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Microcyn™: a novel super-oxidized water with neutral pH and disinfectant activity

C. Landa-Solis[a], D. González-Espinosa[a], B. Guzmán-Soriano[a], M. Snyder[b], G. Reyes-Terán[c], K. Torres[c], A.A. Gutierrez[a,*]

[a]*Instituto Nacional de Rehabilitación, Secretaría de Salud, Mexico, DF, Mexico*
[b]*Department of Environmental Toxicology, University of California at Davis, Davis, CA, USA*
[c]*Instituto Nacional de Enf. Respiratorias, Secretaría de Salud, Mexico, DF, Mexico*

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Summary A new super-oxidized water (SOW) product, Microcyn™, was tested for in vitro antimicrobial and antiviral activities. The effectiveness of this neutral-pH SOW at killing *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Candida albicans* in pure culture was evaluated. One millilitre ($\sim 10^8$ colony-forming units/mL) of each microorganism was subjected to 9 mL Microcyn or sterile water at room temperature for 30 s. Under these conditions, a $\log_{10}$ reduction factor of 8 in the level of all pathogens occurred in the treatment samples. In addition, results of tests with three batches of Microcyn exposed to *Bacillus atrophaeus* spores for 5 min demonstrated complete inactivation of the spores within 2–3 min ($\log_{10}$ reduction factor >4). The effectiveness of Microcyn in reducing human immunodeficiency virus-1 (HIV-1) on hard surfaces (glass) was also evaluated in compliance with Environmental Protection Agency requirements for virucidal claims. After exposure of the tested surfaces to Microcyn for 5 min without agitation, there was a $\log_{10}$ reduction factor >3 in the viral load as measured by both cytopathic effect and antigen p24 of HIV-1 production in MT-2 cultures. Microcyn activity against adenoviral vector type 5 was also analysed under simulated laboratory in-use conditions with viral suspensions. In order to increase the sensitivity of the test, the fluorescent light emitted by AdGFP-infected cells was measured with the use of a flow cytometer. A $\log_{10}$ reduction factor >3 in the viral load was achieved after a 5-min exposure to Microcyn under these strict conditions. These results show that Microcyn exerts a wide antimicrobial spectrum with major advantages over acidic SOWs, including neutral pH, lower free active chlorine (51–85 ppm) and long shelf life (1 year).

* Corresponding author. Tel.: +52 5555 5414 7653.
E-mail address: andresgutz@mexis.com

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Introduction

Super-oxidized waters (SOWs) have been investigated as disinfectants for instruments and hard inanimate surfaces in hospitals.[1] In endoscope disinfection, for example, SOWs have decreased the time, toxicity and costs of material disinfection.[2] The literature also describes the use of SOWs on humans for various indications including the treatment of infectious skin defects or ulcers,[3] mediastinal irrigation after open heart surgery,[4] and treatment of peritonitis and intraperitoneal abscesses.[5] SOWs have also been recommended for handwashing in medical personnel.[6]

In vitro, various SOWs exert distinct microbicidal activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella enteritidis*, *Listeria monocytogenes*, *Mycobacterium avium intracellulare*, *Mycobacterium tuberculosis* and *Candida albicans*.[7–9] In contrast to other common disinfectants (e.g. ethanol), SOWs kill spores of *Bacillus atrophaeus* (formerly *B. subtilis*) and *B. cereus*.[10] Acidic SOWs also modify the antigenicity of the surface protein of hepatitis B virus and the infectivity of human immunodeficiency virus-1 (HIV-1) in a time- and concentration-dependent manner.[11]

Unfortunately, there are marked differences between the various SOWs tested to date (i.e. acidic, neutral or alkaline). It is known that various properties of electrolysed water, including pH, concentration of chlorine and oxidation–reduction potential (ORP), determine the germicide activity, shelf life and corrosion potential of each SOW. Acidic SOWs (pH 2–4) are active microbicidal agents as a result of the high active free chlorine (AFC $>$ 650 ppm) at acidic pHs.[10] Unfortunately, acidic SOWs are corrosive and very unstable agents so their shelf life is extremely short.[12,13] In contrast, the chlorine of SOWs decreases dramatically with the increase of pH from the acidic (pH 2.5) to the alkaline region (pH $>$9.0).[10,14] Although alkaline SOWs also retain antimicrobial properties, they may not be as efficacious as acidic SOWs.

In order to maintain microbicidial activity and to increase tissue compatibility and shelf life, the production of neutral-pH SOWs has been attempted.[15,16] A Californian company has recently launched a neutral-pH SOW called 'Microcyn™'. This is an electrochemically processed aqueous solution manufactured from pure water and salt (NaCl). Electrical energy is used to produce a change in the aqueous solution resulting in a highly oxidized, neutral-pH water with a controlled amount of oxidizing species. Microcyn has a slight concentration of sodium ($<$55 ppm) and chlorine (51–85 ppm), with a pH range of 6.2–7.8 and an ORP $>$ 800. According to the manufacturer's data, Microcyn has a shelf life of one year.

Although Microcyn could be similar to other SOWs, the exact properties of each SOW are different in accordance with the previous statements. The purpose of this study, therefore, was to evaluate the disinfectant activity of Microcyn against various microbes including pathogenic vegetative bacteria, *B. atrophaeus* spores, fungi, adenoviruses and HIV-1 in vitro. Our results indicate that Microcyn is an effective disinfectant for which sporocide activity and appropriate applications are now being validated.

Materials and methods

Production of test substance (Microcyn)

The production of SOW is an electrolytic or redox reaction. Microcyn is produced in a single strength and is made up of 99.99% water with additional ions. Purified water and salt (NaCl) are the sole input materials for the production of Microcyn. The purified water passes through anode and cathode chambers that are separated from a middle salt (NaCl) chamber by ionic membranes in REDOX equipment (Oculus Innovative Sciences, California, USA). The most abundant ions are hypochlorous acid (0.002%) and sodium hypochlorite (0.003%), expressed as free available chlorine (FAC). Additionally, Microcyn contains other ions such as sodium chloride (0.001%), hydrogen peroxide (0.0004%), chlorine dioxide and ozone in extremely trace amounts ($<$ 0.0001%). The resultant output of this process is highly oxidized SOW (ORP $>$ 800) with a neutral pH (6.2–7.8), an FAC between 51 and 85 ppm, and a controlled amount of free radical ions. For all experiments, two to three different batches of Microcyn were used.

Microbial challenge study

These assays were carried out in accordance with the Mexican Official Norm for germicidal testing, NMX-BB-040-SCFI-1999.[17] Any product certified as a germicide must reduce the initial viable bacterial load of *E. coli* and *S. aureus* by 99.9999% in 30 s of exposure at the recommended dose. For this study, certified active cultures of *E. coli* (ATCC #1229), *S. aureus* (ATCC #6538) *Pseudomonas aeruginosa* (ATCC #25619), *S. typhi* (CDC 99) and *C. albicans* (ATCC #10231) were obtained and aliquoted in sterile 0.25 M phosphate buffer (pH 7.1–7.3) to

obtain a final concentration of live micro-organisms of 75–125 $\times 10^8$ colony-forming units (CFU)/mL. In total, 9 mL Microcyn solution was transferred into test tubes for each bacteria tested, utilizing a sterile pipette.

A calibrated time was commenced immediately once a 1-mL suspension of one of the above micro-organism suspensions was added to 9 mL of Microcyn. At the desired times (0.5, 1, 5, 10 and 15 min), the suspension of micro-organisms and Microcyn was gently shaken to obtain a homogeneous suspension, and 1 mL of this suspension was mixed with 9 mL neutralizing solution containing (in g/L): 5 tryptone, 2.5 yeast extract, 2.5 sodium bisulphite, 6 sodium thiosulphate, 0.02 bromo cresol purple, 1 sodium thioglycolate, 5 polysorbate 80, 10 dextrose and 7 soybean lecithin (Sigma, St. Louis, MO, USA). Subsequently, 1 mL of this mixture was plated on to a tryptic soy agar (TSA) plate and spread evenly over the entire plate surface. Two plates per condition were incubated at 35 °C. The number of resulting colonies on each plate was counted after a 48-h incubation period. Appropriate positive controls for each micro-organism were always run in parallel using sterile, deionized water (control).

Test cell cultures

MT-2 cells (human CD4+ lymphocytes) capable of infection by HIV were obtained from the University of California, San Francisco, USA.[18] Tissue culture media, antibiotics and fetal bovine serum were all purchased from Gibco (Carlsbad, CA, USA). Cultures were grown and propagated in HUT medium [RPMI 1640 media supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 1% L-glutamine and 1% streptomycin–penicillin] and used in suspension. On the day of testing, cultures with 85% cell viability were acceptable for the study. Microcyn was filtered with a 0.2-μM membrane for the cell cultures.

The 293 cell line was originally obtained from the American Type Culture Collection (ATCC 1573). Cell cultures were grown and propagated in DMEM-F12 media supplemented with 10% (v/v) heat-inactivated FBS and 1% streptomycin–penicillin at 37 °C and 5% CO_2.

HeLa cells (ATCC CCL-2) were grown and propagated in DMEM media supplemented with 10% (v/v) heat-inactivated FBS and 1% streptomycin-penicillin at 37 °C and 5% CO_2.

HIV-1 strain

The SF33 strain of HIV-1 used for this study was obtained from the University of San Francisco, California, USA (kind gift of Dr Levy's laboratory). Peripheral blood mononuclear cells from a healthy donor were activated with PHA-P (3 μg/mL, Sigma) and human interleukin-2 (20 U/mL, Roche, NJ, USA) in HUT media for three days. Cells were washed and infected with SF33 strain. Supernatant was collected on Days 4 and 6, and tested for the p24 HIV-1 antigen by enzyme-linked immunosorbent assay (ELISA; Beckman Coulter, Fullerton, CA, USA). The supernatant was centrifuged to remove cell and debris at 3000 RPM for 20 min at room temperature. The supernatant was removed and aliquoted, and the virus was stored at −80 °C until the day of use. Frozen aliquots were thawed at 37 °C for 2 min immediately prior to its use.

Construction of replication-defective adenoviral vectors for expressing the green fluorescent protein (AdGFP)

Methods for the construction of replication-defective adenoviral vectors have been described elsewhere.[19,20] These vectors are E1a-, partially E1b- and partially E3-deleted vectors based on human adenovirus type 5. Briefly, a shuttle plasmid containing the green fluorescent protein (GFP) reporter gene under the transcriptional control of pCMV was prepared (pAd-Track). Homologous recombination of this pShuttle plasmid with AdEasy 1 plasmid was carried out in electrocompetent bacteria. Clones that had inserts were tested by restriction endonuclease digestions. Once confirmed, supercoiled plasmid DNA was transformed into DH10B cells for large-scale amplification. Subsequently, 293 cells (ATCC 1573) were cultured in serum-free medium (OptiMEM-GIBCO) and transfected with recombinant plasmid digested with Pac1. Infected cells were monitored for cytopathic effect, collected and lysed with three cycles of freezing and thawing. The resultant viruses (AdGFP) were purified with AdenoPure columns (BD Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. Viruses were quantitated by OD 260/280. The final yield was 1.52×10^{11} plaque-forming units (pfu)/mL.

Virucidal effect of Microcyn against HIV-1 for use on inanimate environmental surfaces

This study was conducted to evaluate the virucidal efficacy of Microcyn against HIV-1 following a 5-min

exposure period. The assay analyzes four diferent conditions as decsribed below for groups A,B,C and D. Except for the control group (uninfected MT-2 cells), five dilutions were tested for all groups (-1 to -5). Four cultures were assayed for each dilution, giving a total of 20 cultures per group. This study was conducted in compliance with the Environmental Protection Agency (EPA) protocol[21] and the US EPA Good Laboratory Practice set forth in 40 CFR part 160.

Preparation of virus films
Films of virus were prepared by spreading 0.2 mL virus inoculum uniformly over the bottoms of 55-cm^2 sterile polystyrene Petri dishes. The virus films were air-dried at room temperature (21 °C) in a biological safety cabinet until they were visibly dry ($\sim$20 min).

Drying effect control (Group A)
A virus film was prepared as described above. The control film was exposed to 2 mL HUT medium for 5 min. The virus was then scraped and diluted.

Treatment of virus films with test substance (Group B)
Separate dried films were each exposed to 2 mL Microcyn for 5 min at room temperature. Following exposure, the plates were scraped to resuspend the contents of the plate. The virus–Microcyn mixture was immediately diluted (10^{-1}) in HUT medium. Serial tenfold dilutions of this resulting suspension were assayed for infectivity.

Direct cytotoxicity control (Group C)
To control for a possible direct cytotoxic effect of Microcyn on MT-2 cells, a 2-mL aliquot of Microcyn was diluted serially (10^{-1} to 10^{-5}) in medium and inoculated into MT-2 cell cultures. Cytotoxicity of the cell cultures was scored simultaneously with the other groups.

Viral viability control (Group D)
To ensure that the virus strain (SF33) was capable of replicating and causing cytopathic effects in all cases, the procedure was repeated with a viral suspension that had remained in HUT medium without being dried. Serial tenfold dilutions (-1 to -5) in HUT medium were used.

HIV-1 infectivity assays
The MT-2 cell line was used as the indicator cell line in the infectivity assays. This line shows a cytopathic effect consisting of syncytia formation when infected with HIV-1. Four microwells were inoculated with 0.2 mL of each dilution of the reconstituted virus suspension from test (reconstituted in Microcyn) and control (reconstituted with control medium) groups. Uninfected cell controls were inoculated with test medium alone. Cultures were incubated at 37 °C and 5% CO_2. The cultures were scored periodically every two days for the presence or absence of a cytopathic effect, as well as the presence of p24-HIV-1 antigen by ELISA (HIV-1 p24 Ag EIA, Beckman Coulter as per the manufacturer's instructions. Calculations of $TCID_{50}$ were made using the formula from Reed and Muench.[22]

Adenovirus infectivity assays

This study was conducted to evaluate the virucidal efficacy of Microcyn against adenovirus encoding the green fluorescent protein gene (AdGFP), following different exposure periods. The test is based on the detection of fluorescence emission from HeLa cells infected with either control or Microcyn-treated AdGFP. A control virus aliquot (1.5×10^8 pfu) was exposed to 1 mL saline solution for 10 min and further diluted in 1 mL of culture medium supplemented with 10% FBS. Virus-Microcyn mixtures were also prepared with AdGFP (1.5×10^8 pfu) exposed to 1 mL of Microcyn for 1, 5 and 10 min and further diluted in 1 mL of supplemented medium. Cells (5×10^5/well) were washed twice with phosphate buffer and further infected with control AdGFP or adenovirus–Microcyn mixtures for 2 h at 37 °C, 5% CO_2. Only 1 mL of the control virus or the virus–Microcyn mixture was used to infect HeLa cells for 2 h (i.e. equivalent to 150 multiples of infection, m.o.i.). At the end of the exposure, Microcyn was inactivated with the addition of 1 mL 10% FBS supplemented medium. At 24 h post infection, cells were harvested and GFP fluorescence was measured on a FACScalibur flow cytometer (Becton Dickinson; Fullerton, CA, USA). Data were acquired and analysed using the CELL Quest software program. Results are expressed as percentage of cells expressing GFP. All experiments were carried out in triplicate.

Sporicidal acitivity of Microcyn

The purpose of these experiments was to assess the activity of Microcyn against spores of *B. atrophaeus*. These assays were based on the method used previously for another SOW.[23] One-millilitre incubations consisted of 0.1 mL of *B. atrophaeus* (ATCC #9372) spore suspension (10^7 spores) and 0.9 mL of Microcyn. Every minute for 5 min, an incubation was neutralized with 9 mL

Table I Effects of Microcyn following a 5-min exposure to human immunodeficiency virus-1 dried on an inanimate surface

SF33 dilution	Dried SF33 and HUT (Group A)	Dried SF33 and Microcyn (Group B)	Cytotoxic control Microcyn (Group C)	Untreated SF33 and HUT (Group D)
Uninfected cells	− − − − −	− − − − − −	NNNN	0000
10^{-2}	+ + + +	0000	NNNN	+ + + +
10^{-3}	+ + + +	0000	NNNN	+ + + +
10^{-4}	0 + + 0	0000	NNNN	+ + + +
10^{-5}	0000	0000	NNNN	0000
10^{-6}	0000	0000	NNNN	0000
$TCID_{50}$	$10^{4.0}$	<10	<10	$10^{4.5}$

Presence (+) or absence (0) of cytopathic effect of serial tenfold dilutions of SF33 on each of four microwells with MT-2 cells. SF33 virus (200 μL) was previously desiccated and reconstituted in HUT or Microcyn (Groups A and B, respectively) or only diluted in HUT medium without drying (Group D). $TCID_{50}$ is the total dilution causing syncytia in half of the wells assayed. NNNN, non-toxic.

tryptic soy broth (TSB) media, 1 mL (10% = 10^6 spore equivalents) was plated on to TSA plates, incubated for 24 h at 35 °C, and any resulting bacteria colonies were counted. For the first-minute samples, dilution series were performed to obtain accurate colony counts.

Results

Bactericidal and fungicidal activities

The efficacy of Microcyn for inactivating *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhi* and *C. albicans* was evaluated. Each strain of approximately 10^8 CFU/mL was inoculated in 9 mL Microcyn or 1 mL sterile, de-ionized water (control) and incubated at room temperature for 30 s and 1, 5, 10 and 15 min. The surviving population of each pathogen at each sampling time was determined on TSA. Under these conditions, an exposure time of 30 s was enough to completely inactivate all pathogens tested in the treatment samples (99.9999% reduction). Thus, a $\log_{10}$ reduction factor of 8 in the level of all pathogens occurred in the treatment samples incubated for only 30 s at room temperature. The counts of all these pathogens in control samples remained the same throughout the incubation. Results indicate that Microcyn is an active bactericidal and fungicidal SOW.

Virucidal activity of Microcyn against SF33 strain of HIV-1

Results of tests with a single batch of Microcyn exposed to HIV-1 for 5 min are shown in Tables I and II. $TCID_{50}$ in the dried virus control (from 200 μL of original suspension) was $10^{4.0}$ by cytopathic effect and $10^{4.5}$ by antigen p24 of HIV-1 production (Table I). Following exposure to Microcyn, test virus infectivity was not demonstrated in the viral suspension at any dilution ($TCID_{50} < 10$). Test substance cytotoxicity upon MT-2 cells was ruled out (Group C), since dilution of Microcyn equal to those used in the tests had no cytopathic effect in the absence of virus ($TCID_{50} < 10$). The viability control (Group D) showed that the virus remains viable when dried, given that the $TCID_{50}$ diminished by less than 10 in this control (Table II). Under these test conditions, Microcyn demonstrated complete inactivation of the HIV-1 as required by the EPA for virucidal claims.

Virucidal activity of Microcyn against adenovirus serotype 5, AdGFP

The efficacy of Microcyn for inactivating adenovirus encoding the GFP gene was evaluated. The test is based on the detection of fluorescence emission from HeLa cells infected with either control AdGFP viruses or Microcyn-treated AdGFP. Infection of HeLa cells was always carried out with 7.5×10^7 pfu/mL (i.e. 150 m.o.i.). In all test conditions, cells appeared normal under light microscopy. As shown in Figure 1, the background fluorescence measured in control HeLa cells was 0.06%. After infection with control AdGFP, 88.51% of HeLa cells expressed GFP. Following exposure to Microcyn, adenovirus infectivity decreased in an inversely proportional manner to the exposure period. Thus, virus treated with Microcyn for 1, 5 and 10 min could only express GFP in 2.8%, 0.13% and 0.09% of HeLa cell cultures, respectively. Considering the autofluorescence and the initial viral load for all tested conditions (i.e. 7.5×10^7 pfu), the infectious titre was 6.6×10^7 pfu in the control AdGFP-HeLa group. In the groups where the virus had been treated with Microcyn, the infectious titres were

Table II Infectivity of human immunodeficiency virus-1 (HIV-1) under different experimental conditions

SF33 dilution	HUT-reconstituted SF33 (Group A)	Microcyn-reconstituted SF33 (Group B)	Untreated SF33 diluted in HUT (Group D)
0			0000
10^{-2}	++++	0000	++++
10^{-3}	++++	0000	++++
10^{-4}	++++	0000	++++
10^{-5}	0000	0000	0+0+
10^{-6}	0000	0000	0000
$TCID_{50}$/200 μL	$10^{4.5}$	<10	$10^{5.0}$

Presence (+) or absence (0) of p24 antigen in supernatant from each of four microwells with MT-2 cell culture inoculated with SF33 strain of HIV-1. SF33 virus (200 μL) was previously desiccated and reconstituted in HUT or Microcyn (Groups A and B, respectively) or only diluted in HUT medium without drying (Group D). $TCID_{50}$ was interpolated with the total dilutions from the original viral suspension that were added to the wells (see Methods).

2.0×10^6, 5.2×10^4 and 2.2×10^4 at 1, 5 and 10 min of virus exposure to Microcyn, respectively. Therefore, the $\log_{10}$ reduction factor was 1.5, 3.1 and 3.5 at 1, 5 and 10 min of viral exposure to Microcyn, respectively. It is important to underline that these values were retrieved from flow cytometry analysis. In contrast, with fluorescence microscopy, no fluorescent cells could be

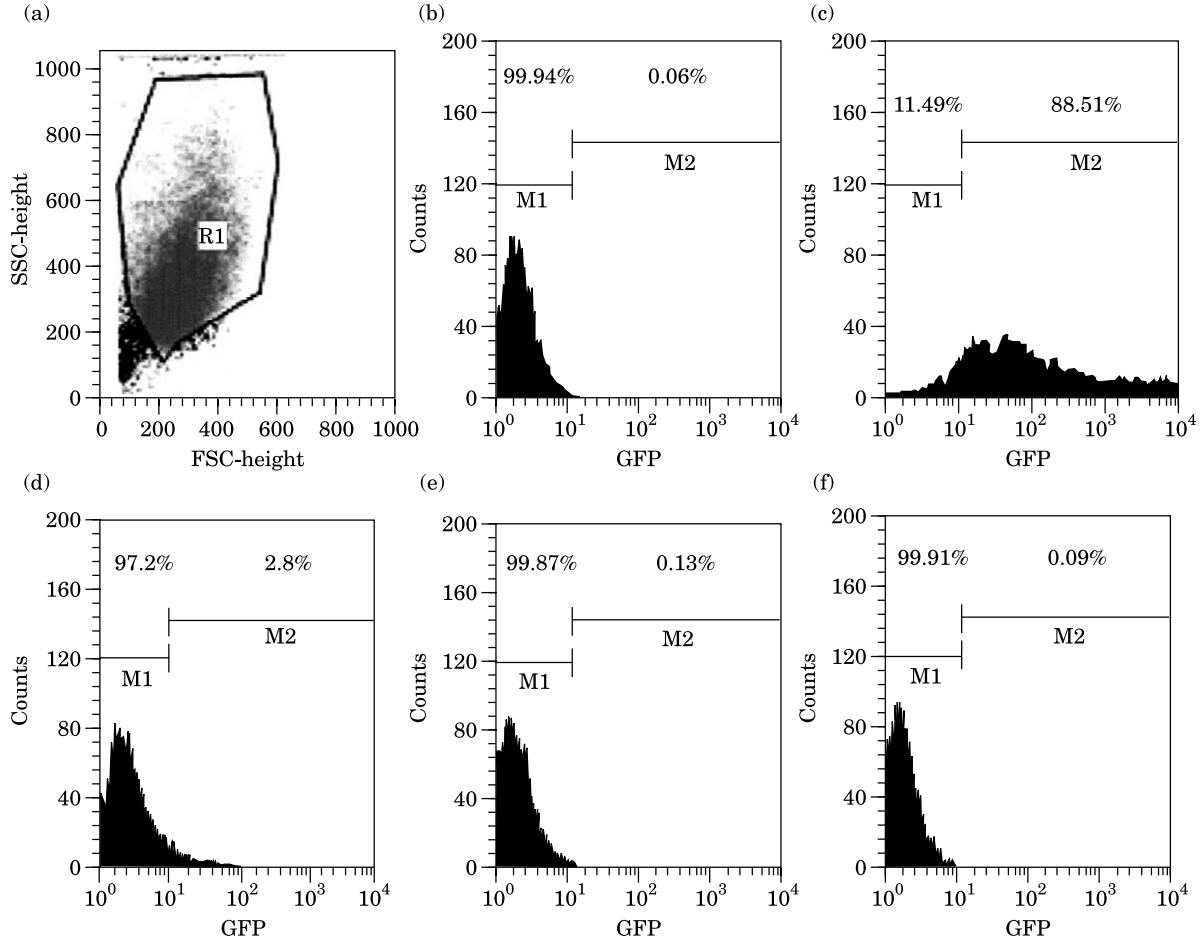

Figure 1 Flow cytometric analysis of the activity of Microcyn against adenovirus type 5. (a) Gating strategy of uninfected HeLa cells on FL1 channel. (b) Autofluorescence of uninfected HeLA cells in M1. (C–F) percentage of non-fluorescent (uninfected) cells in M1 and fluorescent cells in M2. HeLa cells were infected 150 m.o.i. of either untreated AdGFP (c) or Microcyn-treated AdGFP for 1, 5 and 10 min (d, e and f, respectively). Representative experiment of a series of three.

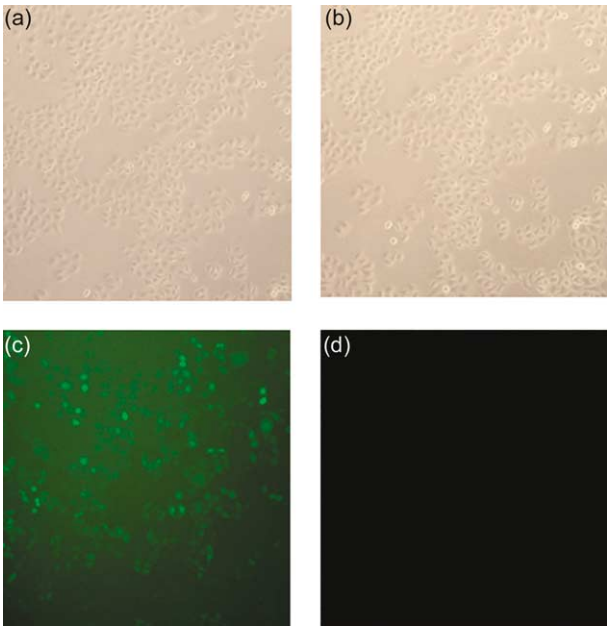

Figure 2 Light and fluorescence microscopy of HeLa cells exposed to 150 m.o.i. of either untreated AdGFP adenovirus or Microcyn-treated AdGFP. Only cells exposed to untreated AdGFP adenovirus were infected and appeared green under fluorescence microscopy (Panels a and c). In contrast, it was impossible to detect any fluorescent cells if the AdGFP had been treated with Microcyn for at least 1 min (Panel d), although cells appeared normal under light microscopy (Panel b). Original magnification×10.

detected if the virus had been treated with Microcyn for at least 1 min (Figure 2). In any case, cells exposed to the untreated or to the Microcyn-treated viruses always appeared normal under light microscopy (Figure 2). Taken together, these results demonstrate that virus exposure to Microcyn for 5 min achieves a $\log_{10}$ reduction factor in the viral load of 3 and that complete inactivation is achieved in 10 min of exposure to Microcyn.

Sporicidal activity of Microcyn against *B. atrophaeus*

Results of tests with three batches of Microcyn exposed to *B. atrophaeus* spores for 5 min are shown in Figure 3. Under these test conditions, Microcyn demonstrated complete inactivation of *B. atrophaeus* spores within 2–3 min. Within 1 min, a $\log_{10}$ reduction factor >1 in spore viability was achieved with Microcyn exposure. At 2 min, the $\log_{10}$ reduction factor was >4 in spore viability. Similar incubations using either 70% ethanol or 70% isopropanol (both in water) showed no reduction in

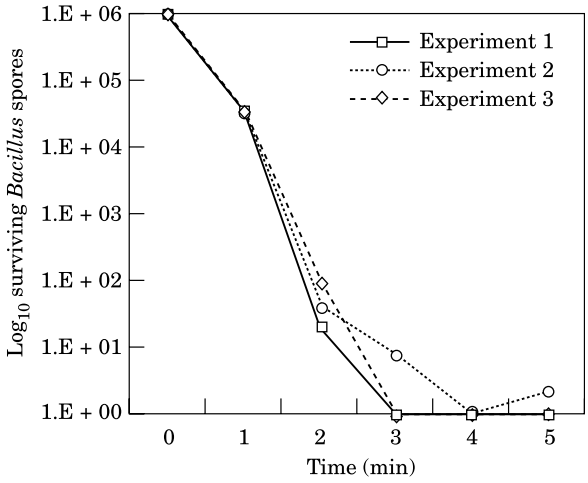

Figure 3 Sporicidal activity of Microcyn against *Bacillus atrophaeus*. 10^7 spores were challenged in 1-mL volumes consisting of 0.1 mL spore suspension and 0.9 mL Microcyn. At the indicated times, the incubations were neutralized with 9 mL TSB media and plate counts (1 mL total volume, 10^6 spore equivalents) were performed to assess surviving spores. The results of three experiments on different batches of Microcyn are shown.

B. atrophaeus spore viability within the incubation period of these tests (data not shown).

Discussion

The antimicrobial activity of Microcyn was tested against bacteria, spores, fungi and viruses under conditions of free organic matter in the laboratory. From our data, it can be concluded that Microcyn exerts a wide antimicrobial spectrum, as reported previously for acidic SOWs.[3,9,11,24–28]

We initially assessed the germicidal activity of Microcyn following the protocol described in the Mexican Official Norm, NMX-BB-040-SCFI-1999.[17] In compliance with the Mexican Norm, Microcyn completely killed all of the *S. aureus* and *E. coli* in 30 s of exposure at room temperature. However, a germicide does not automatically kill other pathogenic bacteria, spores, viruses, tuberculosis or fungi. Furthermore, since bactericides are specific, they are only useful when the pathogens are identified. Thus, we also tested the microbiocidal activity of Microcyn against other pathogenic bacteria and fungi of epidemiological significance. Accordingly, *P. aeruginosa*, *S. typhi* and *C. albicans* were completely destroyed in 30 s of exposure under the same test conditions.

To support claims of high-level disinfection capabilities of Microcyn, sporicidal tests against *B. atrophaeus* were conducted. Microcyn achieved

nearly a 10^5-fold reduction in spore viability within 2 min and a 10^6-fold reduction in 3 min of exposure. The mechanism of spore killing may be related to reactive chlorine and oxygen species damage to the inner membrane.[29] These results compare with those of Sterilox®, a product with three to four times higher available chlorine than Microcyn.[23,26] These results show that Microcyn could be a superior product in that it is pH neutral and has a longer shelf life (discussed below). Tests against spores of *Clostridium sporogenes* as required by the Association of Official Analytical Chemists are now being conducted.

Acidic SOWs have been successful in modifying the infectivity of HIV in a time- and concentration-dependent manner.[11,25] Thus, a test to substantiate virucidal effectiveness of neutral-pH Microcyn was also performed following the EPA protocol for disinfection of inanimate environmental surfaces.[20] For this purpose, we assayed the cytopathic activity and virus replication of HIV-1 (Ag p24 ELISA kit) with untreated and Microcyn-treated viruses. Experimental infection with control HIV-1 exerted a cytopathic effect and Ag p24 protein release into the supernatant in infected MT-2 cultures. In contrast, Microcyn treatment of HIV-1 for 5 min achieved a $\log_{10}$ reduction factor >3 in the viral load as measured in MT-2 cultures by both assays. These results comply with the level of efficacy that the EPA requires for virucidal activity against HIV-1 on inanimate surfaces.[21]

Since claims of virucidal activity for a product must also be restricted to those viruses that have actually been tested (e.g. HIV-1), we also tested the activity of Microcyn against DNA adenovirus type 5. In this case, we simulated laboratory in-use conditions with suspensions of viruses. In order to increase the sensitivity of the test, we also measured the fluorescent light emitted by AdGFP-infected cells with the use of a flow cytometer instead of using fluorescence microscopy. Under these strict conditions, we also achieved a $\log_{10}$ reduction factor >3 in the viral load after a 5-min exposure to Microcyn.

Together, these results show that Microcyn exerts a wide antimicrobial spectrum as stated above for acidic SOWs.[9,22-24] However, Microcyn could have major advantages over them. Firstly, because its shelf life is at least one year according to the manufacturer's label, it is not necessary to prepare Microcyn freshly, as required for acidic SOWs with short shelf lives.[23,25] Therefore, it is not necessary to purchase any equipment to produce SOW on-site or to hire specialized personnel for its operation. Furthermore, the low pH and high AFC level (>650 ppm) in acidic SOWs make them corrosive.[10,23,25] In fact, one company has voided the warranty on its endoscopes if acidic SOWs are used to disinfect them.[30] These obstacles, corrosion and instability, have been major limitations for the use of acidic SOWs in clinical practice in the past and could be overcome by neutral-pH, low-AFC agents such as Microcyn. Validation tests are now being conducted.

However, the concept of SOW to create a high-level disinfectant (or sterilant) maintains its appeal because the basic materials, saline solution and electricity, are inexpensive. In addition, the end product is non-flammable and there are no special requirements for handling or disposal. Therefore, SOWs such as Microcyn are environmentally safe agents and the costs derived from the disposal of toxic chemicals in hospitals could diminish by simply discarding them locally.

Altogether, these data suggest that Microcyn could play a significant role as an effective and safe disinfectant in hospital practice. Additional studies are now being conducted to determine if this solution may be used as an antiseptic and high-level disinfectant.

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