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The effect of Sodium Chloride on the Stability of Disinfecting Solution Aerosol Produced by Ultrasonic Nebulizer

Guang-kuk Hong, Yu-yong Kim, Jin- chol Zho, Ryong-jin Kim, Hui-won Kim*

Department of Daily Food, Institute of Microbiology, State Academy of Sciences, Unjong District, Pyongyang City 355, DPR Korea

***Corresponding author:** Hui-won Kim, Department of Daily Food, Institute of Microbiology, State Academy of Sciences, Unjong District, Pyongyang City 355, DPR Korea

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ABSTRACT

Increasing the aerosol retention time during air disinfection by the disinfecting solution aerosolized is an important way to improve aerosol efficiency. We have proposed and tested the effectiveness of a method for long-term maintenance of sterile liquid aerosols generated using an ultrasonic nebulizer in air. The retention time of aerosols generated by spraying with a mixture of disinfectant and sodium chloride with an ultrasonic nebulizer was considerably longer than that without sodium chloride, which is thought to be very effective in killing microorganisms in the air.

Keywords: Ultrasonic Nebulizer; Aerosol Lifespan; Salt; Sodium Hypochlorite

Introduction

Sodium hypochlorite has been widely used for air or object disinfection since the 1800s because it kills a wide range of microorganisms at concentrations that are almost nontoxic to individuals [1]. Generally, disinfection uses sodium hypochlorite solutions at a concentration of about 5000 ppm. The main active agent of sodium hypochlorite solution is undissociated hypochlorite (HOCl), but the effect is greatly different due to the change in its dissociation degree (HOCl=OCl-+H+) depending on the pH difference of the aqueous solution. Thus, even if the effective chlorine concentration is the same hypochlorite solution, the bactericidal activity is strong in the weakly acidic aqueous solution with a high proportion of HOCl present, and the detergent is strong in the alkaline aqueous solution with a high proportion of OCl-. HOCl is also volatile and OCl- is non-volatile [2,3]. When disinfecting using only aqueous hypochlorite solutions, the disinfection efficiency can be improved by increasing the concentration of disinfectant, but the following problems are limited:

1) The dissociated ions react to produce harmful substances.
2) Resistance of microorganisms to chlorination occurs.
3) The color of the disinfectant object changes and the odor is unpleasant.
4) It is not thoroughly disinfected to the hidden or cornered areas.

This has the disadvantage of using a combination of hypochlorite disinfectant and ultrasound treatment to improve disinfection efficiency without producing toxic products and reducing the concentration used [4], but not completely disinfecting the area covered or in the corner. Therefore, we use the method of spraying the space by atomizing the hypochlorite into the fine-grained phase by an ultrasonic oscillator [5]. Ultrasonic atomizers that generate aerosols for disinfection using ultrasound are being invented, and ultrasonic atomizers can be used to generate a wide range of particles ranging in size from a few microns to about 150 [6]. The effectiveness of drugs commonly used for disinfection depends on their concentration, exposure time,

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and the composition of the contact surface (rough or absorbent surfaces require longer contact times), especially using delivery mechanisms that can maximize the disinfection efficiency of disinfectants. Aerosol delivery is a technique that uses solid particles or droplets suspended in gases [7], and there have been a number of studies on sterilizing airborne bacteria or spores using this technique1 [8-11]. For aerosol disinfection, it is necessary to maintain aerosol in air for a long time to enhance the disinfection efficiency, and to minimize the mass of aerosol particles. To do so, the aerosol particles are reduced in diameter and the aerosol diameter is not increased by maintaining the aerosol stability so that the generated aerosol does not coalesce with each other during random Brownian motion.

The aerosol diameter depends on the device that makes it, such as a rotating disk, pneumatic, spray-type, and ultrasonic type with an injector. Here, the spray using rotary disk, pneumatic or injector is characterized by a high consumption of propellant and long-term air entrainment due to its device characteristics, non-uniform particle size distribution and large particle size. However, ultrasound is the most suitable method for producing the aerosols appropriate for air disinfection due to its uniform particle size distribution and smaller particle size. Currently, the most frequently used ultrasonic wave frequencies for aerosol-generating devices are 1.7-3 MHz, with aerosol sizes varying slightly from drug to drug, typically below 5. A method was developed by a researcher 8 to determine and analyze the target components efficiently by stabilizing the aerosol by neutralizing the charge of the micro-droplets generated by an ultrasonic atomizer using a plus- and minus-charged carrier gas. The long-term air hold of aerosols can provide sufficient contact time with microorganisms, including bacteria and viruses, to achieve effective disinfection. Thus, we have studied the long-term aerosol retention, and we have found that the aerosol is maintained in air for a long time when salt is added. In the literature, no previous studies have used salt as a long-term method for aerosol retention in air. Because salt is relatively low cost and easy to purchase, aerosolization using salt is considered practical. In this paper, we examined the effect of NaCl on the stability of a sodium hypochlorite disinfectant aerosol spray using an ultrasonic nebulizer with a frequency of 2.6 MHz and its disinfection effect on natural bacteria.

Material and Methods

Experimental Apparatuses and Materials

A single ultrasonic sprayer (self-made, working frequency 2.6 MHz, sprayed volume 1.2 L/h), test chamber (transparent glass chamber with height × width × length = 2m × 0.5m × 0.5m), an air ion counter (Air Ion Counter Sapphire 3M-Yantar LLC, NPF "Yanttar"), a thermostat (Honeywell Home, Amazon.com), settle plates (Product Number 146799, Tryptic Soy Agar), sodium hypochlorite with an active chlorine content of 10000 ppm, NaCl with a purity of more than 97%.

Method to Evaluate Aerosol Stability

A transparent glass chamber modeled with a height × width × length = 2m×0.5m×0.5m was constructed. By placing different concentrations of saline water in the ultrasonic nebulizer and then after aerosol generation, the time of aerosol loss was investigated. The aerosol loss time was estimated by recording the time after filling the glass box with aerosol and the time until it disappeared. As a control, aerosols atomized from the water with no salt by ultrasonic nebulizer were used.

Method to Evaluate Air Disinfection Effectiveness

The experimental site used an air-free room with a volume of 60 m^3 and air was sampled by passive sampling using an IMA standard system [12]. These results ($CFU/m^2/h$) were then transformed into CFU/m^3 according to a literature [13]. TVC (Total Viable Count) was recorded using trypsin soy agar (TSA, Product Number 146799) and the plates were incubated for 48 h at an average temperature of 36 ± 1 °C.

The air disinfection efficiency, i.e. the disinfection rate, was evaluated by the following formula:

$$\text{Disinfection rate (\%)} = (C_0 - C / C_0) \times 100$$

Where

C_0 - number of bacteria present in air before treatment

C - number of bacteria present in air after aerosol spraying

Ion Counting Method of Ultrasonic Aerosol

Air ion counter (Air Ion Counter Sapphire 3M-Yantar LLC, NPF "Yanttar") was used to measure the number of electrically charged particles (ions) present in aerosols produced by ultrasonic nebulizer.

Statistical Analysis

All experiments and analyses were performed in triplicate and the results were evaluated using one-way ANOVA (one-way ANOVA). Statistical significance of differences between means (p=0.05) was determined using Fisher's LSD test. The data obtained for statistical analysis were transferred in Microsoft Excel spreadsheet software (Microsoft Corp., Redmond, WA, USA). Analysis of variance was performed using IBM SPSS STATISTICS 22 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Safety of Aerosols with the Addition of Salt

The level of factor A (NaCl concentration, %) was set to $A_1 = 0$, $A_2 = 0.25$, $A_3 = 0.5$, $A_4 = 1$, $A_5 = 2$, $A_6 = 5$, and the level of factor B (temperature, %) was set to $B_1 = 5$, $B_2 = 10$, $B_3 = 15$, $B_4 = 20$, $B_5 = 25$, $B_6 = 30$, and the time of aerosol loss was measured at each level combination (Table 1). Next, ANOVA was performed on the aerosol loss time data with concentration of sodium chloride solution and temperature (Table 2).

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It can be seen that there is a significant difference in the interaction between NaCl concentrations, temperatures and NaCl concentration and temperature with respect to aerosol loss time (Table 2). In space, aerosols disappear immediately after the salt is not added, but they are present for a long time when the salt is added. Also, the time of the presence of salt-containing aerosols in the space increases with higher salt concentrations and lower temperatures ($p < 0.05$). The reason for not increasing the brine concentration by more than 5% is that higher brine concentration causes higher viscosity and higher load on the ultrasonic nebulizer due to reduced aerosol generation, while the minimum retention time is 124.36 min, which is sufficient for disinfection.

Table 1: Aerosol loss time versus concentration and temperature of sodium chloride solution ($\overline{X} \pm SE$, min).

NaCl Concentration, %	Temperature, ^{0}C					
	5	10	15	20	25	30
0	$0.06^a \pm 0.01$	$0.06^a \pm 0.02$	$0.05^a \pm 0.01$	$0.04^a \pm 0.008$	$0.02^a \pm 0.006$	$0.01^a \pm 0.007$
0.25	$90.20^b \pm 4.3$	$81.60^b \pm 4.08$	$70.00^b \pm 1.79$	$54.20^b \pm 1.74$	$35.80^b \pm 2.15$	$11.80^b \pm 2.15$
0.5	$215.20^c \pm 23.43$	$183.80^c \pm 18.82$	$170.20^c \pm 17.43$	$149.80^c \pm 17.31$	$125.60^c \pm 17.56$	$93.60^c \pm 17.00$
1	$255.00^c \pm 13.43$	$243.40^c \pm 18.82$	$227.40^d \pm 17.43$	$204.00^d \pm 17.31$	$182.60^d \pm 17.56$	$154.00^d \pm 17.00$
2	$277.00^d \pm 24.21$	$267.20^d \pm 24.24$	$251.80^e \pm 24.04$	$232.00^e \pm 33.89$	$208.60^{de} \pm 24.82$	$177.00^e \pm 13.89$
5	$285.60^d \pm 14.23$	$272.80^d \pm 13.92$	$257.60^e \pm 3.97$	$238.60^e \pm 33.80$	$214.40^e \pm 23.85$	$185.40^e \pm 14.14$
Average	224.60	209.76	195.40	175.80	153.40	124.36

Note: The different letters in the same column show significant differences at the $p < 0.05$ level.

Table 2: ANOVA results of aerosol loss time data with concentration and temperature of sodium chloride solution.

ANOVA Two-Factor with replication						
Source of Variation	SS	df	MS	F	P- value	F crit
NaCl Conc. Effect	912978.246	5	182595.6492	1941.898715	2.73987E-75	2.341827531
Temperature Effect	86668.1631	5	17333.63262	184.342612	1.40598E-39	2.341827531
Interaction	19201.7835	25	768.07134	8.168413403	1.16431E-12	1.659359908
Error Item	6770.119698	72	94.02944025			
Total	1025618.312	107				

Efficiency of Air Disinfectant with the Addition of Salt

The aero-disinfection efficiency of aerosol generated by the addition of different concentrations of sodium hypochlorite to the ultrasonic nebulizer was compared with that of aerosol generated by the addition of different concentrations of sodium hypochlorite and different concentrations of salt. The level of factor A (NaCl concentration, %) was set to A_1=0, A_2=5, and the level of factor B (NaOCl concentration, %) was set to B_1=500, B_2=1000, B_3=2000, B_4=4000, B_5=8000, B_6=10000, and the level of factor C (time, min) was set to C_1=10, C_2=20, C_3=30, C_4=40. The number of planar bacteria (CFU/plate) at each level combination was measured to examine the bactericidal effect of aerosols according to Eq. (1) (Table 3) and ANOVA analysis (Table 4). It can be seen that there are significant differences between NaOCl concentrations, between times and between NaOCl concentrations and between times (Table 4). Also, no disinfection effect was observed without salt addition (Table 3). This is because the sodium hypochlorite aerosol generated by the ultrasonic aerosol generator disappears immediately without the addition of salt, and therefore, no sodium hypochlorite aerosol exists in the air. The effect of aero-disinfection is greater with higher concentration of sodium hypochlorite, and the disinfection rate of natural bacteria is more than 99% when the concentration of sodium hypochlorite is 8000 ppm, incubation time 30 min, and spray dosage of 8 ml/min.

Table 3: Disinfection rate (%) of the disinfect aerosol against natural bacteria (cfu/m^3).

NaCl Addition	NaOCl Concentration, ppm	Time, min			
		10	20	30	40
A	500-10000	3668(0)	3996(0)	4267(0)	5612(0)
B	500	4582±30.9 (21^a±0.8)	3364±42.5 (42^a±1.1)	2195±54.1 (62.15^a±1.4)	2194.7±42.5 (62.16^a±1.1)
	1000	4292±34.8 (26^a±0.9)	3016±46.4 (48^a±1.2)	1925.02±65.7 (66.81^a±1.7)	1922.7±81.2 (66.85^a±2.1)
	2000	3944±58 (32^{ab}±1.5)	2842±92.8 (51a^{ab}±2.4)	1501.04±127.6 (74.12^{ab}±3.3)	14.92.92±96.7 (74.26^b±2.5)
	4000	3596±61.8 (38^b±1.6)	2494±69.6 (57^b±1.8)	1068.36±81.2 (81.58^{be}±2.1)	1065.46±69.6 (81.63^{be}±1.8)
	8000	834.04±77.3 (85.62^c±2.0)	281.88±73.4 (95.14^c±1.9)	5.8±0.77 (99.90^c±0.02)	5.22±0.38 (99.91^e±0.01)
	10000	804.46±42.5 (86.13^c±1.1)	259.84±0.38 (95.52^c±0.01)	5.22±0.15 (99.91^c±0.004)	4.64±0.31 (99.92^e±0.008)

Note: A: without salt, B: with salt 5% in concentration. Numbers in parentheses show the disinfection rate (%) against natural bacteria of aerosols. The different letters in the same column show significant differences at the $p < 0.05$ level.

Table 4: Results of ANOVA analysis of the effect of NaCl on the disinfection efficiency of sodium hypochlorite aerosol.

ANOVA Two-Factor with replication						
Source of Variation	SS	df	MS	F	P-value	F crit
NaOCl Effect	249007	5	49801.39	2142.317	3.66E-55	2.408514
Time Effect	118243.7	3	39414.58	1695.506	1.11E-48	2.798061
Interaction	20741.77	15	1382.785	59.48356	8.97E-26	1.880175
Error Item	1115.832	48	23.24651			
Total	389108.3	71				

The results of this study show that the aerosol stability is significantly different with or without the addition of salt when the sodium hypochlorite is sonicated to generate aerosols. This is because when NaCl is aerosolized by ultrasound, the salt aerosol particles self-discharge the electrostatic electricity generated by sweeping each other in Brownian motion in the air. Thus, it can be expected that aerosol particles soon subsided due to their large volume and their heavy mass as a result of coagulation (coalescence) with each other. To demonstrate this, we measured the charge when the aerosol was generated by adding saline water to the ultrasonic aerosol generator (Table 5). The aerosol of the non-salted water was charged with a large amount of charge, while the salted water had little charge (Table 5). To stabilize the aerosol produced by the ultrasonic atomizer, it is one way to overcome the electrically charged phenomena due to the Lennard effect. The previous study [14] reported an aerosol generator for ease of measurement or analysis of the target component by stabilizing the aerosol by neutralizing aerosol droplets using carrier gas. However, in this method, separate devices are required to generate and transport carrier gas. We propose a method to stabilize aerosols by adding salt to the sample solution without adding a special device to the ultrasonic atomizer.

Table 5: Charge of Salt Solution Aerosols ($\overline{X} \pm SE$, count/cm^3).

NaCl Concentration %	Anion content count/cm^3	Cation content count/cm^3
0	300000^a ± 243	16 ± 2
0.25	10000^b ± 98	12 ± 2
0.5	2000^c ± 65	9 ± 1
1	344^d ± 13	8 ± 1
2	254^e ± 10	5 ± 1
5	182^f ± 7	0

Note: The different letters in the same column show significant differences at the $p < 0.05$ level.

Conclusion

The aerosol generation by the addition of salt to the liquid increases the aerosol retention time in space to a considerable extent. When 5% NaCl was added to the sodium hypochlorite at 8000 ppm and aerosol was generated, the bactericidal effect on natural bacteria present in the air was 99.9% after 30 min. The long-term aerosol retention method has potential applications in other areas as well as disinfection.

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