.

Keywords: Anolyte, Catholyte, Virkon^s, Candida Albicans, Antimicrobial Activity

Introduction

In the medical and veterinary practice, as well as in microbiological laboratories, regular and safe surfaces disinfection is required to reduce the number of microorganisms and prevent contaminations and infections (Zhelev et al., 2017). Conventional methods in this regard use aggressive and toxic chemicals, are not effective in the long run and can hardly be standardized (Kühn et al., 2003). One of the most resistant to the disinfection microorganism is *Candida albicans*. In order to search for effective antiseptics and disinfectants, different chemical products have been tested against it. Some of them are effective, such as the Sterilox® disinfectant (Shetty et al., 1999), 0.2%, 1% and 2% chlorhexidine gluconate, 5.25% sodium hypochlorite (NaOCl) (Vianna et al., 2004) and others. Effective antiseptics against *C. albicans* are also few. For this purpose, some essential oils have been successfully tested, such as that from *Ocimum gratissimum* (Nakamura et al., 2004) and from *S. officinalis* L. (Sookto et al., 2013). The leaf extracts of some South African plants such as *C. dentata*, *T. sambesiaca* and *T. phanerophlebia* also show significant inhibitory activity against *C. albicans* (Shai et al., 2008). Other tested agents did not show the expected effect, such as 0.05% sodium hypochlorite (Barnabé et al., 2004).

Electrochemically activated aqueous solutions (EAAS) are today known as safe and perspective agents with application capabilities as antiseptics and disinfectants. The electrochemical activation process consists in the electrolysis of slightly concentrated aqueous solutions of inorganic salts. EAAS are divided into two groups: anolytes and catholytes. Anolytes have strong oxidizing properties with a pH in the range of 2.5 to 8.5 and an oxidation-reduction potential (ORP) of +600 to +1200 mV. They contain hypochlorous acid, which is a strong bactericide. The catholyte solutions are antioxidants having a pH in the range of 10.5 to 12.0 and an ORP of -600 to -900 mV (Fukuzaki, 2006; Tasheva et al., 2010). The development of technologies for the production of EAAS began in the 1970s by a team of the Tashkent Research Institute under the leadership of Prof. Alekhin (Miroshnikov, 2002). Their broad-spectrum biological activity has been established and a number of studies have been carried out for their application in various fields of human activity, including as antimicrobial agents. Gurgulova et al. (2010, 2011), Popova et al. (2016a, 2016c) and others found significant bactericidal activity of such solutions against Gram-negative and Gram-positive microorganisms, including spore-forming bacteria. However, the data regarding their fungicidal action are scarce. In a 2010 study, Tasheva et al. for the first time in Bulgaria reported the antimycotic action of anolytes on two *C. albicans* field strains. The authors

found this effect only in anolytes obtained by electrochemical activation of aqueous solutions of sodium carbonate and sodium chloride at concentrations of 0.14 and 0.18% but not of a solution of 0.18% sodium acetate.

Electrochemical activation has been developed as a rapid and effective method of producing hypochlorite and, according to some studies EAAS are more effective than conventional disinfectant solutions. Many application options have been proposed, including hospital disinfection, waste water treatment, routine disinfection of drinking water and biological decontamination. Helme et al. (2010) recommend anolyte of 0.5% NaCl as a highly effective but non-corrosive disinfectant that has countless scientific, medical, military and public health applications The activation of water by physical means stimulates a new scientific approach in microbiology, especially in antimicrobial methods. Heredia-Rojas et al. (2012) presented evidence of biological effect of electrically activated water samples with the antifungal Amphotericin B.

Since the EAASs are obtained by electrolysis of aqueous solutions of various salts and with different baseline concentrations (primarily 0.1-0.5%), whereby the efficacy is different, the aim of the present study was to investigate the effect on *Candida albicans* of anolytes and catholytes derived from aqueous solutions of NaCl and Na_2CO_3 at a concentration of 0.5%.

Materials and Methods

Anolytes: • Anolyte 1 neutral, prepared with a combination of 0.5% NaCl and 0.5% Na_2CO_3. • Anolyte 2, prepared with 0.5% NaCl.

Katholytes: • 1 neutral, prepared with a combination of 0.5% NaCl and 0.5% Na_2CO_3. • Katholyte 2, prepared with 0.5% NaCl.

The physical performances pH, oxidation-reduction potential (ORP) and temperature of the investigated EGAR were determined using the Manual multi-parameter analyser Consort C1010 (Consort bvba, Belgium) for pH, mV and temperature measurement.

Control. Virkon^S, applied at a final concentration of 0.5%, was used.

Microorganisms. A pure culture of Candida albicans ATCC 10231 (NBIMCC 74), obtained from the Bulgarian National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC) was used in the study, as well as a clinical strain of C. albicans 4 isolated from a parrot throat with signs of pharyngitis.

Nutrient media. Colorex Chromogenic Orientation Candida arap (HiMeida Laboratories Pvt. Ltd. Mumbai India) was used.

Experimentalsettings.

● Antimicrobial activity study of anolytes and catholytes. To 9 ml of each of the anolytes and catholytes tested, a C. albicans suspension of 10^7 cells/ml in a 1 ml volume was added, resulting in a final concentration of 10^6 cells/ml. The following controls were applied - sterile distilled water (without anolyte or catholyte) with the same content of each of the strains tested, as well as 100% anolyte or catholyte without microorganisms.

● Examination of the antimicrobial activity of **Virkon^S**, used as a control to compare the effect of anolytes and catholytes. One ml C. albicans suspension at a concentration of 10^7 cells/ml was added to 9 ml of a 0.5% solution of Virkon^S in sterile distilled water, resulting in a final concentration of 10^6 cells/ml. The following controls were set - sterile distilled water (without Virkon^S) with the same content of each of the strains tested, and a 0.5% solution of Virkon^S in sterile distilled water without microorganisms. After homogenization at 500 turnovers per 1 min on a Vortex apparatus (Heidolph - Labimex, Bulgaria) and various time intervals for the EAAS and Virkon^S action (5 min, 10 min, 15 min, 20 min, 30 min, 60 min, 90 min and 120 min) cultures were

made from each of the samples on Colorex Chromogenic Orientation Candida agar which were cultured at 37° C for 24-96 hours under aerobic conditions. The growth of the microorganisms tested treated with the examined solutions as well as of the controls was taken into account after the cultivation. The most massive growth was scored by four characters plus (++++), and the negative finding (100% inactivation) - with the minus sign (-).

Results

The physical indicators pH, ORP and temperature of the investigated EAASs are presented in Table 1.

Table 1. Physical indicators of the studied anolytes and catholytes

Starting composition		**pH**	**ORP, mV**	**t° C**
Aqueous solution of sodium chloride (0.5%) and sodium carbonate (0.5%)	Anolyte 1 - first day	6,63	870	19,8
	after 1 week	6,59	23	18,6
	after 2 weeks	6,81	12	20,5
	after 4 weeks	7,03	0	19,7
	after 8 weeks	7,16	-9	21,8
	Katholyte 1 - first day	11,58	-852 mV	19,5 ^{0}C
	after 1 week	11,47	-261	18,4 ^{0}C
	after 2 weeks	11,59	-275	20,2
	after 4 weeks	11,59	-271	19,3
	after 8 weeks	11,58	-273	21,5
Aqueous sodium chloride solution 0.5%	Anolyte 2 - first day	2,76	1200 mV	19,7 ^{0}C
	after 1 week	2,74	250,3	18,7 ^{0}C
	after 2 weeks	2,80	247	20,6
	after 4 weeks	2,81	246	19,8
	after 8 weeks	2,81	249	21,6
	Katholyte 2 - first day	11,59	- 960 mV	18,6
	after 1 week	11,59	-276,7	18,5
	after 2 weeks	11,64	-273	20,7
	after 4 weeks	11,64	-273	19,7
	after 8 weeks	11,64	-270	21,8

ORP - oxidation-reduction potential

The data in the table show that in the first week after the preparation of the EAASs, their ORP decreased significantly, but this change was negligible over the next 7 weeks. The pH changes of the tested solutions over the 2 month period were also very low.

The results of testing the antimicrobial activity of freshly prepared EAAS on *C. albicans* ATCC 74 are summarized in Table 2.

Table 2. Growth of *C. albicans* ATCC 74 on Candida Chrome agar after different treatment intervals of freshly prepared EAASs

Sample No	Type of activated solution	Growth on Candida Chrome agar							
		Time of impact - min							
		5	10	15	20	30	60	90	120
1	anolyte of Na_2CO_3	-	-	-	-	-	-	-	-
2	anolyte of NaCl	-	-	-	-	-	-	-	-
3	catholyte of Na_2CO_3	++++	+++ +	+++ +	+++	+++	++	+	-
4	catholyte of NaCl	++++	+++ +	+++ +	++	+	-	-	-
5	0.5% Virkon^S	-	-	-	-	-	-	-	-
6	untreated control	++++	+++ +	+++ +	+++ +	+++ +	+++ +	+++ +	++++

Legend: – = absent of growth (100 % inactivation); + = presence of growth

The data in the table show that *C. albicans* ATCC exhibits high sensitivity to the two tested anolytes and died under their influence for a 5 min interval. The susceptibility of the strain was the same and to the Virkon^S control disinfectant, but not to the two catholytes. The catholyte with Na_2C0_3 inactivated *C. albicans* ATCC after more than 90 min and that with NaCl for over 30 min.

The tested clinical strain of *C. albicans* 4 exhibited similar sensitivity to freshly prepared anolytes and catholytes. It was killed by each of both anolytes for a 5-min interval, as well as when exposed to Virkon^S.

Its sensitivity to the catholytes turns out to be weak - the catholyte with Na_2CO_3 inactivated it in more than 90 min, and that of NaCl - for over 60 min. Although most microorganisms died up to 20 minutes, single cells remained viable for much longer time. These results can be seen in Table 3.

These antimicrobial properties of the tested EAAS were kept to a maximum degree after 2 weeks when stored in the dark at room temperature. The results obtained in the two strains tested were completely analogous and are presented together in Table 4.

Table 3. Growth of *C. albicans* 4 on Candida Chrome agar after different treatment intervals of freshly prepared EAASs

Sample No	Type of activated solution	Growth on Candida Chrome agar							
		Time of impact - min							
		5	10	15	20	30	60	90	120
1	anolyte of Na_2CO_3	-	-	-	-	-	-	-	-
2	anolyte of NaCl	-	-	-	-	-	-	-	-
3	catholyte of Na_2CO_3	++++	+++ +	+++ +	+	+	+	+	-
4	catholyte of NaCl	++++	+++ +	+++ +	+	+	+	-	-
5	0.5% Virkon^S	-	-	-	-	-	-	-	-
6	untreated control	++++	+++ +	+++ +	+++ +	+++ +	+++ +	+++ +	++++

Legend: – = absent of growth (100 % inactivation); + = presence of growth

Table 4. Growth of *C. albicans* ATCC 74 as well as of *C. albicans* 4 on Candida Chrome agar after various intervals of exposure to the EAASs stored for 2 weeks

Sample No	Type of activated solution	Growth on Candida Chrome agar							
		Time of impact - min							
		5	10	15	20	30	60	90	120
1	anolyte of Na_2CO_3	-	-	-	-	-	-	-	-
2	anolyte of NaCl	-	-	-	-	-	-	-	-
3	catholyte of Na_2CO_3	++++	+++ +	+++ +	+	+	+	+	-
4	catholyte of NaCl	++++	+++ +	+++ +	+	+	+	-	-
5	0.5% Virkon^S	-	-	-	-	-	-	-	-
6	untreated control	++++	+++ +	+++ +	+++ +	+++ +	+++ +	+++ +	++++

Legend: – = absent of growth (100 % inactivation); + = presence of growth

Storage under these conditions for an interval of 4 and 8 weeks did not affect the antimicrobial properties of the two tested anolytes (Table 5), but in the catholytes there was a decrease in this activity. Both strains of *C. albicans* survived more than 2 hours under their influence as shown in Table 5.

Table 5. Growth of *C. albicans* ATCC 74 as well as of *C. albicans* 4 on Candida Chrome agar after various intervals of exposure to the EAASs stored for 4 and 8 weeks

Sample No	Type of activated solution	Growth on Candida Chrome agar							
		Time of impact - min							
		5	10	15	20	30	60	90	120
1	anolyte of Na_2CO_3	-	-	-	-	-	-	-	-
2	anolyte of NaCl	-	-	-	-	-	-	-	-
3	catholyte of Na_2CO_3	++++	+++ +	+++ +	+++ +	+++	+++	+++	+++
4	catholyte of NaCl	++++	+++ +	+++ +	+++ +	+++	+++	+++	+++
5	0.5% Virkon^S	-	-	-	-	-	-	-	-
6	untreated control	++++	+++ +	+++ +	+++ +	+++ +	+++ +	+++ +	++++

Legend: – = absent of growth (100 % inactivation); + = presence of growth

Discussion

The results obtained by us demonstrating the high fungicidal activity of EAASs are consistent with the studies of Tasheva et al. (2010). These authors found significant differences in the fungicidal potential of the anolytes they used. Their data show that anolytes obtained by electrochemical activation of aqueous solutions of combinations of sodium carbonate and sodium chloride with relatively low concentrations of 0.14 and 0.18% have a fungicidal action against *C. albicans* field strains over a period of 15 min. Anolyte obtained with 0.18% sodium acetate has no fungicidal action against the same strains. The experiments we conducted confirm these results. From our data it is clear that anolytes obtained with 0.5% sodium chloride and 0.5% sodium carbonate kill fungi within 5 min. Helme et al. (2010) determine the anolyte obtained by electrochemical activation with 0.5% NaCl as four times more efficient than the commercially available NaOCl. In the studies of Tasheva et al. (2010) aqueous solutions of sodium carbonate and sodium chloride were used in lower concentrations - 0.14 and 0.18%, whose fungicidal action is slower - within 15 min. These differences indicate that the fungicidal action of the solutions is directly related to the type and concentration of the inorganic salts used. When it is 0.5% or higher, the antimycotic effect is very fast - within 5 min. These results enable us to recommend the tested anolytes for use as new, modern, environmentally friendly disinfectants.

Our studies strongly suggest that EAASs activated with NaCl, as well as with Na_2CO_3, can be used with great success as antiseptics and disinfectants against *C. albicans*. This disinfection is efficient and safe. The tested anolytes can be used as a safe, harmless and environmentally friendly means of destroying *C. albicans*. This is of great practical importance, since the *Candida* genus is characterized by considerable resistance to the

action of chemical disinfectants. By used by us electrochemically activated aqueous solutions of inorganic salts and the established good biocidal action with respect to the studied fungal strains could notably increase the potential for effective control of the infections caused by them. This fact becomes even more important due to the absence of anolyte side effects for the environment, for humans and animals, which are often subject to fungal invasion. This opinion coincides with the recommendations given by Miroshnikov (2002) and others for the use of EAAS as broad-spectrum biocides. Our results confirm those received by other authors in support of the possibility of using anolytes, both in medical practice and in other areas of human activity. Such conclusion is reached also by Robinson et al. (2010) and others, according to which electrochemically activated aqueous solutions have a significant potential for biocidal action while at the same time they are harmless to living organisms.

These and other literature data reported antimicrobial activity of anolytes, but there was no conclusive evidence of such activity of the catholytes. In our previous studies (Popova et al., 2018), we found a bactericidal action of catholyte prepared with 0.4% NaCl and 0.4% Na_2CO_3 without dilution and diluted 50%, as well as with 0.8% NaCl without dilution, but not of the catholyte prepared without salts. The results of a present study show that catholytes prepared with somewhat higher concentrations of NaCl and Na_2CO_3 have not so well expressed antimycotic activity. After storage for 2 months, the EAAS retained to a large extent these properties. This is consistent with the results of our previous studies demonstrating that the antimicrobial activity of the anolytes is maintained even after storage for weeks at room temperature (Popova et al., 2016b, Popova et al., 2018). Obviously, an important role for the antimicrobial activity of the solutions we studied except for ORP has their hydrogen ion concentration which changes very little during the eight week study period. The data from our studies confirm our previous results that despite the change in the ORP parameter of the tested solutions, which is essential for their antimicrobial activity, they retain this activity for a long time.

Anolytes turn out to be a sure means of safe decontamination of *C. albicans* containing materials, even when the solutions have been stored for two months.

Conclusions

Anolytes obtained by electrochemical activation of aqueous solutions of 0.5% NaCl and a combination of 0.5% NaCl and 0.5% Na_2CO_3 exert a fungicidal action against *Candida albicans* from two strains - ATCC 74 and clinical, within 5 min. Their activity is identical to that of the Virkon^S control disinfectant. Anolytes are a perspective antimicrobial agent with very high activity against *C. albicans*.

The catholytes exhibit weak antimicrobial properties. The catholyte, obtained from a combination of 0.5% NaCl and 0.5% Na_2CO_3, inactivates *C. albicans* in more than 90 min and from 0.5% NaCl - for more than 30 min (*C. albicans* ATCC) and the clinical strain - for over 60 min. The antifungal activity of the catholyte prepared with NaCl alone outweighed that of the obtained with a combination of 0.5% NaCl and 0.5% Na_2CO_3. The antimicrobial properties of the tested anolytes are fully preserved over a period of 2 months when stored in the dark at room temperature but of the catholytes - for not more than 2 weeks.

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