Low Temperature Plasma Vacuum Sterilization of Medical Devices by using SterAcidAgent®: Description and Distinctive Characteristics

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Abstract: This article presents a description and distinctive characteristics of the new method of low-temperature sterilization. This method based on using a mixture which is consists of peroxide and organic acid as the sterilized agent (SterAcidAgent®). This study shows that SterAcidAgent® composition has reduced the concentration of hydrogen peroxide, it also has increased bactericidal property of the mixture. We conducted studies of the sterilizing activity of 5-carboxylic low molecular weight acids, investigated the effect of basicity and hydroxyl group in the alpha position on sterilizing activity, and proposed a potential composition for a new line of sterilizing agents.

1 INTRODUCTION

The low-temperature plasma sterilization method is used as an alternative to gas sterilization based on ethylene oxide or formaldehyde vapor (Zhao and Li, 2018). High toxicity of ethylene oxide is a reason for its increasing usage limitation as well as the strict requirement of subsequent prolonged ventilation of sterilized products (Dianfeng, 2016).

Plasma sterilization is carried out at low temperatures (up to 60 °C) in a dry atmosphere (Plewa and Yousfi, 2014). A pair of a 60% hydrogen peroxide aqueous solution (peroxide) H_2O_2 and its low-temperature plasma is used as a sterilizing agent (Xaplanteris and Filippaki, 2019). This combination of these factors provides the sterilization process estimated time to be reduced to 35-45 minutes.

According to manufacturers, a vast scope of instruments and medical devices are not recommended or eligible to be sterilized in high temperature and humidity conditions (Suanpoot and Sornsakdanuphap, 2016). These tools include surgical, traumatological, ophthalmic, dental (excluding burs), microsurgical instruments, optical fibers, laser and optical fibers, electrical cords and cables, electrical and electronic devices (Li and Hang, 2016), electrophysiological catheters (Esmond and Winfrey, 2016), pens instruments (Ahn and Chae, 2016), breathing circuits, plastic containers and many other. Implementation of plasma sterilizer appears to be a decent option to such tools, especially effective in sterilization of heat-sensitive materials products and materials prone to active corrosion. Also, the plasma sterilization method could be used to sterilize hard-to-reach and finished surfaces. However, wear and maintain performance of instruments with thin and sharp working parts could be reduced through plasma sterilization in a longer period in comparison to autoclave sterilization method (Smolyakov and Romadanov, 2015).

Using this method makes it possible to sterilize the internal surfaces of the channels of medical devices, such as endoscopes (diameter up to 1 mm and length up to 3000 mm). Plasma sterilization cause no harm to the environment since hydrogen peroxide leaves only non-toxic components (oxygen and water) after utilization.

The method of low-temperature plasma sterilization is embodied in the line of low-temperature plasma sterilizers represented by Steriplaz® models manufactured by Lidkor LLC.

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These sterilizers have usable volumes of sterilization chambers of 50, 80 and 126 litters (Fig.1).

Figure 1: Model Line of Steriplaz®.

Limitation of plasma technology is based on the evaluation of technology itself in numerous system configurations (Oshita and Kawano, 2015). Thus, there is an explanation of increased interest in the plasma used for the microorganism inactivation as a substitute for other non-thermal sterilization procedures (Suanpoot and Han, 2015). Publications (Jeništa, 2016) evidence this fact and highlight several advantages (Yang and Yan, 2015) and disadvantages (Gil'man, 2003) (for example, the need for extensive research to determine the most plasma-resistant microorganisms (Deilmann and Thei, 2008)) associated with method (Xu and Wang, 2019). This article summarizes the results of plasma sterilization using SterAcidAgent®. The microbiological assessment shows the effectiveness of microorganism's inactivation technology based on SterAcidAgent®.

2 METHODS

2.1 Technical Implementation of Developed Sterilization Method

All acids are water-soluble, prepared in non-explosible concentrations, and stored in accordance with peroxide storage rules. All reagents were purchased by Lidkor LLC. The experiments were performed on a Steriplaz-120 brand sterilizer, and bactericidal tests were performed on biological and chemical indicators manufactured by Lidkor LLC.

Fig.2 shows a simplified diagram of a plasma sterilization device, where:

1 - a sterilization chamber;
2 - a sterilizing agent evaporator;
3 - a vacuum pump;
4 - a high-frequency generator;
5 - a container with a sterilizing agent;
6 - a pump of sterilizing agent;
7 - a sterilizing agent measuring cup;
8 - valve for injection of a sterilizing agent;
9 - valve of a vacuum pump;
10 - atmospheric valve;
11 - chamber walls;
12 - concentration equalization slit.

The designed method may be divided into 7 main stages, which are described below and referred to the elements of a simplified diagram of the plasma sterilization device (Fig. 2) and an influence of sterilization chamber pressure on time (Fig.3).

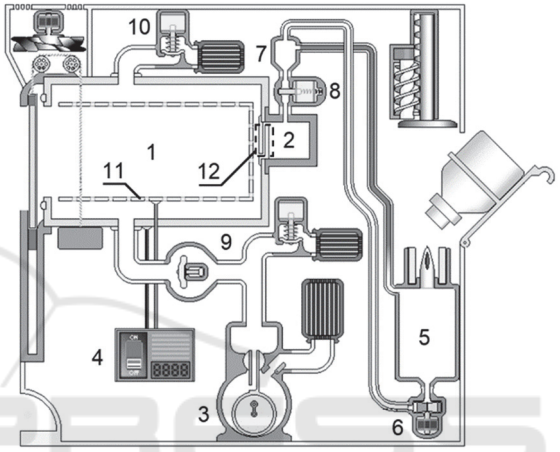


Figure 2: Simplified Diagram of a Plasma Sterilization Device.

2.1.1 Evacuation

The sterilization chamber and evaporator heating initiate the start of operation of the sterilization device. Sterilization chamber (Fig. 2, 1) is heated in the range from 45 to 50 degrees Celsius and of the sterilizing agent (Fig. 2, 2) is heated to 110 degrees Celsius. When the device is ready for sterilization, the operator places the object into the chamber and fills the container with a sterilizing agent (Fig. 2, 1).

Atmospheric pressure valve (Figs. 2, 10) closing and vacuum pump starting proceed automatically, providing the first target pressure (at least 25 Pa) (Figs. 2, 3).

At this stage, pressure reduces linearly to the value of at least 200 Pa. After reaching that point, the pressure inside the chamber decreases exponentially to the first target pressure value. The evacuation process shown in curve 3 may be divided into two parts. The first part is a low vacuum (linear), where the main influence is caused by the chamber volume. The second part is high vacuum (nonlinear), where the vacuum time depends on the inner surface area of

the sterilization chamber. The inside chamber pressure is represented on curve 3 (Fig.3, 3).

Camera pre-drying cycle is displayed on curves 1 and 2. Curve 1 shows evacuation to 100 Pa, after which the RF generator is turned on to the maximum vacuum value. Curve 2 shows the opening of the atmospheric valve and increasing the pressure to atmospheric value. If additional camera drying is required the user can select the camera pre-drying cycle manually.

2.1.2 Injection of a Sterilizing Agent

Hydraulic pump operation start provides the supply of the sterilizing agent in the measuring cup (Fig. 2, 6), after which the sterilizing agent is injected into the evaporator (Fig. 2, 2) by the opening of the measuring cup valve (Fig. 2, 8).

Curve 4 (Fig.3, 2) shows the change in pressure inside the chamber (Fig.2, 1) when opening the atmospheric valve (Fig.2, 10). An immediate increase in pressure up to no more than 400 Pa occurs as a result of the injection valve opening (Fig.2, 8) since the measuring cup (Fig.2, 7) has connection with the atmosphere.

2.1.3 Diffusion of the Sterilizing Agent

In the evaporator (Fig.2, 2), the sterilizing agent changes its aqueous state of aggregation to gaseous. Using the concentration equalization slit (Fig.2, 12) is essential to reach the sterilizing agent concentrations alignment between the evaporator (Fig.2, 2) and the chamber (Fig.2, 1) (or the penetration of the sterilizing agent into the chamber (Fig. 2, 1)).

Diffusion initiates with the sterilizing agent subside on the sterilized object. The diffusion process of an organic acid-based sterilizing agent is presented in more detail in the second paragraph described below.

Concentration gradient and sterilizing agent diffusion inside the chamber (Fig.2, 1) are the reason for the alignment of concentrations between the chamber and the evaporator as shown in Curve 5 (Fig.3, 5). Initially, the pressure increases exponentially due to the pressure gradient inside the chamber. The linear part of the curve corresponds to the sterilizing agent evaporation and an increase in the temperature inside the chamber as a result.

2.1.4 Opening of the Atmospheric Valve and Subsequent Diffusion of the Sterilizing Agent

Some of the objects, like endoscopes and tubular systems, have cavities inaccessible for penetration of the sterilizing agent. Thus, an increase in pressure to 1000 Pa occurs by opening the atmospheric valve for penetrating the sterilizing agent into the tubular systems (Fig.2, 10) and allow the sterilizing agent to persist action on bacteria on the surface of the sterilized object.

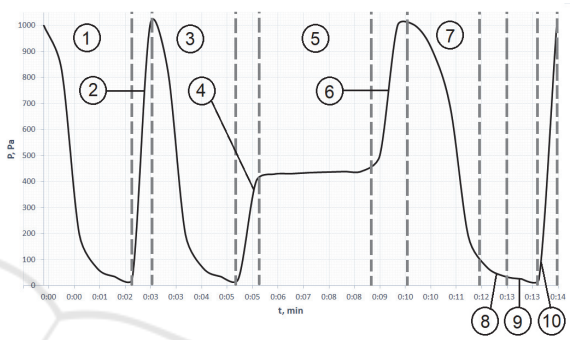


Figure 3: Graph of the Pressure Versus Time of the Plasma Sterilization Process.

The change in pressure inside the chamber (Fig.2, 1) when opening the atmospheric valve (Fig.2, 10) is represented on curve 6 (figure 3).

The engagement of the RF generator (Fig.2, 4) generates a short-term increase in pressure associated with the transfer of plasma energy to condensed water, thereby contributing to the evaporation process.

The effect of radiofrequency discharge on a sterilizing agent is presented in more detail in the third paragraph, described below.

The evacuation process is shown in curve 7 (Fig.3) is similar to the process in curve 3.

The introduction into the plasma chamber promotes a brief increase in pressure, shown in curve 8 (Fig.3). The plasma inside the chamber can effectively destroy any pathogen present in the working area (Qi-Kang and Si-Jing, 2019). As reported in recent studies (Stulić and Vukušić, 2019), it is known that atmospheric pressure plasma is highly destructive towards microorganisms (Li and Zhou, 2019), which makes it an object of potential use for various biological and medical purposes.

The optimal pressure range for an effective breakdown of the spark gap lays between 30 to 100 Pa. This increasing pressure step in sterilization process is displayed on the curve 8 (Fig.3). However,

the breakdown of the gaseous medium relies on assorted factors. Pressure, temperature, and composition of the working mixture and the distance between the electrodes makes the contribution into breakdown properties.

2.1.5 Exposition

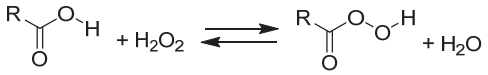
The RF generator is disabled by the control unit (Fig.2, 4). The plasma burns out during plasma exposure occurring in chamber. The mechanism of the UV-radiation formation is presented in more detail in the fourth paragraph, described below.

2.1.6 Opening the Atmospheric Valve

This stage returns an atmospheric pressure to the chamber. Pressure equalization between the atmosphere and the chamber is realized due to the concentration gradient. An abrupt increase in pressure to atmospheric is displayed on curve 10 (Fig. 3).

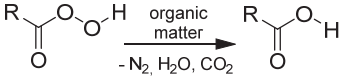
2.2 Diffusion Process of an Organic Acid-based Sterilizing Agent

Since the second half of the twentieth century, a wide scope of oxidation reactions is realized through the organic peracids action (Ki and Masur, 2019). Currently, organic peracids are regularly used in diverse bleaching and cleaning products and disinfectants (Li and Ma, 2019). Organic peracids could be obtained by the reversible reaction of hydrogen peroxide with organic acids (1), but the yields do not exceed 10% without catalyst.



(1)

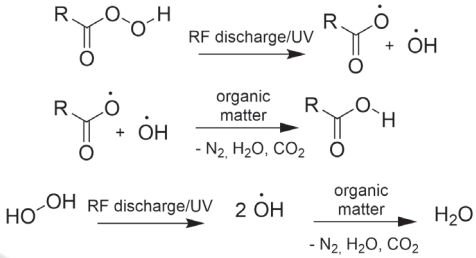
Substituent R in α-position to carboxyl group determines the oxidizing potential of organic peracids. Higher oxidative activity, in comparison to hydrogen peroxide, and, as a result, more effective sterilization, is provided by more powerful electron-withdrawing substituent R. The peracids affect organic matter similarly to other strong oxidizing agents, which means the carbon dioxide, nitrogen, and water in reaction products. Original carbon acid is regenerated through the oxidation process (2).



(2)

2.3 The Effect of RF Discharge on a Sterilizing Agent

All proceeding reactions undergo the same reaction centre – peroxyl group, prone to homolytic decomposition, making effect of RF Discharge on peracids and hydrogen peroxide similar. The O-O bond cleavage proceeds when exposed both to RF discharge as well as UV radiation produced. Obtained radicals are significantly more active oxidants comparing to starter compounds, which leads to increased sterilization activity (3).



(3)

It's noteworthy, that obtaining radicals are highly unstable, that provides not only effective oxidative sterilization but also self-oxidation to carbon dioxide and water, which has a positive influence on sterilization camera cleanness.

2.4 The Mechanism of UV Radiation Formation

Inactivation or removal of biological contaminants (pathogens) under influence of non-thermal plasma has been reported in papers (McEvoy and Rowan, 2019). Thus, moderate processes for gentle biological deactivation could be realized through plasma action as reported for sensitive products (Homola and Pongrac, 2019), medical instruments (Chumakov and Taranchuk, 2018) and implants (Souza and Ferreira, 2012). UV radiation has a lower antimicrobial effect than direct plasma treatment (George and Barrett, 2019). Moreover, biological deactivation using non-thermal plasma can include processes involving active radicals or ions (Vasilets and Gutsol, 2009).

3 RESULTS AND DISCUSSION

Chemical indicators CI PCD and biological indicators BI PCD with a class G culture (Stearothermophilus culture of Lidkor LLC) were used for effectiveness evaluation of the sterilizing agent. For each sterilization iteration, two chemical and two

biological indicators were used. Result was considered as a successful in the case of positive results of both biological indicators.

A portion of acid and the required amount of water was added to 60% hydrogen peroxide to prepare solutions with given mass concentrations. The resulting solutions were kept in the dark for 10 days to form a sufficient amount of peracids.

As part of the study, a set of low molecular weight carboxylic acids including acetic, propionic, lactic, oxalic and citric acid were selected for the experiments. During the study, the concentration of acids ranged from 5 to 15% in increments of 2.5%, the concentration of hydrogen peroxide changed from 50 to 20% in increments of 5% and from 20 to 10% in increments of 2%.

The critical concentrations at which sterilization was possible (the minimum acid concentration at the minimum peroxide concentration) are shown in Table 1.

Table 1: Critical Concentrations.

Acid	Acetic	Propionic	Lactic	Oxalic	Citric
Acid/ Peroxid, %	12.5/20	15/20	15/16	15/20	15/10

Figure 4: Selection of the Optimal Composition.

Obtained results for chosen original carbon acids are provided in Tables I-VI. Each table represents the sterilization activity of carbon acid and hydrogen peroxide mixture (Fig.4). “Plus” and “minus” signs in the table cells mean effective and ineffective sterilization respectively based on indicator response. Sterilization is considered complete if both results are “plus”. It is noteworthy that hydrogen peroxide aqueous solution without carbon acids persist effective sterilization only in concentrations above 52,5%.

The results of the experiments on the selection of the optimal composition and a comparative table are shown in Table 2-7.

Sterilization activity should be associated with peracid oxidative potential; hence it should be determined by the carboxyl group substituent. Actually, an increase in sterilization activity correlates with an increase of the electron-withdrawing group influence for the series of propionic, acetic and lactic acids.

Table 2: Propionic Acid Sterilization Potential.

Peroxid \ Acid	5,0	7,5	10,0	12,5	15,0
45	+/+	+/+	+/+	+/+	+/+
40	+/+	+/+	+/+	+/+	+/+
35	+/-	+/+	+/+	+/+	+/+
30	-/-	+/+	+/+	+/+	+/+
25	-/-	-/-	+/+	+/+	+/+
20	-/-	-/-	-/-	+/-	+/+

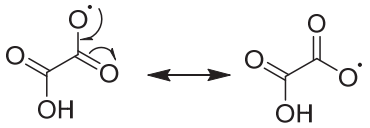
Table 3: Oxalic Acid Sterilization Potential.

Peroxid \ Acid	5,0	7,5	10,0	12,5	15,0
45	+/+	+/+	+/+	+/+	+/+
40	+/+	+/+	+/+	+/+	+/+
35	+/-	+/+	+/+	+/+	+/+
30	-/-	+/+	+/+	+/+	+/+
25	-/-	-/-	+/+	+/+	+/+
20	-/-	-/-	-/-	+/-	+/+

Table 4: Acetic Acid Sterilization Potential.

Peroxid \ Acid	5,0	7,5	10,0	12,5	15,0
45	+/+	+/+	+/+	+/+	+/+
40	+/+	+/+	+/+	+/+	+/+
35	+/+	+/+	+/+	+/+	+/+
30	+/+	+/+	+/+	+/+	+/+
25	-/-	+/-	+/+	+/+	+/+
20	-/-	-/-	-/-	+/+	+/+

Almost similar results for oxalic and acetic acids could be explained with oxalic radical increased stability due to resonance structures set (4). The highest efficiency was performed by lactic and citric acids in accordance with the assumption.



(4)

Table 5: Lactic Acid Sterilization Potential.

Peroxid \ Acid	5,0	7,5	10,0	12,5	15,0
45	+/+	+/+	+/+	+/+	+/+
40	+/+	+/+	+/+	+/+	+/+
35	+/+	+/+	+/+	+/+	+/+
30	+/+	+/+	+/+	+/+	+/+
25	-/-	+/-	+/+	+/+	+/+
20	-/-	-/-	-/-	+/+	+/+

Thus, we divide the acid set into two groups, applicable and non-applicable for further investigations as a sterilization agent. Propionic and oxalic acids are considered to be non-applicable due to propionic acid poor efficiency and oxalic acid higher toxicity in comparison with acetic acid.

Table 6: Citric Acid Sterilization Potential.

Peroxid \ Acid	5,0	7,5	10,0	12,5	15,0
45	+/+	+/+	+/+	+/+	+/+
40	+/+	+/+	+/+	+/+	+/+
35	+/+	+/+	+/+	+/+	+/+
30	+/+	+/+	+/+	+/+	+/+
25	+/+	-/-	+/-	+/+	+/+
20	-/-	-/-	-/-	+/-	+/+

Table 7: Lactic, Citric and Acetic Acid Sterilization Potential in Conditions of Low Peroxide Concentration.

Peroxid \ Acid	Lactic, 15%	Citric, 15%	Acetic, 15%
18	+/+	+/+	-/-
16	+/+	-/-	-/-
14	-/-	-/-	-/-
12	-/-	-/-	-/-
10	-/-	+/+	-/-

Most substantial results are recorded for citric acid, even with unexplainable at this moment's gaps in sterilization facilities. Also, a high activity in low peroxide concentration was performed by acetic and lactic acids which could lead to the decreased corrosive effect on glass parts of medical equipment. That makes acetic, lactic and citric acid regarded as possible compounds for an agent of commercial use. Moreover, no papers or patents content citric acid as a possible sterilization agent, which makes it a very interesting object of further investigations.

A method for experimental mixtures effectiveness evaluation was developed and consist in usage of pair of chemical and biological indicators (Fig.5). The chemical indicator is a test strip with five applied layers of the active colored substance. Upon successful sterilization, all colored strips change their color to pale yellow, a special table for color comparison is provided. Evaluation of the sterilization effectiveness using chemical sterilizers is approximate, but express. Thus, it has the role of a rapid detector of errors that could possibly occur during the sterilization process. The biological indicator represents a combination of a plastic capsule containing a G. Stearothermophilus bacterial culture (ATCC 7953) and incubator. This indicator is more accurate and is used for the final assessment of sterilization efficiency, but incubation time requires 24 hours to complete.

A pair of biological and chemical indicators are placed in both sterilizer compartments for experimental sterilizing agent testing. After leak-proof covers are removed, the capsule with a biological indicator is placed into a Teflon lumen load with a silicone hose 1000 mm long and 1 mm in diameter, simulating endoscopes.

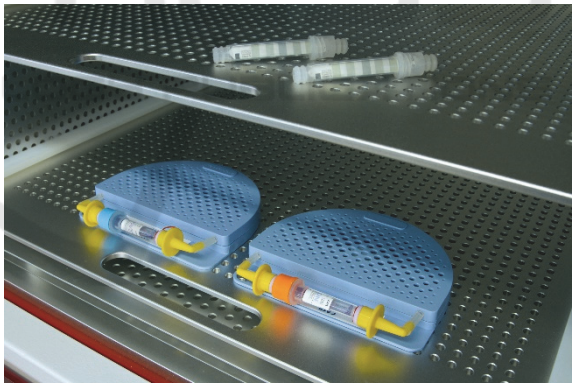


Figure 5: Pairs of Chemical and Biological Indicators.

After the sterilization cycle is complete, the glass ampoule is crushed, providing a nutrient medium access to oxygen. A broken ampoule, along with a control broken ampoule are instantly placed in an incubator for 24 hours at a temperature of 55 degrees Celsius (Fig.6). Successful sterilization provides the growth of a bacteriological culture causing a chemical indicator to turn pale yellow under the influence of the bacteria waste products. Sterilization is considered successful only if both biological indicators persist in their color.

4 CONCLUSIONS

This article presents a new method of low-temperature plasma sterilization using a mixture of hydrogen peroxide and low molecular weight carboxylic acids as a sterilizing agent as well as a method for the bactericidal activity evaluation based on combination of chemical and biological indicators. The sterilization process includes both chemical oxidative and physical effects due to low-temperature plasma and ultraviolet radiation, determining the complex nature of the method. The carboxylic acid usage in the composition of sterilizing agents allowed a 5-6-fold reduction in the working concentration of a hydrogen peroxide solution, thereby reducing the corrosive effect on the glass components of expensive medical equipment.

Figure 6: Biological Indicator Incubator.

Bactericidal activity of hydrogen peroxide and carbon acid mixtures was investigated, results are provided in Table 2-7. Final monitoring of sterilizer effectiveness was carried out by the bacteriological method, consisting in biotests based on the deactivation of test culture spores. This paper represents the results based on the germination of crops in an incubator. However, additional bacteriological tests required as a part of the validation of a new type of Steriplaz® sterilizer.

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