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A new ozone-based method for virus inactivation: preliminary study

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Abstract. The nebulization technique reported here could be used to inactivate viruses with ozone in large volumes of body fluids, such as plasma, partial blood and perhaps whole blood in a short time. Coliphage MS2 was used as a model because it is safe, easy to handle and more resistant to chemical disinfections than viruses such as HIV. The theoretical curves and experimental points, describing ozone inactivation of MS2, form a semi-sigmoid of congruent data. There was a $>7\log_{10}$ reduction in MS2 viability and the possibilities of minimizing the ozone concentration required to kill viruses are indicated.

The analysis was expanded to account for the interaction of ozone with a virus suspension in the shape of a thin film from the experimental findings of Bolton *et al*. We again find a semi-sigmoid of congruent data for their case, i.e. describing ozone inactivation of the influenza A virus (WSN strain) and the vesicular stomatitis virus versus time. For the method of nebulization, the exposure time of droplets with ozone is a few seconds, whereas for the thin film method the exposure time is measured in hours.

1. Introduction

A consortium involving the Canadian Department of National Defence, Agriculture Canada and the Canadian Red Cross Society has conducted preliminary studies on ozone inactivation of viruses in blood. One of the authors (MMK) was a member of this consortium and was asked to develop an ozone delivery system to better facilitate interactions between the gas (ozone) and the body fluids, because the two existing techniques used by the consortium, (i) 'hollow fibre' (Wells *et al* 1991) (US patent of the date **91** 1205) and (ii) 'conventional bubbling' by Müller Medical Inc. (West German patent 1068428), convey inconsistent ozone transfer to test fluids. Müller's method also produces excessive bubbling and with both methods the consistency of the treated fluid is not satisfactory. In Müller's system ozone is administered extracorporeally, i.e. ozone is foamed through a small sample of venous blood (10 ml) removed from a patient by phlebotomy, exposed for 3 to 30 min to ozone, and immediately injected intramuscularly back into the patient. This technique is referred to as autoheamotherapy.

In the proposed method, the fluids are thoroughly nebulized or atomized, i.e. dispersed into minute droplets to create a fine 'rain' to fall through a controlled atmosphere of O_3/O_2 and/or O_3/inert gas mixture. Electric and magnetic fields can be superimposed over the space through which the droplets are passed. The preliminary experimental work shows that this method yields consistent results. By comparison with Müller's method, the system described in this paper would ozonate test fluids at a rate of at least 20 ml min^{-1}.

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Many industrial applications (e.g. powdered milk, powdered eggs, instant coffee and the carburation of liquid fuels in cars and jet planes) are based on the nebulization principle described in this paper.

Our work is grouped into five parts: (i) theoretical analysis of the ozone penetration into the biological fluids, (ii) comparison between the nebulization and the thin film method of Bolton *et al* (1982), (iii) experimental examination of the nebulization method by inactivating MS2 coliphage and *Bacillus subtilis* in the selected reactor configuration as a function of ozone dosage, (iv) theoretical examination of the findings by Bolton *et al* (1982) and (v) formulation of the suitability of surrogate viruses for subsequent evaluation, particularly the lentivirus group of retroviruses.

2. Theoretical formulation

2.1. Nebulization

In general, ozone is transferred to the interior of the fluid by diffusion. The rate of ozone absorption in an aqueous solution of droplets must be compensated by the addition of ozone from outside the sphere. Razumovskii and Zaikov (1982), have shown that in zero-order approximation the kinetics of the ozone–liquid interaction follows an exponential law

$$\frac{\partial q/\partial t}{q} \approx -\frac{W}{H}.$$

W is the specific rate of gas-fluid feed (litre gas per litre solution) and H is the coefficient of Henry's law. Let us now apply their experimental observation to a droplet: the ozone penetrates the surface area of the droplet with a diffusion velocity, v, and in time, Δt, it will occupy the volume of $4\pi R^2 v\Delta t$. R is the radius of the droplet and the droplet has the volume of $(4/3)\pi R^3$. Since W is the rate of feed, we see that $W = 3\alpha v/R$. By definition, the diffusion velocity, v, is approximately $D/\Delta x_d$, where Δx_d is the characteristic diffusion length ($= 2(Dt)^{1/2}$). If $t \to \infty$ and O_3 becomes Q_0, the total amount of ozone absorbed according to the above equation, $q(X)$, is

$$\frac{q(X)}{Q_0} = 1 - e^{-\sqrt{X}} \tag{1}$$

where X is the normalized time equal to $9\alpha^2 Dt/R^2$ and $\alpha = 1/H$. As a numerical example, the diffusion constant, D, for water is 2.14×10^{-5} cm^2 s^{-1} and for glucose 0.52×10^{-5} cm^2 s^{-1}. For 1% ozone concentration in oxygen at 20 °C for water, α is 0.386.

We find that the observation of Razumovskii and Zaikov is the well known Fick's law, which satisfies the classical mass diffusion–heat conduction (transport) law. In one dimension (of x), this is

$$\frac{\partial q}{\partial t} = D\frac{\partial^2 q}{\partial x^2}. \tag{2}$$

In zero-order approximation equation (2) implies that the absorption of ozone by the fluid corresponds to the same law as the flow of heat from the surroundings into the biological fluid.

For a droplet (sphere), equation (2) was solved (see the appendix). The total amount of ozone absorbed, $q(X)$, is

$$\frac{q(X)}{Q_0} = 1 - e^X \operatorname{Erf}\sqrt{X}. \tag{3}$$

Erf is the complementary error function. In figure 1, equation (3) is given as a full line. If the droplet before landing meets the requirement that $X < 1$, equation (3) can be approximated by equation (1) (see figure 1). If $X \ll 1$, equation (1) can be reduced further to

$$\frac{q(X)}{Q_0} = \sqrt{X} = \frac{3\alpha\sqrt{Dt}}{R} \tag{4}$$

giving

$$R = \frac{3\alpha Q_0}{q_0}\sqrt{Dt} \sim Q_0\sqrt{D} \sim \text{const}\, Q_0. \tag{5}$$

When the time of flight is fixed, the time, t, becomes a constant quantity, t_0, equal to the droplet's time of flight. To inactivate a virus by ozone, we assume that the dosage must exceed a certain minimum value. If a single species of viruses is used, $q(t)$ becomes $q_0 =$ constant. Q_0 is the saturation concentration of ozone in the liquid in accordance with Henry's law. If Q is the concentration in the chamber, then $Q_0 = \alpha Q$ (for $t \to \infty$). In equation (5), Q is the only variable. The biological fluid is characterized by the diffusion constant, D, and the absorption coefficient, α. The virus is specified by q_0.

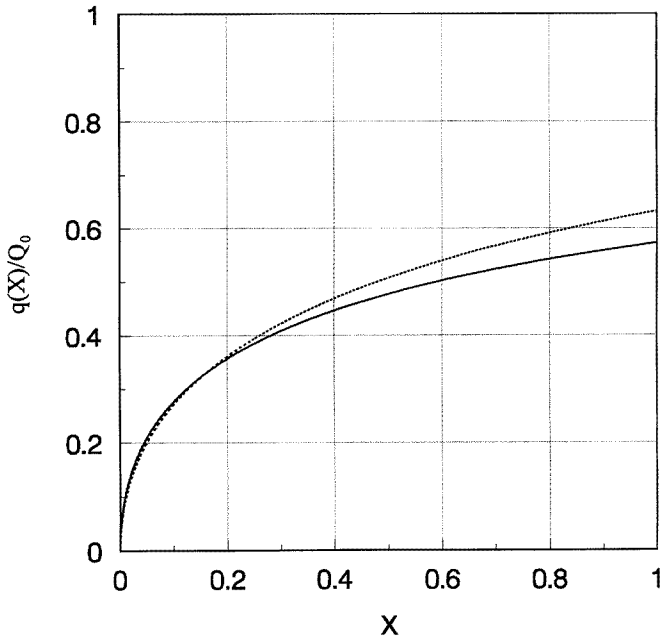

Figure 1. The full curve is equation (3): $q(X)/Q_0 = 1 - \exp X\ \text{Erf}X^{1/2}$. The broken curve is equation (1): $q(X)/Q_0 = 1 - \exp(-X)^{1/2}$.

To relate this analysis to virus inactivation in the fluid's droplets, the distribution of the droplets in a spray can be described by the Gaussian function (represented by the left-hand side of equation (6), having mean drop size, m, and standard deviation, σ. When a virus titre of 2×10^7 plaque forming units (pfu) per ml is added, on average, each droplet of a mean size of 30 to 40 μm contains a single virus. When the droplets are subjected to the ozone atmosphere, the viruses present in the smaller droplets will be the first to be

inactivated. The relative number of inactivated viruses is

$$\frac{1}{\sigma\sqrt{2\pi}}\int_{-\infty}^{x}\{\exp[-(t-m)^2/(2\sigma^2)]\}\,\mathrm{d}t=\frac{1}{2}\left[1+\mathrm{Erf}\left(\frac{x-m}{\sigma\sqrt{2}}\right)\right]. \tag{6}$$

From $-\infty$ to $+\infty$, the integral has the value of 1, and Erf is the error function (see Abramowitz and Stegun (1968)). Therefore, the relative number of viruses surviving the ozone treatment is

$$s=\frac{1}{2}\left[1-\mathrm{Erf}\left(\frac{x-m}{\sigma\sqrt{2}}\right)\right]=\frac{1}{2}\left[1-\mathrm{Erf}\left(\frac{-m+\mathrm{const}\,Q_0}{\sigma\sqrt{2}}\right)\right]. \tag{7}$$

Here x represents a large size droplet (containing a virus) in the distribution that is not affected by the ozone treatment. R of equation (5) is substituted in the middle part of equation (7) as $x=2R$, and the theoretical survival curves are produced following the path given by Kekez *et al* (1996).

2.2. Thin film method

The thin film method of Bolton *et al* (1982) also produces consistent results. The biological fluid is suspended in the shape of a thin film on the surface of a rotating culture bottle and the ozone is drawn through the bottle at a constant rate. The total amount of ozone absorption in the thin film is also given by equation (3) but here $X=\alpha^2Dt/(\Delta x^2)$, where Δx is the thickness of the film. If $X\gg 1$, equation (3) with new X becomes

$$\frac{q(X)}{Q_0}=1-\frac{1}{2\sqrt{X}}=1-\frac{\Delta x}{2\alpha\sqrt{Dt}} \tag{8}$$

giving

$$\Delta x=2\alpha\left(1-\frac{q_0}{Q_0}\right)\sqrt{Dt}. \tag{9}$$

Equation (3) (approximation) and equation (8) (exact solution) are compared in figure 2. We see that the approximation is valid only for $X>1$. In the Bolton *et al* (1982) experiments the ozone concentration in the chamber, Q, is the parameter and the time, t, is the main variable. Their time is measured in hours, hence $X>1$. For their experimental conditions, the titre of viruses is large (10^8 pfu ml^{-1}). It is useful to assume that the thickness of the film approaches that of a monolayer; therefore, Δx is also proportional to the diameter of the virus, x, having its own mean size, m, and standard deviation, σ. Substituting equation (9) in the second bracket of equation (7), the relative number of the viruses, s, surviving the ozone treatment is obtained when the ozone exposure time, t, is a variable quantity:

$$s=\frac{1}{2}\left[1-\mathrm{Erf}\left(\frac{-m+\mathrm{const}\sqrt{t}}{\sigma\sqrt{2}}\right)\right]. \tag{10}$$

2.3. Comparison between the nebulization method and the thin film method

To make comparison for a given (constant) volume, we consider that the cubic volume of the thin film $(\Delta x)^3$ is equal to the equivalent volume of the sphere $(4/3)\pi R^3$. This gives $R=\Delta x(3/4\pi)^{1/3}$. However, the normalized time, X, for the sphere has the multiplier of 9 in its definition in comparison to X used in the thin film case. This implies that the nebulization method is faster by a factor of $9(3/4\pi)^{1/3}=5.58$ in comparison to the thin film method.

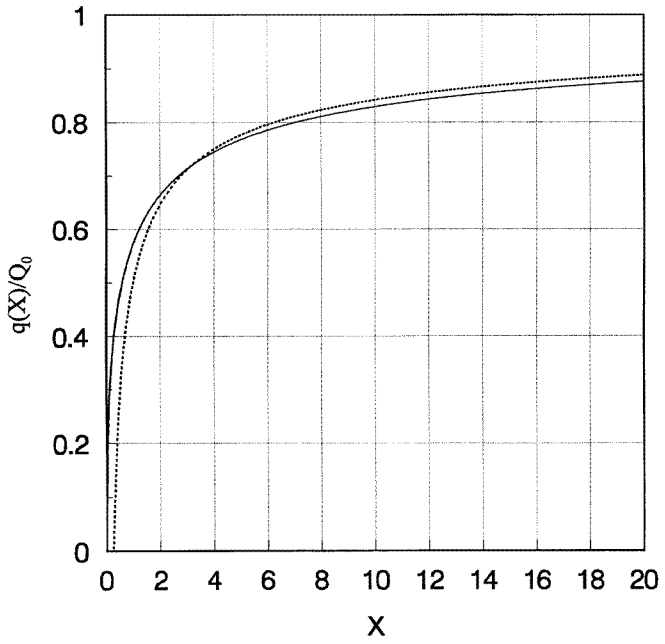

Figure 2. The full curve is equation (3): $q(X)/Q_0 = 1 - \exp X \ \mathrm{Erf} X^{1/2}$. The broken curve is equation (8): $q(X)/Q_0 = 1 - 1/2X^{1/2}$. The comparison applies when $X > 1$.

3. Basic methodologies and rationales for their selection

3.1. Nebulization

In our experimental work, the fluid is atomized into small droplets by a nebulizer. The droplets are injected into an ozonation chamber and allowed to fall through a controlled atmosphere containing ozone as indicated in figure 3; therefore the exposure time is a function of the height of the ozonation chamber. The properties and composition of the fluid affect the ability of the nebulizer to atomize. The method proposed is fluid specific, requiring an appropriate nozzle for each fluid. Since the absorption is an exponential function of the radius (see equation (1)), the dosage of ozone received by any viral contaminants is related to the droplet size and the reactor design. To treat plasma and/or serum it is necessary that the droplets are as small as possible, in order for the ozone to eliminate effectively the viruses inside the droplet. To make the apparatus compact, it is also advisable to minimize the time of flight, i.e. to shorten the height of the inactivation chamber. Reduction of the effective dosage will also minimize additional damage to desirable components of the test fluids. The experiment used a commercial ozone generator (model GTC-0.5, Griffin Technics, New Jersey).

3.2. Selection of test fluid

A major concern with the ozone treatment process is virus inactivation without unacceptable damage to desirable components of the treated product(s). Since the likely damage to whole blood is greater and more difficult to assess because of its cellular components, in this study we propose to test only plasma, a major source for blood products. Although

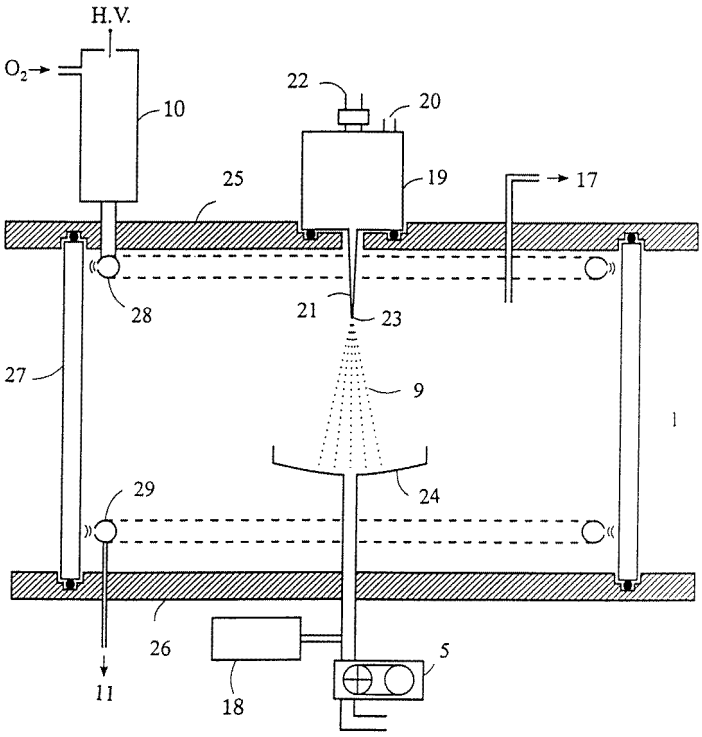

Figure 3. The ultrasonic nebulizer system.

it is recognized that the possibility of damage to proteinaceous material as well as the possibility of generating toxins during the process exists, we consider that the assessment of these factors is beyond the scope of this exploratory study.

3.3. Selection of nebulizer

In this work, the nebulizers considered were: a compressed gas atomizer (or pressure jet atomizer) and twin fluid atomizer; an ultrasonic nebulizer and a rotary nebulizer. Of these, the ultrasonic nebulizer is preferable due to the low velocity of the incoming spray, and the experimental chamber with such a nebulizer appears to yield the most compact design for an equivalent ozone dose. Very fine atomization is achieved at low velocity (1–3 m s^{-1}), making this device ideal for use with the flow rates of 10 to 400 ml min^{-1}.

The nebulizer system in figure 3 consists of a piezoelectric resonant device (19), whose transducer discs are energized by high-frequency electrical signals applied to the terminals (20). This results in the propagation of pressure waves in both directions along the nozzle (21). The liquid inlet is at point (22) and the spray originates at the atomizing surface (23). The droplets are collected at a dish (24), and a peristaltic pump (5) removes the liquids from this dish (24). The inactivation chamber is formed between the two flanges (25 and 26) and a glass cylinder (27). Ozone is injected into the chamber via an annular ring (28) through 24 small-diameter openings to ensure that the ozone stream is slow-moving. The ozone outlet (29) feeds the used gas to the vent decomposer (11).

3.4. Application of electric and magnetic fields

The flight characteristics of the droplets can be augmented and controlled electronically. Each droplet is charged by an electrostatic field. A high voltage is applied to the dish (24) and the nozzle (21) acts as a ground electrode, ensuring that the droplets do not fuse in flight. To further control the speed of the droplets, another electrode in the form of a ring could be placed in the space between the nozzle (21) and the dish (24) to create a classical triode structure. If this process is used for whole blood, a magnetic field could be applied to increase the flight time of the droplets by making them follow a helical path, due to the iron in red blood cells. The magnetic field is produced by putting a coil around the glass cylinder (27).

3.5. Separation of ozone from oxygen

When ozone is generated by electrical discharges, a gas mixture (O_3–O_2 or O_3–air) is produced in which the ozone concentration is typically 1 to 5% by volume. Organic matter is prone to oxidation and it may be important in some applications to minimize the oxidative stress caused by oxygen radicals. Work is in progress to develop a low-temperature distillation (adsorption–desorption) technique to attain an ozone-to-oxygen ratio of about 1 to 5 (i.e. 20 to 30% by volume over silica gel with a low impurity level of Fe) during the adsorption cycle. Later, during the desorption phase (when ozone is separated from oxygen), an inert gas (e.g. N_2, He, etc) will carry ozone to the inactivation chamber.

3.6. Control of droplet size

The size and distribution of the droplets are determined by a Doppler velocity apparatus. This apparatus can also help measure the time of flight, optimize the flow rate and determine the droplet's evaporation rate.

4. Experimental results

4.1. Whole blood

Preliminary experiments were conducted to demonstrate some aspects of the proposed concept. A drop of blood was released from a 10 ml syringe with a needle (gauge 14–20) by knocking the needle with a finger, and these drops were allowed to fall freely over a distance of 5 to 10 cm until enough blood collected in a Petri dish for the analysis. There was minimal (<2%) or no haemolysis of the red blood cells due to free fall and exposure to air. With the first (compressed gas) atomizer built, 87% of the total number of the red blood cells present in whole blood survived the nebulization treatment.

4.2. Tests with bacteriophage MS2

An ultrasonic nebulizer was used in this study. A bacteriophage, the tailless icosahedral RNA-coliphage MS2, suspended in bovine serum, was used to optimize a 'viral inactivator'. MS2 was used as a model here because it is safe, easy to handle, and can grow to titres up to 10^{13} pfu ml^{-1} in a suitable bacterial host. MS2 phage, being a small (27 nm in diameter), icosahedral and non-enveloped virus, survives better in the environment and is generally more resistant to inactivation by chemical agents when compared with larger, enveloped viruses such as HIV. The titre of active MS2 in the serum/plasma sample can be accurately

measured by counting the number of plaques produced in an *Escherichia coli* lawn. The effectiveness of the ozone treatment for the inactivation of the test virus in treated serum samples was indicated by the reduction in the number of plaques as a function of ozone dosage.

The phage was diluted to a final concentration of about 2×10^7 pfu ml^{-1} for the data shown in figure 4. Dose–response curves of MS2 inactivation by ozone were obtained with the virus suspended in (i) Dulbecco's phosphate buffered saline (PBS), (ii) a 10% solution of bovine serum in PBS, and (iii) a 25% bovine serum in PBS. A comparison of the experimental points and the theoretical curves for MS2 shows that the curves and points form a semi-sigmoid of congruent data (figure 4). The increase in bovine serum concentration only results in translating the curve to the right.

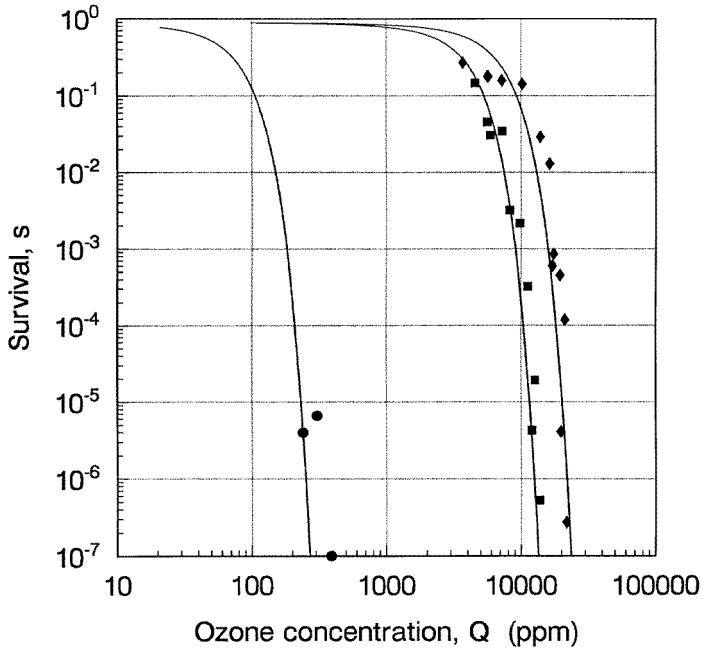

Figure 4. Dose–response curves of MS2 inactivation by ozone as a function of ozone concentration using the nebulization method. MS2 was suspended in (i) Dulbecco's phosphate buffered saline (PBS), (ii) a 10% solution of bovine serum in PBS and (iii) a 25% bovine serum in PBS. The experimental points are: ●, PBS; ■, 10% serum; ♦, 25% serum. Data points are the average of three experiments performed in triplicate. Theoretical curves are described by the following expressions.
PBS: $s = 0.5\{1 - \mathrm{Erf}[0.0241(-36.38 - \mathbf{0.7}Q)]\}$
10% serum: $s = 0.5\{1 - \mathrm{Erf}[0.0241(-36.38 - \mathbf{0.014}Q)]\}$
25% serum: $s = 0.5\{1 - \mathrm{Erf}[0.0241(-36.38 - \mathbf{0.008}Q)]\}$
when the ozone exposure time used was about 1 s.

The theoretical curves were computed using equation (7) for the measured value of $m = 36$ μm, $\sigma = 29$ $(\mu\mathrm{m})^{-1}$. To get a good fit with the experimental points, a set of values for the 'constant' of equation (7), named here β, was carried out. We find that β has a value of 0.7, 0.014 and 0.008 for PBS, 10% serum and 25% serum respectively. From equation (5) we see that β is proportional to the square root of the diffusion constant. The

ratio of β for 10% serum to β for 25% serum is 1.75, implying that the diffusion constant at 25% serum is decreased by a factor of 3 with respect to the diffusion constant at 10% serum concentration. To get a more accurate evaluation of the change in the diffusion constant, it is necessary to use equation (1) instead of equation (5) in determining the value of R. Hence, we have estimated that q_0 (for an air-borne virus to be inactivated) has a value of 20 ppm, when the time of ozone exposure is a few seconds.

4.3. Tests with the spores of Bacillus subtilis

An ultrasonic nebulizer was used in this study. The system was examined for its ability to inactivate the spores of *Bacillus subtilis* (ATCC 19659). Such spores are routinely used to assess the sporicidal activity of liquid and gaseous chemicals and as biological indicators to validate the performance of steam and gas sterilizers. The bacterium was grown in Columbia Broth (Difco, Detroit, Michigan, USA) diluted ten-fold in deionized water. In this medium, there was nearly a 100% sporulation after 48 h at 37 °C. The spores were washed three times in deionized water and suspended in the test medium to a final concentration of 10^8 colony forming units/ml. When *B. subtilis* spores were suspended in PBS and nebulized in the presence of ozone, there was $>5 \log_{10}$ reduction in their viability.

4.4. Thin film method of Bolton et al (1982)

4.4.1. Test with vesicular stomatitis virus. If equation (10) is to be applied to these results, detailed knowledge of the virus distribution is required. From Wagner (1975) and Fields *et al* (1996) we see that there is considerable heterogeneity in particle size. The typical infectious B virion is a bullet-shaped (B particle) cylinder, 180 ± 10 nm in length and 65 ± 10 nm in diameter at the blunt end. If this volume is approximated by a sphere, it will have a radius of 50.65 nm. For the predominant defective truncated (T) virions having about one-third the length (65 nm) of the infectious B virions, we find the equivalent radius to be 36 nm. The molecular weight of the infective VS B-virion was estimated to be 4.4×10^6 daltons at the upper extreme and 3.2×10^6 daltons at the lower end. For the density of 1.31 g cm^{-3}, this corresponds to the equivalent radius of 9.9 nm at the lower end. Defective VS virions have a molecular weight of $(0.7\text{–}1.2) \times 10^6$ daltons which corresponds to the equivalent radius of 5.9 to 7.1 nm.

If half of all the particles are occupied by the infectious B viruses in the Gaussian type distribution curve we have from the definition of the error function that $(x - m)/(\sigma\sqrt{2}) = 0.477$ as $\mathrm{Erf}\,(0.477) = 0.50$. For the mean radius of the infectious B virion, m, of 50.6 nm, we get $x - m = 14.8$ nm. Here, x corresponds to the mean radius of the defective truncated (T) virion of 36 nm. This gives $1/(\sigma\sqrt{2}) = 0.032$ nm^{-1}. Putting the values for m and $1/(\sigma\sqrt{2})$ into equation (11), the theoretical curves for ozone inactivation versus time are obtained. The results are given in figure 5, again showing a semi-sigmoid of congruent data.

4.4.2. Test with influenza A virus (WSN strain). The information about the distribution of this virus can be obtained from the work by Compans and Choppin (1975) and Fields *et al* (1996). The majority of particles are spherical with a diameter of 80–100 nm, making the average radius 45 nm. The purity, homogeneity and approximate composition of population of virus particles were not conclusively established. However, to derive the theoretical curves, we assume that the relative distribution for influenza A virus is the same as for

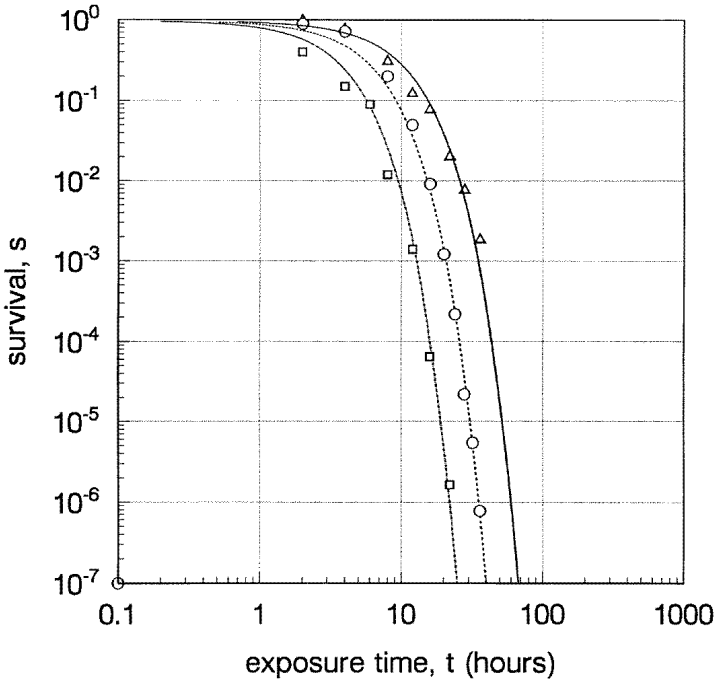

Figure 5. Ozone inactivation of vesicular stomatitis virus versus time. Experimental points obtained by Bolton *et al* (1982) were replotted on the log–log scale. The thin film method was used. The virus suspension was exposed to: □, 0.64 ppm of ozone; ○, 0.16 ppm; △, 0.00 ppm. Full curves are the theoretical curves for:
0.00 ppm: $s = 0.5\{1 - \mathrm{Erf}[0.0323(-50.65 + \mathbf{20}(\mathrm{time})^{1/2})]\}$
0.16 ppm: $s = 0.5\{1 - \mathrm{Erf}[0.0323(-50.65 + \mathbf{26}(\mathrm{time})^{1/2})]\}$
0.64 ppm: $s = 0.5\{1 - \mathrm{Erf}[0.0323(-50.65 + \mathbf{33}(\mathrm{time})^{1/2})]\}$.

vesicular stomatitis virus. This means that $1/(\sigma\sqrt{2})$ equals 0.032 nm^{-1}; the results are given in figure 6. We suggest that the slightly higher resistance (manifested by higher value of q_0 used in equation (9)) to inactivate influenza A viruses in comparison with vesicular stomatitis viruses, may be attributed to the fact that they have a segmented genome. There are eight separate segments which make up the full genetic complement of the influenza virus.

5. Selection of test viruses for future study

To assess the ability of ozone applied by the nebulization method for inactivating viruses in biological fluids, it is necessary to spike the test samples with relevant viruses and examine their inactivation as a function of ozone dosage. Relevant human viruses may include the hepatitis viruses, parvoviruses (including B19), the herpes viruses (including herpesvirus 2 and cytomegalovirus) and retroviruses (including type C and HIV). However, safety issues as well as practical difficulties of working with some of these agents precluded their use at the preliminary stages of such a study. It is also difficult at this stage to reliably extrapolate to mammalian viruses from studies on bacteriophage(s). Therefore, experiments will be performed using a panel of surrogate viruses in proper containment facilities.

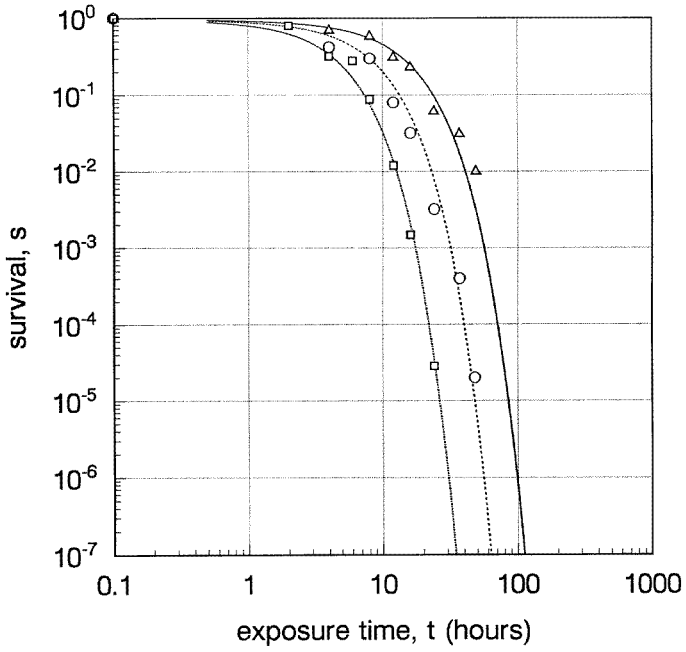

Figure 6. As in figure 5, but for influenza A virus (WSN strain). Full curves are the theoretical curves for:
0.00 ppm: $s = 0.5\{1 - \mathrm{Erf}[0.0323(-45 + \mathbf{15}(\mathrm{time})^{1/2})]\}$
0.16 ppm: $s = 0.5\{1 - \mathrm{Erf}[0.0323(-45 + \mathbf{20}(\mathrm{time})^{1/2})]\}$
0.64 ppm: $s = 0.5\{1 - \mathrm{Erf}[0.0323(-45 + \mathbf{27}(\mathrm{time})^{1/2})]\}$.

6. Conclusions

The technique reported could be used to inactivate viruses with ozone in large volumes of body fluids such as plasma, partial blood and perhaps whole blood in a short period of time. To enhance the absorption of ozone in the fluid, the method atomizes the fluid into small droplets that are sprayed into an atmosphere of ozone which kills viruses. It is shown by Carpendale and Freeberg (1991) that ozone inactivates HIV at non-cytotic concentrations.

The current work offers many opportunities to minimize the ozone concentration required to kill viruses. It is necessary to decrease the droplets size as much as possible in order that the virus inactivation is done at low ozone concentration; however, if a very small droplet size is chosen, the use of hydrocyclone technology to collect droplets becomes necessary.

An objection to our approach has been raised that we have neglected the chemical reaction of ozone with liquid. For example, when ozone diffuses in a liquid and reacts with it irreversibly, equation (2) must be modified according to a first-order reaction. This means that, on the left hand side of equation (2) we must add the term βq to account for this reaction. Here, β is a constant greater than zero. After solving this new expression, the total amount of ozone absorbed versus normalized time again reaches the semi-saturation for $X > 1$ in a similar fashion as in figure 2, but at smaller value of $q(X)$. The fundamental relationship of both equations (4) and (8) is maintained and the validity of equation (4) is expanded over the large domain of X.

Acknowledgments

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Appendix

Carslaw and Jaeger (1989, p 349 (III)) quote the problem of a sphere of radius a and perfect conductor of specific heat c_1, surrounded by an infinite region of diffusivity D, specific heat c and contact resistance $(1/h)$. When the heat source Q is absent, the problem becomes that given on p 350 (IV). With the conductor at the initial temperature T_0, and with contact resistance approaching zero, $h \to \infty$, the integral #21 on p 350, describing the cooling of the sphere, reduces to

$$T = \frac{2kT_0}{\pi} \int_0^\infty \frac{[\exp(-u^2 Dt/a^2)]u^2}{(u^2-k)^2 + k^2u^2}\,\mathrm{d}u$$

where $k = 4\pi a^3 \rho c / M_1 c_1$. Here, M_1 is the mass of the sphere. This integral can be evaluated by partial fraction expansion of the polynomial part of the integrand. The denominator is a quadratic in u^2 with two roots:

$$v = u^2 = k\left(1 - \frac{k}{2}\right) \pm \frac{k^2}{2}\sqrt{1 - \frac{4}{k}}.$$

For large values of k, the square root can be expanded to first order, giving the following value for the two roots: $v_1 = 0$ and $v_2 = -k^2$. Hence, the integral becomes

$$T = \frac{2kT_0}{\pi} \int_0^\infty \frac{\exp(-u^2 Dt/a^2)}{u^2 + k^2}\,\mathrm{d}u. \tag{A1}$$

From Gradshteyn and Ryzhik (1965, p 338, #3.466 (1)), we have

$$\int_0^\infty \frac{\exp(-\mu^2 x^2)}{x^2 + \beta^2}\,\mathrm{d}x = \mathrm{Erf}(\beta\mu)\frac{\pi}{2\beta}\exp(\beta^2\mu^2)$$

and the solution of equation (A1) is

$$T = T_0\,\mathrm{e}^X \mathrm{Erf}\sqrt{X} \tag{A2}$$

where

$$\sqrt{X} = \beta\mu \quad \mu = \sqrt{\frac{Dt}{a^2}} \quad \beta = k = \frac{3\rho c}{\rho_1 c_1}$$

since

$$\frac{D_1}{D} = \frac{\rho^2}{\rho_1^2} \quad \frac{c}{c_1} = \alpha$$

we get

$$X = \frac{9\alpha^2 D_1 t}{a^2}.$$

Here, D_1 is the diffusion constant inside the sphere. If the sphere were to be be heated, equation (A2) would read

$$\frac{T}{T_0} = 1 - e^X \operatorname{Erf}\sqrt{X}.$$

For the analogies between heat and mass transfer, we need to substitute T by Q. Hence, the equation above becomes equation (3) given in the main text.

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