Pharmacognosy Communications
Volume 2 | Issue 1 | Jan-Mar 2012
www.phcogcommn.org

Research Article

Colloidal Silver (CS) as an Antiseptic: Two opposing viewpoints

Ian Cock[1,2], Shimony Mohanty[1,2], Alan White[2], Michael Whitehouse[3*]

[1]Environmental Futures Centre, Griffith University, Nathan, Qld. 4111, Australia. [2]Biomolecular and Physical Sciences, Griffith University, Nathan, Qld. 4111, Australia. [3]School of Medicine, Griffith University, Gold Coast, Qld. 4222, Australia.

ABSTRACT: Introduction: Despite its long history as an antiseptic, the image of CS has been badly 'tarnished' by opportunistic promoters, lack of quality controls (QC), deliberate misinformation and reprehensible scare tactics. This article evaluates some commercial colloidal silver (CS) preparations for their efficacy as antiseptic agents. **Aims:** We examined the potential medicinal value of commercially available CS preparations testing them by a) various chemical and physical criteria and also b) in vitro assays for bio-efficacy and safety. **Methods:** Antibacterial activity of CS preparations was determined by disc diffusion growth inhibition assays against a panel of pathogenic bacteria and fungi. Toxicity (LC50) was assessed by the *Artemia franciscanna* nauplii bioassay. **Results:** Of the 12 CS preparations tested, 10 (83%) showed antimicrobial activity, albeit with varying specificity and efficacy. Argyrol and HLY displayed the broadest specificity, inhibiting the growth of all 14 bacteria tested (100%). These particular preparations also inhibited the growth of 3 (100%) and 2 (67%) of the fungal species tested respectively. The other preparations had varying degrees of efficacy and specificity. In general, only low concentrations of CS were required to achieve antibiotic activity, with MIC values ≤ 5µg/ml for some preparations against some microbial species. In contrast, 2 colloidal gold preparations were completely devoid of antimicrobial activity. All CS preparations were either nontoxic or displayed low toxicity in the *Artemia franciscanna* nauplii bioassay, further confirming their potential as antiseptics for medicinal use. **Conclusions:** The commercial CS preparations varied widely in their potential utility as complementary medicines. The establishment of quality controls for both antimicrobial efficacy and incipient toxicity to animal cells are badly needed. However, this study does demonstrate the effective antiseptic activity of certain CS preparations indicating that they should be seriously considered as medicinals for topical use e.g. treating burns, periodontitis, thrush etc.

Key words: silver nanoparticles, colloidal silver, inorganic pharmacognosy, antimicrobial, Ag^{o}, Ag^{+}

INTRODUCTION

So often there is a singular lack of pharmacognosy – literally knowing your drug – when procuring, or even preparing, an antiseptic 'colloidal silver' (CS) preparation. The history of silver pharmacology is confused by lack of standardized preparations other than topical silver nitrate used as a caustic or soluble disinfectant, that rapidly stains surrounding tissues black.[1]

CS preparations for topical and/or oral use were subsequently developed to overcome the astringency of, and staining by, simple silver salts[2] while retaining the long-known antiseptic properties of metallic silver.[3] The older preparations, pre-1910, usually contained high levels of oxidized silver (Ag^{+}), not precipitated by isotonic salt solutions, made from silver nitrate and various carrier proteins or derived polypeptides. Argyrol® is one such product still extant today and formerly much used as an eye disinfectant to prevent neonatal blindness.

Finely dispersed silver metal (Ag^{o}) preparations were originally made as pigments (green-yellow) for medieval glass, manufactured by including a silver salt with a reducing agent in the glass-making process. In the 19th Century aqueous metallic/zerovalent silver colloids/hydrosols were prepared *chemically* by reducing silver salts with organic reductants e.g. sodium citrate (the so-called Carey Lea's preparation, 1889[4]), sodium tannate, etc. Their antiseptic properties were generally of less interest than their physical properties e.g. preparing mirrors, for electronic applications.

Since 1911, the availability of silver metal dispersions prepared *electrolytically* from silver rods/plates provided bio-accessible silver in the zerovalent state (Ag^{o}) and proved

*Correspondence:
(Prof) M Whitehouse, PO Box 68, Stones Corner, Qld. 4120.
E-mail: whitehousemd@spin.net.au; Fax: +617 3349 3006
DOI: 10.5530/pc.2012.1.9

life-saving in World War I e.g. treating 'trench fever', a rickettsial infection.[5]

Until the availability of sulfonamides (1930s) and antibiotics (1940s), CS preparations were widely used whenever less effective chemosterilants (alcohol, hypochlorite, iodine, phenols) proved inadequate. Some questionable activities then contrived to legally remove CS from the public domain in the USA and ensure only patented anti-infective organic pharmaceuticals could be sold to the public.

With the resurgent interest in do-it-yourself (DIY) medicine, electrolytic CS generators have become widely available for home use.[3, 6] Such generators produce variable mixtures of soluble Ag^+ and Ag^o particles, together with some less soluble oxidised Ag products e.g. oxide, carbonate. These CS products usually contain only low quantities of silver of the order 5-50 ppm (mg/L). Depending on the qualities of the silver electrodes (<99.99 per cent pure) and of the water used as electrolyte, significant minor impurities may sometimes contaminate these DIY preparations e.g. As, Cu, Mg, Pb.

Currently the US Food and Drug Administration (FDA) and the Australian Therapeutics Goods Administration (TGA) both proscribe the medicinal use of CS and prohibit making any claims regarding its efficacy. These administrations dogmatically proclaim that CS is both inefficacious *and* toxic (surely an enigma). Nevertheless, the TGA does recognise the value of CS for sterilising water, surely a medicinal property but apparently not an (illegal) claim. (Another enigma.) However, many CS preparations are available in Australia, New Zealand, UK and USA from pharmacies, health food stores and internet sales. Their labels usually indicate only the total silver content. Some also carry such disingenuous statements as, 'We are not allowed by law to tell you what this product is good for'.

It is important for the FDA, TGA and other drug regulatory bodies to have available some practical criteria for sensibly assessing CS products and that these criteria are available to the general public. Otherwise, the present absurd restrictions will be mindlessly perpetuated, despite the accepted use of silver antibiosis by NASA, the US Army, various NGO's and other frontline agencies facing infections in the field. The current study examined the antiseptic properties of some locally available commercial CS preparations against a panel of microbial agents, to see whether their continuing usage as antiseptic agents might be justified.

MATERIALS AND METHODS

Colloidal silver samples

CS samples were commercial products (mostly from Brisbane health stores) with the exception of 3 reference samples. Two of these were prepared chemically and supplied as 10 nm and 50 nm sized nanoparticles (Nano Composix, San Deigo CA). The third reference CS was prepared electrolytically with a mean particle diameter of 33 nm (kindly donated by H. Laroo, Security Research, Ipswich Qld). For antimicrobial testing, this was concentrated to 135 ug/ml.

Colloidal gold preparations

One gold colloid preparation was obtained commercially from a local Brisbane health store. A second colloidal gold preparation was prepared by reducing $AuCl_4$ with sodium citrate and contained particles with a mean diameter of 37 nm.[7]

Physical and chemical characterisation of colloidal preparations

One CS sample (Argyrol branded as Argyrex) was obtained as a semi-solid and readily dispersed into distilled water.

Aqueous samples were routinely examined for the following properties:

- Total silver content as ppm (mg/L) determined by atomic absorption spectroscopy (AAS) or by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
- pH and electrical conductivity
- colour and plasmon absorption over the range 410-430 mm
- light scattering at 532 nm, measured at right angles (90 degrees) in arbitary units/ppm using a 5 milliwatt green laser and USB 2000 photometer (Ocean Optics, Dunedin, FLA)
- Free/contaminating silver ions (Ag^+) by measuring the amount of silver either a) removed by precipitation with KSCN or after shaking with a suspension of the cation exchanger Na^+ Amberlite IR-120 (Rohm and Haas, Philadelphia, Pa) or b) by cyclic voltammetry to measure electro-reducible species.
- Product stability: presence/absence of visible precipitate after standing at room temperature in the dark for periods up to three months.
- Median particle size of the dominant population was measured using a Nicomp 370 instrument (Particle Sizing Systems, Santa Barbara CA) and are expressed as the median value (by number weighting) of the dominant species (≥ 99.5 %).

Some samples were further examined for contamination by other metals or arsenic by ICP-MS. Samples which showed zero silver content by AAS (due to matrix effects) were specifically re-analysed for silver by ICP-MS

Antimicrobial screening

Test microorganisms

All microbial strains were obtained from Michelle Mendell and Tarita Morais, Biomolecular and Physical Sciences,

Griffith University, Australia. Stock cultures of *Aeromonas hydrophila, Alcaligenes faecalis , Bacillus cereus, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas fluorescens, Salmonella newport, Serratia marcescens, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus pyogenes* were subcultured and maintained in nutrient broth at 4 °C. Stock cultures of *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* were subcultured and maintained in Sabouraud media at 4 °C.

Evaluation of antimicrobial activity

Antimicrobial activity was determined using a modified disc diffusion method previously described.[8, 9] Briefly, 100 µl of the test bacteria/fungi were grown in 10 ml of the appropriate fresh broth until they reached a density of approximately 10^8 cells/ml of bacteria or 10^5 cells/ml for fungi (as determined by direct microscopic determination). One hundred microliters of microbial suspension was spread onto agar plates prepared with the broth in which they were maintained.

CS were tested using 5 mm sterilised filter paper discs. Discs were impregnated with 10 µl of the test sample, allowed to dry, then placed on the microbially inoculated agar plates. The plates were allowed to stand at 4 °C for 2 hours before incubation with the test microbial agents. Plates inoculated with *Alcaligenes feacalis*, *Aeromonas hydrophilia*, *Bacillus cereus*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Proteus mirabilis, Pseudomonas fluorescens*, *Serratia marcescens*, and *Candida albicans* were incubated at 30 °C for 24 hours, then the diameters of the inhibition zones were measured in millimetres. Plates inoculated with *Escherichia coli*, *Salmonella newport*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were incubated at 37 °C for 24 hours, then the diameters of the inhibition zones were measured. *Aspergillus niger* inoculated plates were incubated at 25 °C for 48 hours before measuring the zones of inhibition. All measurements were to the closest whole millimetre. Each antimicrobial assay was performed in triplicate. Mean values are reported in this study. Standard discs of ampicillin (2 µg), chloramphenicol (10 µg) and nystatin (100 µg), obtained from Oxoid Ltd. Australia served as positive controls for antimicrobial activity. Filter discs impregnated with 10 µl of distilled water were used as negative controls.

Determining Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the colloidal silver samples were determined by a modified disc diffusion method[10, 11] across a range of doses. The samples were serially diluted in deionised water. Discs were impregnated with 10 µl of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was performed as outlined above and graphs of the zone of inhibition versus concentration were plotted for each CS preparation. Linear regression was used to calculate the MIC values.

Toxicity Screening

Reference Toxins for Toxicity Assay

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 1.6 mg/ml solution in distilled water and serially diluted in artificial seawater (see below) for use in the *Artemia franciscana* nauplii bioassay. Mevinphos (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate), obtained from Sigma-Aldrich as a mixture of cis (76.6%) and trans (23.0%) isomers, was prepared as a 4 mg/ml stock solution in distilled water. This was serially diluted in artificial seawater for use in the bioassay.

Evaluation of Toxicity

Toxicity was measured using the *Artemia franciscana* nauplii lethality assay originally developed by Meyer *et al*[12] for screening phytotoxins. The assay was modified as previously described.[13, 14] Briefly, *Artemia franciscana* Kellogg cysts were obtained from North American Brine Shrimp, LLC, USA (harvested from the Great Salt Lake, Utah). Synthetic seawater was prepared using Reef Salt, AZOO Co., USA. Seawater solutions at 34 g/l distilled water were prepared prior to use. 2 g of *A. franciscana* cysts were incubated in 1 L synthetic seawater under artificial light at 25°C, 2000 Lux with continuous aeration. Hatching commenced within 16-18 h of incubation. Newly hatched *A. franciscana* (nauplii) were used within 10 h of hatching. Nauplii were separated from the shells and remaining cysts and were concentrated to a suitable density by placing an artificial light at one end of their incubation vessel and the nauplii rich water closest to the light was removed for biological assays. Seawater (400 µl) containing approximately 43 (mean 43.5, n = 248, SD 12.8) nauplii were added to wells of a 48 well plate and immediately used for bioassay. The CS preparations were diluted in seawater for toxicity testing. 400 µl of the samples and the reference toxin were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 Lux). A negative control (400 µl seawater) was run in at least triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead nauplii counted. The nauplii were considered dead if no movement of the appendages was observed within 10 seconds. After 72 h all nauplii were sacrificed by adding acetic acid and counted to determine the total number per well. The LC_{50} with 95% confidence limits for each treatment was calculated using probit analysis.[15]

RESULTS

Characterisation of commercial CS samples

Silver content ascertained by AAS varied from 64 % (Courtenays Original CS) to 207 % (Burke-Hale) of the

quantities displayed on the labels (Table 1). A few samples (notably some originating from Malaysia) displayed zero Ag content by AAS but had an Ag content plus or minus 10% the content indicated on the label when re-examined by ICP-MS (unpublished results). This disparity may be due to matrix effects inherent in the AAS assay. These preparations were considered atypical and were not included in the subsequent antimicrobial/toxicity studies.

pH was also quite variable, ranging from 8.1 to 10.7. Further CS preparations tested prior to these studies displayed even greater variability, with pH values as low as 4.7. These low pH CS preparations were considered atypical and were not included in the subsequent antimicrobial/toxicity studies.

Most samples were colourless, some opalescent and only a few had distinctive yellow-brown tints. Semi-quantitative determinations of scattering of green laser light gave values ranging from 6 (Nanosized Silver) to 98 (HLY) arbitrary units/ppm silver.

The content of free ionic silver was difficult to ascertain as some CS samples appeared to have a very high Ag^+ content (>80% by conventional analysis); but still caused significant light scattering due to the presence of colloidal particles. This may be explained by the fact that even reference CS samples (prepared by the chemical reduction of Ag^+) were variably removed by low speed centrifugation techniques used in separating the Ag^+.

Conductivities ranged from 18 (HLY) to 2540 µS (Argyrol); those with high conductivity generally showing strong absorption for Na^+ in the AAS assay. Other impurities detected included calcium (often high), arsenic, lead and copper indicating use of impure water or silver metal for preparing CS. Notably contaminated samples were excluded from bioassays. Only one sample, contaminated with Pb, significantly exceeded current Australian standards for permissible metallic constituents in drinking water and was not included in subsequent antimicrobial/toxicity studies.

Antimicrobial activity

Antimicrobial activity of each CS preparation (10 µl) was tested in the disc diffusion assay against a panel of 14 bacterial, 2 fungal and 1 yeast species (Table 2). Of the 12 CS preparations tested, 10 (83 %) were found to be have inhibitory activity, albeit with varying specificity and efficacy. Argyrol and HLY displayed the broadest specificity, inhibiting the growth of all 14 bacteria tested (100%). These preparations also inhibited the growth of 3 (100 %) and 2 (67 %) of the fungal species tested respectively. Two other CS preparations (Silver Magic and Suttons Bali Belly Buster) also displayed broad antibacterial activity, inhibiting the growth of 11 (78 %) and 9 (64 %) of the 14 bacterial species tested respectively. Both these latter CS preparations displayed greater inhibitory activity towards Gram-negative bacteria than to Gram-positive bacteria. The Silver Magic sample inhibited the growth of 9 of the 10 Gram-negative bacteria (90 %) tested but only 2 of the 4 (50 %) of the Gram-positive bacteria tested. Similarly, Suttons Bali Belly Buster inhibited the growth of 7 Gram-negative bacteria (70 %) and only 2 (50 %) Gram-positive bacteria respectively.

Other CS preparations showed varying degrees of antimicrobial activity and specificity. Suttons Original CS also had a relatively broad specificity for Gram-negative bacteria, inhibiting the growth of 6 of the 10 Gram-negative bacteria (60 %) tested but did not inhibit the Gram-positive bacteria. Ionic silver (as $AgNO_3$), Courtnays CS, Nano Xact (10 nm), Burke's CS and Holland and Barrett CS inhibited 4 (40 %), 3 (30 %), 2 (20 %), 2 (20 %) and 0 (0 %) of the Gram-negative bacteria respectively. Neither the silver nitrate nor Courtnays CS preparation inhibited the growth of any of the Gram-positive bacteria tested, whilst Nano Xact (10 nm), Burke's CS and Holland and Barrett CS each inhibited a single (25 %) Gram-positive bacterium. Both the Nano Silver preparation and the Nano Xact (50 nm) failed to inhibit the growth of any bacteria.

These preparations varied widely in terms of total silver content (ppm) (Table 1). It is therefore likely that some preparations may appear to have low efficacy due to the doses tested. For example, the Holland and Barrett CS preparation inhibited the growth of only a single bacterium. However, this particular preparation had a very low silver content (2.5 ppm) compared to the other commercial CS products which was below the MIC of many of the other CS preparations. So it is possible that this preparation (Holland and Barrett) might have displayed broader specificity at a higher silver content. In general only low concentrations of CS were required to achieve antibiotic activity, with MIC values ≤ 5µg total silver/ml for some preparations against some microbial species. In contrast, 2 nanoparticulate colloidal gold preparations (30-70 nm diameter) were completely devoid of antimicrobial activity. Neither of these colloidal gold preparations was effective against any of the bacteria or fungi tested.

Toxicity Studies

All CS preparations were serially diluted in artificial seawater for toxicity testing in the *Artemia franciscanna* nauplii lethality assay (Table 3). For comparison, the reference toxins potassium dichromate and Mevinphos were also tested. Both reference toxins were rapid in the induction of toxicity, with mortality noted within 3 hours of exposure (unreported results). Potassium dichromate was particularly toxic with LC50 values at 24 h of 86.3 µg/mL. In contrast, none of the CS preparations induced mortality significantly above that of the seawater control within the first 24 h of exposure.

Table 1: Characterisation of (a) some commercial CS products (shown in green), (b) some reference CS preparations (shown in grey) and (c) some colloidal gold products (shown in yellow).

Physical Properties	F & B	Orig CS	BBB	SM	IS	NzS	H & B	Bion	Argyrol	NX 10	NX 50	HLY	Gold Colloid	Gold (37nm)
Total Silver Content (from company)	9-14	16-20	NS	25-30	10	10	3	3	NR	20	20	13.5	NR	NR
Total Silver Content (measured)	9.1	12.8	18.5	38.8	10.7	10	2.8	6.2	14500	20	20	13.5	NR	NR
Conductivity (µSiemens)	105	72	80	113	56	1096	60	97	2540	777	635	18	365	NR
pH	9.1	9.4	8.4	8.2	8.5	10.7	8.9	9	8.7	8.7	8.4	8.1	10.1	NR
Light Scattering Index	15	19	14	10	29	6	7	15	7	24	45	98	5	NR
Median Nanoparticle Size (µm)	33±4.0	33±4.1	32.6±4.2	33±4.1	33 ± 4.1	73.5±9.7	33±4.1	33±4.1	33±4.0	32.5±4.2	33.1±4	33±4.1	286±58	32.6±4.2

F & B = First and Best, **Courtenay**, Montville Qld Australia; Orig CS = Original CS, **Suttons**, WA Australia; BBB = **Bali Belly Buster, Suttons**, WA Australia; SM = **Silver Magic** (extra strength), Lowood Qld Australia; IS = **Ionic Silver**, Mineral Solutions, USA; NzS= **Nanonized Silver**, Trust Nature, Malaysia; H & B = Silver protein (dairy), **Holland and Barrett**, UK; Bion = Bionaid $Ag.H_2O$, **Burke Hale**, USA; **Argyrol** = Mild Silver Protein SS 10% Argyrex, USA; NX 10/50 = **NanoXact**, Nano Composix, San Deigo USA; **HLY** = electrolytic preparation, Hans Laroo, Ipswich Australia. Nanoparticle sizes are expressed as median values (± SD) of the dominant population (≥99.5%). NR = not recorded.

Table 2: Relative antimicrobial potencies of (a) 9 commercial (shown in green) and 3 reference (shown in grey) CS samples, (b) 2 gold hydrosols (shown in yellow) and (c) 4 standard antibiotic discs (shown in blue).

	MIC (µg/ml)													Inhibition by Antibiotics			
	Courtnays	Suttons	Suttons Bali Belly Buster	Silver Magic	Ionic Silver	Nano Silver	Holland and Barrett	Burke's	Argyrol	Nano Xact (10nm)	Nano Xact (50nm)	HLY	Gold Colloid	Gold (37nm)	Ampicillin (2 µg)	Chloramphenicol (10 µ g)	Ciprafloxicin (2.5 µg)	Nystatin (100 µg)
Gram negative rods																		
Aeromonas hydrophilia	6.3	7.4	0.1	17.4	10.7	–	–	–	7.1	–	–	2.5	–	–	+	+	+	NT
Alcaligenes faecalis	–	4.1	–	0.3	10.7	–	–	6.2	4.5	20	–	6.6	–	–	+	+	+	NT
Citrobacter fruendii	–	13	0.6	7.7	–	–	–	6.2	8.4	–	–	1	–	–	+	+	+	NT
Escherichia coli	–	–	–	12.2	–	–	–	–	4.9	–	–	9.1	–	–	+	+	+	NT
Klebsellia pneumoniae	7.6	0.8	0.3	10.2	–	–	–	–	1	–	–	4.1	–	–	+	+	+	NT
Proteus mirabilis	–	–	–	0.3	–	–	–	–	0.7	–	–	2.5	–	–	+	+	+	NT
Pseudomonas fluorescens	9.1	12.8	15.4	0.6	10.7	–	–	–	16	–	–	0.2	–	–	+	+	+	NT
Salmomella newport	–	12.8	0.5	11.8	–	–	–	–	8.4	20	–	1.6	–	–	+	+	+	NT
Seratia marescens	–	–	0.8	–	6.7	–	–	–	18.5	–	–	5.2	–	–	–	+	+	NT
Shigella sonnei	–	–	0.8	0.6	–	–	–	–	7.1	–	–	6.1	–	–	+	+	+	NT
Gram positive rods																		
Bacillus cereus	–	–	12.9	5.5	–	–	–	–	4.6	–	–	13.7	–	–	+	+	+	NT
Gram positive cocci																		
Staphylococcus aureus	–	–	–	–	–	–	2.5	6.2	1.5	20	–	10.2	–	–	+	+	+	NT
Staphylococcus epidermidis	–	–	–	–	–	–	–	–	16.1	–	–	2.7	–	–	+	+	+	NT
Staphyloccocus pyogenes	–	–	12.9	0.6	–	–	–	–	2.2	–	–	11.3	–	–	+	+	+	NT
Fungi																		
Aspergillus niger	–	–	–	–	–	–	–	–	1207	–	–	–	–	–	–	–	+	–
Candida albicans	–	–	–	–	–	–	–	–	278.8	–	–	135	–	–	–	–	+	+
Yeast																		
Saccharomyces cerevisiae	–	–	–	–	–	–	–	–	129.9	–	–	135	–	–	–	–	+	+

Numbers indicate the mean MIC values of triplicate determinations; – indicates no inhibition; + indicates that the antibiotic standard disc inhibited microbial growth and an MIC was not obtained (as only a single dose disc was tested); NT indicates that the antibiotic disc was not tested against an individual bacterium.

Table 3: LC50 (95% confidence interval) for brine shrimp nauplii exposed to (a) 9 commercial (shown in green) and 3 reference (shown in grey) CS samples, (b) 2 gold hydrosols (shown in yellow) and (c) positive (potassium dichromate and mevinphos) and negative (seawater) controls.

	LC50 (µg/mL)														Control (µg/mL)		
	Courtnays	Suttons	Suttons Bali Belly Buster	Silver Magic	Ionic Silver	Nano Silver	Holland and Barrett	Burke's	Argyrol	Nano Xact (10nm)	Nano Xact (50nm)	HLY	Gold Colloid	Gold (37nm)	Potassium Dichromate	Mevinphos	Seawater
24 hour LC50	–	–	–	–	–	–	–	–	–	–	–	–	–	–	86.3	1346	–
48 hour LC50	–	–	–	–	–	–	–	–	–	–	–	46.4	–	–	80.4	505	–
72 hour LC50	3.8	5	1.5	–	4.5	–	–	–	–	–	–	3.1	–	–	77.9	103.9	–

Numbers indicate the mean LC50 values of triplicate determinations; –denotes values that were not obtained as ≥ 50 % mortality was not obtained at this time point for any concentration tested.

Indeed, only the HLY preparation induced high enough mortality for the determination of an LC50 (46.4 µg/mL) within the first 48 h of exposure. Whilst an increased induction of mortality above that of the seawater control was also evident for the Courtneys, Suttons, Suttons Bali Belly Buster and Ionic Silver preparations at 48 h, the mortality was below 50%. Therefore it was not possible to determine an LC50 for these CS preparations and they were considered of low toxicity at 48 h. Mortality induction was above 50% for the Courtneys, Suttons, Suttons Bali Belly Buster and Ionic Silver preparations at 72 h enabling the determination of LC50 values. However, Artemia nauplii toxicity studies usually only reports LC50's at 24 h and/or 48 h. Therefore the reporting of a 72 h LC50 may be considered excessive. All CS preparations tested in this study can therefore be considered either nontoxic (Silver Magic, Nano Silver, Holland and Barrett, Burkes, Agyrol and the two Nano Xact CS preparations) or of low toxicity (Courtneys, Suttons, Suttons Bali Belly Buster, Ionic Silver and HLY CS preparations).

Neither of the gold hydrosols tested in this study induced mortality above that of the seawater control at any concentration/time point tested. It was therefore not possible to determine LC50 values for the gold hydrosols and they are therefore also considered nontoxic.

DISCUSSION

CS preparations have been used as medicinal agents for centuries. In ancient times, metallic silver was used as a disinfectant to purify and store drinking water.[16] More recently, silver has been used for similar purposes on the Apollo spacecraft,[17] NASA space shuttles[18] and the MIR space station.[19] In the 19th century, silver preparations were used as antiseptics post surgery, in dentistry, as well as for the prevention of ophthalmia neonatorum in newborn children.[16] CS proved life-saving in World War I and gained widespread acceptance as an antiseptic agent.[5] In the 1920's the US Food and Drug Administration (FDA) approved CS for wound treatment. Later, the discovery of sulphonamide, penicillin, and macrolide antibiotics led to a decline in the use of CS antibiotics. However, the development of super-resistant bacterial strains has rekindled some interest in colloidal silver as a medicinal agent. Despite the long history of effective usage of silver preparations as antiseptic agents as well as their acceptance by the FDA in the 1920's, the US FDA and the Australian Therapeutics Goods Administration (TGA) have recently reversed this approval. They now prohibit individuals making any claims regarding the efficacy of commercial CS preparations,[20, 21] offering next to no scientific evidence to justify this position.

The present study has verified the antimicrobial properties of a selection of commercially available CS preparations tested in vitro against a panel of pathogenic bacteria and fungi. Indeed, 83% of the CS preparations tested were able to inhibit the growth of 1 or more bacterial species. Numerous recent publications have also demonstrated the antimicrobial activity of laboratory synthesised colloidal silver preparations. A poly-N-vinyl-2-pyrrolidone (PVP)

stabilised silver nanoparticle preparation displayed potent antibacterial activity against *S.aureus* and *E. coli*.[22] Silver nanoparticles synthesised by the inert gas condensation method were effective at inhibiting the growth of *E. coli*, albeit at a higher MIC (60 μg/ml) than seen in our study or by Cho et al.[22] In an interesting recent report, the bacteria *Klebsiella pneumonia* was used to reduce aqueous Ag^+ to produce a biogenic CS preparation which was an effective inhibitor of *S.aureus* and *E. coli*. [23] This same report also documented the ability of these biogenic silver nanoparticles to increase the efficacy of various other antibiotics, highlighting the potential of CS/antibiotic co-treatments.

By contrast, other studies have questioned the efficacy of CS preparations as antiseptic agents.[24, 25] The CS preparations tested in one of these studies[24] were produced from silver salts by chemical reduction. Studies in our laboratory have also shown that chemically produced CS preparations may have lower antibacterial activities than CS produced by other methods.[26] The other study[25] used CS preparations with low silver content (5 ppm). Our results confirm the low efficacy of CS preparations with similarly low concentrations (e.g. the Holland and Barrett CS preparation with only 2.5 ppm silver).

Bacteria may also develop resistance to ionic silver preparations.[27] Several studies have tested CS preparations against antibiotic multi-resistant bacterial strains. It is possible that the low efficacies reported from some of these studies are related to the bacterial strains tested. Presumably, it is these opposing reports on the efficacy of CS as antimicrobial agents that led to both the FDA and the TGA prohibiting general claims regarding the efficacy of all CS preparations.[20, 21] Furthermore, these administrations proclaim CS is also toxic without considering whether the toxicities are due to contaminant Ag^+, rather than colloidal Ag^0.

It is likely that the opposing viewpoints of the efficacy of CS as antimicrobial agents may be due to the nature of the CS preparation itself. The term "colloidal silver" is a blanket designation used to refer to at least 4 different types of product:

1. Silver impregnated proteins prepared from silver salts and stable in NaCl.
2. Particulate silver metallic dispersions prepared by:
   a. electrolytic dissolution of metallic silver rods.
   b. chemical reduction of silver salts with excess reductant ensuring virtual absence of free Ag^+.
   c. (dry) sintering of metallic silver.

It is very important to understand that whilst all of these products may be marketed as CS, not all are colloidal suspensions of metallic silver and the different products may have very different physical properties as well as bio-efficacies. The quality of CS preparations will vary between products and may also vary from batch to batch of the same product. So CS preparations may vary widely in terms of efficacy and indeed, probable safety. Currently, there are no industry standards for manufacturers to adhere to and no guidelines and government regulation of the industry (aside from prohibiting claims of efficacy).

Historically the original types of CS were the silver-protein/polypeptide products prepared by reacting silver salts (Ag^+) with a polypeptide 'carrier' (class 1 above). The Argyrol and the Holland and Barrett products examined in this study are 2 examples of silver-protein preparations still available today. Argyrol had the broadest antimicrobial specificity of any CS preparation tested in this study. While Argyrol inhibited the growth of all bacterial and fungal species tested, it was not particularly potent: the recorded MIC's against some of microbial species is relatively high. For example the MIC of Argyrol for *S. marcescens* (18.5 ppm) is quite high compared to other CS preparations tested. This is even more apparent for the antifungal activity of Argyrol. Whilst Argyrol was the only preparation which inhibited all 3 fungal species tested, in all cases the MIC was quite high (≥ 100 ppm). It is possible that similar activity of the other CS preparations was not evident either because their silver content was either too low or they contained the wrong size of particles for antifungal activities.

The most commonly available class of CS products seem to be the electro-CS preparations (class 2a above). These are usually prepared by low voltage electrolysis using silver electrodes in deionised water yielding dispersed metallic silver preparations, ranging from 2-150 ppm total Ag. Ideally, this procedure would produce pure silver hydrosols suspended in water without contaminants. The clusters of silver atoms constituting the metallic nanoparticle in these preparations generally carry a positive electrical charge.[28] Most of the CS preparations examined here were electro-CS preparations. The most effective reference CS preparation with antimicrobial activity (HLY) was produced by electrolysis.

The third type of CS preparations (class 2b) are produced chemically by adding reagents (e.g. reducing agents) to soluble silver salts (Ag^+) to produce a hydrosol (water dispersible) form of zerovalent metallic silver (Ag^0). As with the other types of CS, the concentration of these CS preparations can vary widely, depending on the preparative procedures employed. The resultant silver particles generally carry a negative electrical charge,[28] the particles being readily precipitated by charged cations (e.g. Al^{3+}, La^{3+}). However, even these CS preparations will invariably contain non-

silver contaminants (e.g. buffers, reductants), which may affect their stability, efficacy and toxicity.

The fourth class of CS preparation (class 2c above), sometimes referred to as "powdered silver", is obtained when a silver wire is disintegrated by a high voltage electrical discharge analogous to an old photographic flash bulb. The resultant silver 'dust' is either dispersed into aqueous milieu, or added directly to creams and salves for topical usage. No "powdered" CS preparations were tested in this study. Therefore we can not comment on their antimicrobial potential or toxicity.

Most of the commercial CS preparations tested (7 of the 12 tested) were completely nontoxic at the concentrations supplied. Of the few preparations that were toxic (5 of the 12 tested), toxicity was low and then only after extended exposure. This finding opposes the opinion of the FDA and TGA. In a 2007 report, the TGA describes 4 cases of silver toxicity resulting from the ingestion of "homemade" CS preparations.[21] However, this report significantly failed to define the nature of these CS preparations (i.e. purity and how they were made), nor the levels of CS ingested. This same report also states that the TGA has received no reports of toxicity associated with "legitimate therapeutic goods containing presentations of silver that remain appropriate". Whilst not citing examples of toxicity linked to CS treatment, the FDA issued a ruling in 1999 stating that CS preparations are not recognised as safe or effective.[20] As with the TGA report, no discrimination was made in this ruling between the different classes of CS products, the physiochemical properties of the colloids (size, ionic state etc), nor the dosage and method of administration. A further report by a US government agency (US Dept of Energy) is also sometimes cited as proof of the toxicity of CS preparations.[29] This study reported that exposure to high doses of silver (specifically silver nitrate and silver oxide) may result in a range of symptoms including irritation of the skin, eyes, gastrointestinal and respiratory tracts, and mucous membranes as well as more serious complications, and even death at very high doses. However, whilst this report is cited as proof of the toxicity of CS, the author makes it very clear that she is reporting on the toxicity of silver in general, not CS specifically.

The only reports of CS toxicity in humans relate to the consumption of CS (not its topical use) and these describe relatively mild responses. The worst adverse reaction documented from the medicinal usage of CS is argyria, a condition characterised by a bluish discoloration of the skin.[2, 30, 31] Argyria is primarily a cosmetic condition which causes no discomfort and has no other known side effects. With argyria the skin discolouration may be misdiagnosed as cyanosis, methaemoglobinaemia or haemochromatosis leading to inappropriate treatment. It is possible that CS ingestion may interfere with the absorption and thus bioavailability of some drugs, thus reducing their effectiveness. Further studies are needed to examine the consequences arising from using CS orally. Interestingly, we were unable to find any reports of argyria directly attributed to the usage of CS preparations produced by electrolysis, which may contain a lesser content of impurities.

The antibacterial activity[32] and toxicity[33] of silver nanoparticles have previously been related to the size of the CS particle rather than to the concentration of silver alone. The correlation of toxicity with particle size may be fortuitous as previous studies indicate that antimicrobial efficacy may have an inverse relationship with toxicity. Whilst previous studies indicate that CS toxicity decreases with decreasing particle size, efficacy appears to increase with decreasing size.[31] Thus it is likely that a CS preparation with small particle size would be likely to have greater antibacterial activity, yet lower toxicity, than preparations containing larger metallic particles. Furthermore, production of these small particles by protocols which diminish the chances of contamination (e.g. electrolysis) may produce somewhat safer preparations.

Our studies confirm the dependency of efficacy on particle size. Two preparations of different particle size at the same concentration from the same supplier (Nano Xact) were tested in these studies. Only the CS preparation with the smaller particles (10 nm) inhibited the growth of any bacteria, whilst the 50 nm CS preparation was completely ineffective. Furthermore, antimicrobial efficacy can also depend upon the shape of the CS nanoparticles. In some instances, triangular nanoparticles may be more effective antimicrobial agents than spherical nanoparticles, which in turn are more effective than rod shaped particles.[34] Further studies are required to examine the relationship between colloid size and shape with antimicrobial efficacy and safety.

As efficacy and safety may be related to the various factors described above (concentration, particle size, the charge on the nanoparticulate silver, possible contaminations), quality control of CS preparations is required to ensure reproducible medicinal properties. Further studies are needed to determine the optimum conditions to produce non-toxic, higher efficacy CS preparations. Rather than placing a blanket ban on statements concerning the antibacterial activity of CS, regulatory bodies such as the FDA and the TGA may be better servants of thier communities by establishing Quality Control guidelines and allowing (instead of forbidding) further research on silver pharmacognosy; so helping biomedical scientists to learn more about the safe and effective usage of CS, a product that has long been used and will no doubt continue to be used as a topical antiseptic.

CONCLUSIONS

The commercial CS preparations tested in this study varied widely in their potential utility as complementary medicines. Quality controls for their content, antimicrobial efficacy and incipient toxicity to animal cells are certainly needed. This study clearly demonstrated the effective antiseptic activity of some CS preparations, indicating that they should still be seriously considered as medicinals for topical treatments e.g. burns, periodontitis, thrush. Concerns about safety (previously raised by the US FDA and the Australian TGA) need to be debated on scientific grounds. Claims about inefficacy are meaningless without attempts to define composition, concentration and bio-evaluation.

ACKNOWLEDGEMENTS

We are indebted to Prof. J. Ng, National Centre for Environmental Toxicology, Coopers Plains, Australia for ICP-MS analyses.

REFERENCES

1. Higginbottom J. An essay on the application of the lunar caustic: in the cure of certain wounds and ulcers. London: Longman Rees Orme Brown & Green, 147 pp; 1826. Available from Kessinger Publishing: www.kessinger.net
2. Hill WR, Pillsbury DM. Argyria. The pharmacology of silver. Baltimore: Williams & Wilkins Co. 1939.
3. Hancock GL. Colloidal Metallic Silver, 2nd edition. Kalbar: Kalaya Publications. 2011. Available from www.kalayaproducts.com.au
4. Frens G, Overbeek JThG. Carey Lea's colloidal silver. Kolloid Z.uZ.fur Polymere 1969; 233:922-929.
5. Sweet JE, Wilmer HP. A treatment for trench fever. The Lancet 1919; 252-254.
6. Jefferson W. Colloidal silver today. The all-natural wide-spectrum germ killer. Summertown TN USA 38483. Healthy Living Publications 2003, pp63.
7. Keating CD, Musick MD, Keefe MH, Natam MJ. Kinetics and thermodynamics of Au colloid monolayer self-assembly. J Chemical Education 1999; 76: 949-955.
8. Cock IE. Antimicrobial activity of *Aloe barbadensis* Miller leaf gel components. The Internet Journal of Microbiology 2008; 4 (2).
9. Cock IE. Antimicrobial activity of selected Australian native plant extracts. The Internet Journal of Microbiology 2008; 4 (2).
10. Cock IE, Kukkonen L. An examination of the medicinal potential of *Scaevola spinescens*: Toxicity, antibacterial and antiviral activities. Pharmacognosy Research 2011; 3 (2): 85-94.
11. Vesoul J, Cock IE. An examination of the medicinal potential of *Pittosporum phylliraeoides*: Toxicity, antibacterial and antifungal activities. Pharmacognosy Communications 2011; 1 (2): 8-17.
12. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica 1982; 45: 31-34.
13. Cock IE. High performance liquid chromatographic separation and identification of a toxic fraction from *Aloe barbadensis* Miller leaf gel using the Artemia naulpii bioassay. The Internet Journal of Toxicology 2008; 4 (2).
14. Ruebhart DR, Wickramasinghe W, Cock IE. Protective effect of the antioxidants vitamin E and Trolox against *Microcystis aeruginosa* and microcystin-LR in *Artemia franciscana* nauplii. Journal of Toxicology and Environmental Health, Part A 2009; 72 (24): 1567-1575.
15. Finney, D.J. 1971. Probit Analysis, 3rd ed., Cambridge University Press, Cambridge.
16. White RJ, An historical overview on the use of silver in modern wound management. British Journal of Nursing, 2002; 15(10): 3-8.
17. Albright CF, Nachum R, Lechtman MD, Electrolytic silver ion generator for water sterilisation in Apollo spacecraft water systems. Apollo applications program, NASA Contract Rep, 1967; Report Number NASA-CR-65738.
18. http://www.sti.nasa.gov/tto/Spinoff2004/er_1.html, Water treatment systems make a big splash. 2004, Accessed 30 September, 2011.
19. Conrand AH, Tramp CR, Long CJ, Wells DC, Paulsen AQ, Conrand GW, Ag^+ alters cell growth, neurite extension, cardiomyocyte beating, and fertilised egg constriction. Aviation, Space and Environmental Medicine, 1999; 70(11): 1096-1105.
20. Federal Register, Over-the-counter drug products containing colloidal silver ingredients or silver salts. Department of Health and Human Services (HHS), Public Health Service (PHS), Food and Drug Administration (FDA). Final rule. Federal Register 1999; 64 (158): 44653-44658.
21. Therapeutic Goods Association, 2007, Australian adverse drug reactions bulletin, 26 (5), http://www.tga.gov.au/pdf/aadrb-0710.pdf. Retrieved 4 Oct 2011.
22. Cho K, Park JE, Osaka T, Park SG, The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrachimica Acta, 2005; 51: 956-960.
23. Shahverdi AR, Fakhimi A, Shahverdi HR, Mianaian S, Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. Nanomedicine: Nanotechnology, Biology and Medicine, 2007; 3: 168-171.
24. van Hasselt P, Gashe BA, Ahmad J, Colloidal silver as an antimicrobial agent: fact or fiction. Journal of Wound Care 2004; 13 (4): 154-155.
25. Spratt DA, Pratten J Wilson M, Gulabivala K, An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. International Endodontic Journal 2001; 34 (4): 300-307.
26. White A, Cock IE, Mohanty S, Laroo H, Whitehouse M. Unpublished results 2011.
27. Silver S, Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS microbiology Reviews 2003; 27: 341-353.
28. Key FS, Maass G. Ions, atoms and charged particles. Silver Colloids 2001: http://www.silver-colloids.com/Papers/IonsAtoms&ChargedParticles, accessed 27 October 2011.
29. Faust RA. Toxicity Summary for Silver. Oak Ridge Reservation Environmental Restoration Program, Oak Ridge 1992.
30. Bouts BA. Argyria. New England Journal of Medicine 1999; 340: 1554.
31. Legat FJ, Goessler W, Schlagenhaufen C, Soyer HP. Argyria after short-contact acupuncture. Lancet 1998; 352: 241.
32. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ. The bactericidal effect of silver nanoparticles. Nanotechnology 2005; 16 (10): 2346-2353.
33. Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicology in Vitro 2005; 19: 975-983.
34. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Applied Environmental Microbiology 2007; 27 (6): 1712-1720.