



## **How Much, How Safe**

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### **Method**

#### **Static Tryptic Soy Broth Test (for the determination of silver release)**

In this test, the release of silver from nylon-based wound care dressings is measured in 250 mL of tryptic soy broth (the standard concentration is 30 grams tryptic soy broth media per 1.0 L of sterile distilled-deionized water) as a function of time.

1. 50 cm<sup>2</sup> of silver-coated bandage material was placed in a 250 mL Teflon Erlenmeyer flask containing 250 mL of tryptic soy broth made with sterilized distilled- deionized water and autoclaved. The flask had been capped with Whatman BugStopper10 filter vents. The filter device prevents bacteria or viruses from entering or exiting the culture vessel while at the same time allowing the free passage of air and gases through the vent layer. It has a filter rating of 99.9% bacterial filtration efficiency (BFE) and viral efficiency (VFE).
2. Each flask and contents were placed in a heated Lab-Line Environ platform orbital shaker to stir the solutions at 200 rpm and maintain a temperature of 310 K throughout the test.
3. 1.0 mL aliquots of the solutions were removed from the flasks at the following intervals: 1hr, 2 hr, 4 hr, 24hr, 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs, and 168 hrs and stored in amber vials to which 50 uL of 6.0M nitric acid had been added as a preservative. The samples were stored in a refrigerator at 40°C while awaiting analysis.
4. The solutions were then filtered through 0.45 micron syringe filters immediately prior to analysis.

5. The concentration of silver in ug/mL for each solution was determined through atomic absorption spectrophotometry using flame (air-acetylene) analysis and plotted as a function of time.

All samples were filtered through a 0.22  $\mu\text{m}$  syringe filter prior to analysis, and analyzed for silver ion concentration by flame atomic absorption using the 328.1 nm line of a Perkin-Elmer Lumina Ag Lamp in a Perkin-Elmer Aanalyst 300 Atomic Absorption Spectrometer. The instrument was calibrated with distilled, deionized water acting as a blank, and a 4.00 ug/ml and 12.00 ug/mL standard solution prepared by dilution of the standard silver solution. A calibration curve was obtained with a  $r^2$  value of at least 0.99. It should be noted that the calibration concentrations are outside the linear ranged of the silver hallow cathode lamp used in AAS. However, the Perkin-Elmer Aanalyst 300 Atomic Absorption Spectrometer utilizes a microprocessor with a complex curve-fitting software program that compensates for being outside the linear range. This technique was published in Spectrochimica Acta, 39B, p 829, 1984 by Unvala and Barnett, and is entitled ,” A Callibration for Atomic Absorption.” *The technique is recommended by the instrument manufacturer and discussed in “Analytical Methods for Atomic Absorption Spectrometry, Release E”, published by Perkin-Elmer Instruments, Inc in 2000.* The slit width of the instrument was 0.7 nm. The flame was an acetylene-air flame (lean blue, oxidizing). The limit of detection of silver ion was 0.9 ng/mL. Duplicate measurements were performed for each sample.

**Preparation of 4.00 ug/mL and 12.00 ug/mL standard solutions:** The solutions were prepared using a NIST traceable  $\text{Ag}^+$  certified standard solution (987 ug/mL). First, a 100 ug/mL stock solution was prepared by pipetting 10.13 mL of the 987 ug/mL standard solution into a 100 mL volumetric flask and diluting to the mark with distilled, deionized water. To prepare 100 mL of the 4.00 ug/mL standard solution, 4.00 mL of the 100 ug/mL stock solution was pipetted into a 100 MI volumetric flask and diluted to the mark with distilled, deionized water. The same procedure was followed to prepare the 12.00 ug/mL solution, except that 12.00 mL of the 100 ug/mL stock solution was used

## Results

Silverlon® Wound Contact Dressing (WCD-22) has been demonstrated to release 35-50 ug/mL of silver in Tryptic Soy Broth test medium after 24 hours of exposure at 310K and 50-60 ug/mL of silver after 7 days of continuous exposure under the same conditions as shown in Table 1.<sup>1</sup> In these test conditions, a 50 cm<sup>2</sup> piece of WCD-22 is placed in 250 mL of sterile Tryptic Soy Broth as outlined earlier.

Immersion Time (Hours)	Concentration of Silver (ug/mL)		
	Trial #1	Trial #2	Mean
1	6.950	6.252	6.601
2	20.060	14.920	17.490
4	28.840	18.270	23.555
8	32.170	24.110	28.140
16	37.990	37.070	37.530
24	47.100	38.130	42.615
48	48.960	45.480	47.220
72	57.200	54.780	55.990
120	53.270	54.880	54.075
192	54.710	54.670	54.690

## Discussion

The chloride ion present within TSB precipitates free silver ion and serves as a challenge to the formation of free silver ion in the media. In a similar fashion, chloride ion would limit the formation of free silver ion *in vivo*, during actual use of a silver wound dressing. However, TSB was shown to increase the amount of soluble silver in release studies over that of distilled water or normal saline.<sup>2</sup> An equilibrium most likely exists of free ionic silver and complexed silver due to the composition of TSB, which is comprised of pancreatic digest of casein, papaic digest of soybean meal, phosphates, dextrose, and sodium chloride. Complexed silver would encompass silver chloride and any complex silver ions formed with free amino acids and oligopeptides present in the test medium from the protein digests. Since TSB contains enough sodium chloride (5.0g/L) to precipitate all free silver, there exists a maximal concentration of free ionic silver, related to the  $K_{sp}$  of silver chloride and the concentration of chloride. Equation 1 illustrates this phenomenon.

**Equation 1.**  $K_{sp}$  Calculation: Concentration of ionic silver from silver chloride in TSB

$$K_{sp} \text{ of AgCl} = 1.8 \times 10^{-10}$$

$$[\text{Cl}^-] = 5.0 \text{ g/L} = 0.086 \text{ mol/L}$$

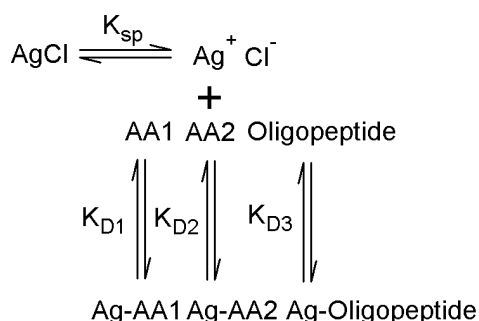
$$1.8 \times 10^{-10} = [x][x + 0.086]$$

$$[Ag^+] = [x] = 2.1 \times 10^{-9} \text{ mol/L} = 2.3 \times 10^{-7} \text{ g/L} = 0.23 \text{ ug/L} \rightarrow \mathbf{0.23 \text{ ppb}}$$

The maximum concentration of free ionic silver in TSB is approximately 0.23ug/L if, theoretically, there were no other complexing side reactions.

Silver release studies involve submerging a bandage in a solution and measuring silver levels as a function of time. Since Silverlon<sup>®</sup> silver wound dressings release approximately 10<sup>5</sup> x more silver (50-60 mg/L in 24 hours as determined by Atomic Absorption measurements) when placed in TSB than the calculated value, it is reasonable to conclude that the increase in measured silver levels is due to the formation of soluble silver complexes, not an increase in the formation of free ionic silver.

**Scheme 1.** Theoretical example of complex equilibria of silver in TSB.



AA represents any amino acid that can form a complex ion with silver, such as cysteine.

While the formation of soluble silver complexes shifts the equilibrium of silver chloride to the right and increases the release of silver from the wound dressings, the complexes do not increase the concentration of free ionic silver. All free ionic silver would be complexed with amino acids or oligopeptides. Since the  $K_D$  values of these complex ions must be considerably smaller than the  $K_{sp}$ , these complexes would not dissociate to the same extent that silver chloride dissociates and therefore they regenerate substantially less ionic silver.

Since WCD-22 has been plated (and measured by atomic absorption spectrometry) to contain 20% Silver by mass, the total level of available silver on a 50 cm<sup>2</sup> piece of dressing would contain approximately 235 mg of silver. If the entire silver load was released into 250 mL of Tryptic Soy Broth under the test condition noted above, the resultant solution would have a silver concentration > 900 ug/mL. Since only 50-60 ug/mL of silver is actually measured after 7 days of immersion in TSB, less than 6.5% of the available silver is being released from the dressing, maintaining over 93% of the silver as a reservoir on the dressing which would serve to prevent bacterial growth on the dressing itself in vitro and in vivo.

Furthermore, due to equilibria that form between free silver ion and complexing agents as noted above, the release of silver ion from the dressing would be a self-limiting, self-leveling process which would prevent further loss of solubilized silver from the dressing.

Experimental evidence, shown in Figure 1, shows the sustained concentration of total silver to be approximately 55 ug/mL in TSB once equilibrium has been established.<sup>1</sup>

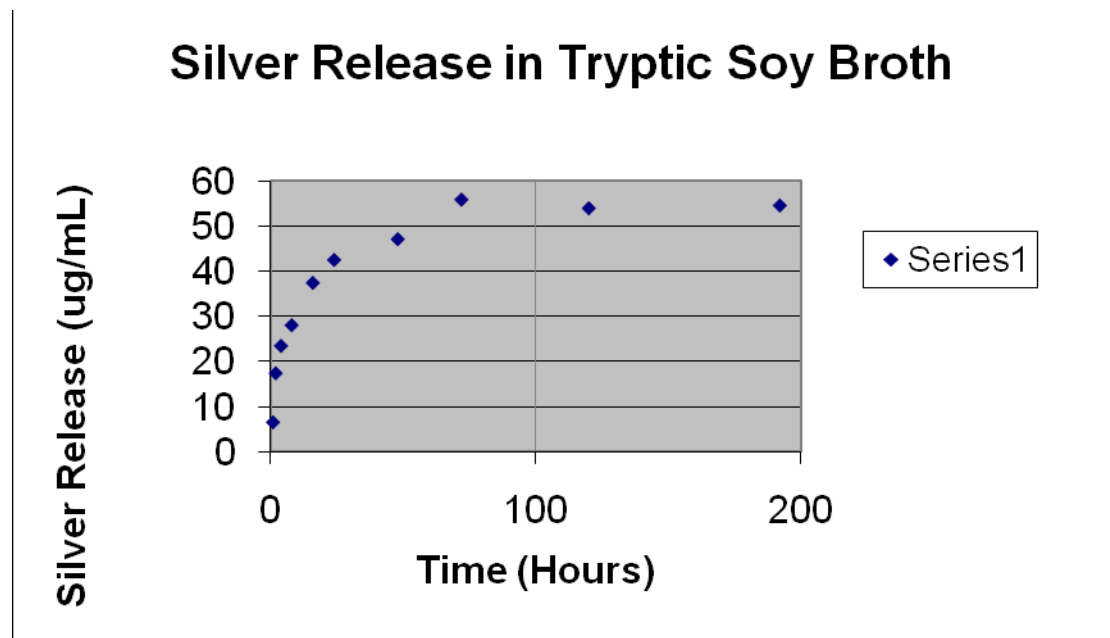


Figure 1: Graph of Atomic Absorption data showing Silver Release in Tryptic Soy Broth

The consideration of an effective, practical silver-based dressing must take into account the quantity of silver released from the dressing, the mechanism of delivery, and the ultimate fate of the silver metal surface.

The scanning electron micrographs of WCD-22 taken before and after exposure to TSB (Figure 2) show that the silver surface remains essentially intact. Small sections of silver have abraded from the fiber surface, while some crystals appear on the surface of the fibers. Energy dispersive X-ray analysis (EDX) has revealed that these crystals are silver chloride. The crystals are most likely formed as a result of the 5.0 g/liter of sodium chloride that is present within the TSB, and serve as a source of ionic silver initially released from the dressing. The electron micrographs shown in Figure 3 (before and after exposure to TSB) for Acticoat, a nanocrystalline silver-based wound care product consisting of alternating layers of silver-coated polyethylene mesh with rayon/polyester sandwiched between the silver-coated mesh, *shows that the silver surface is substantially disturbed* after immersion. Silver has been stripped from significant portions of the polymer, scratches on the silver are readily apparent, and flakes of non-adhered silver are scattered over the surface of the dressing.

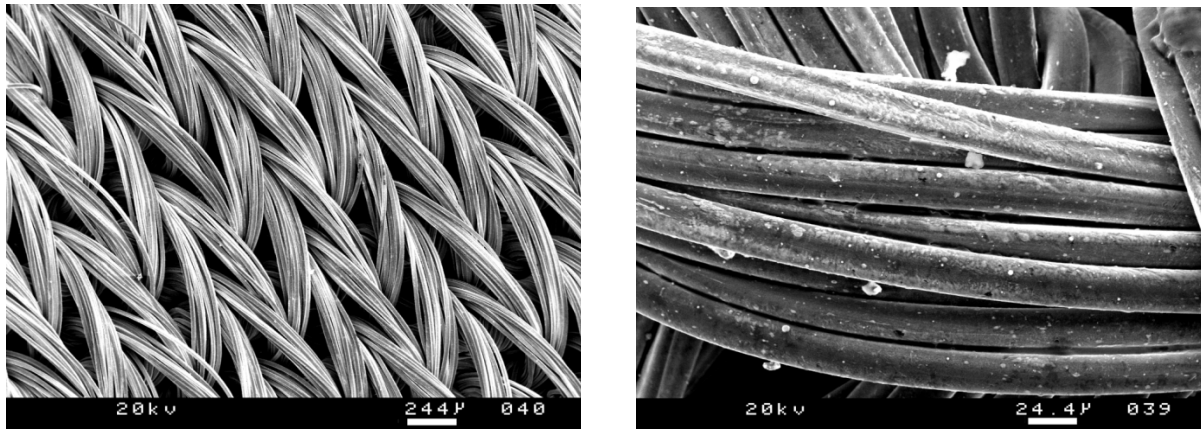


Figure 2: Scanning electron micrographs of WCD-22 before (left) and after (right) 8-day immersion in Tryptic Soy Broth

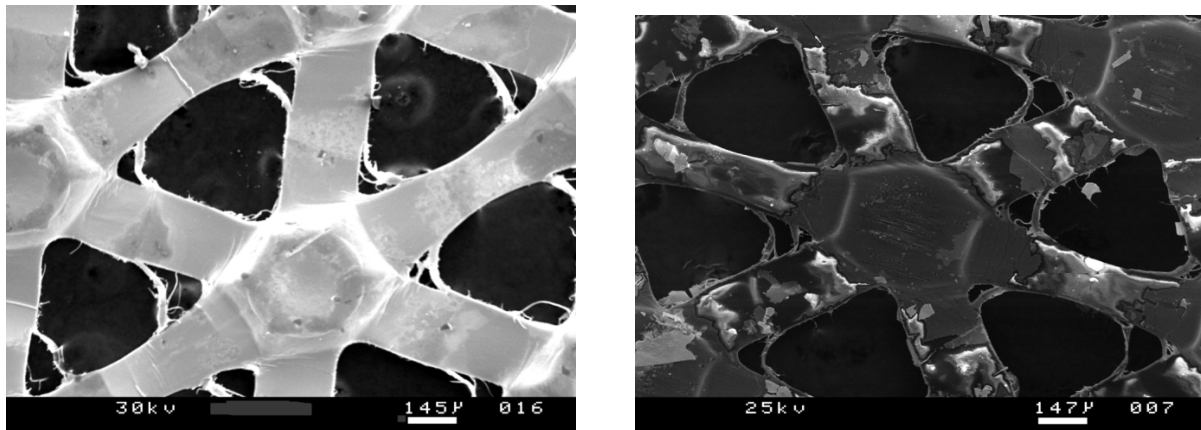


Figure 3: Scanning electron micrographs of Acticoat, a nanocrystalline silver product, before (left) and after (right) 8-day immersion in Tryptic Soy Broth

Silver was evident as small particles and flakes dispersed throughout the test solutions. The lack of silver metal adhesion during testing by immersion in TSB was noted for Acticoat. This is of concern given the reports in the literature of localized cutaneous argyria with permanent skin tattooing. Suzuki, et al. have reported the case of a 41 year old female with pigmented blue-black macules present on her forehead, neck and chest.<sup>3</sup> Subsequent analysis demonstrated that these discolorations were caused by silver released from implanted acupuncture needles. In another less extreme case, localized cutaneous argyria was reported by Legat, et al. in a 39 year old woman who had undergone 5 short sessions of acupuncture in her ears.<sup>4-5</sup> Each session was no longer than 30 minutes in length, thus the needles had not been in contact with the skin any longer than three hours. After 10 years, the patient noticed a blue-black macule present in her left

ear. The tissue was excised, with numerous brown-black granules noted in the surrounding tissue, and analyzed to reveal the presence of silver. Legat speculated that during one of the acupuncture sessions a small particle of a needle might have deposited silver in the ear, which then manifested itself, years later, as a blue-black macule in the skin.

The silver metal coating of WCD-22 was uniform and strongly adherent. On the other hand, upon immersion, Nanocrystalline Acticoat, displayed silver-sloughing that was readily apparent with visual and microscopic analysis. Thus, WCD-22 shows self-limiting silver release levels in solution that have been demonstrated to be clinically efficacious, but importantly WCD-22 was not observed to release metallic silver particles. The observed silver flakes released from Acticoat with nanocrystalline silver have the potential to accumulate in un-equilibrated manner in a wound environment and cause localized argyria.

In support of the efficacy of safety of the use of silver-based wound dressing, a 2004 review study by Lansdown and Williams<sup>6</sup> on silver safety concluded that the “risks of lasting tissue damage or functional disorders are low,” and that “silver exhibits low toxicity in the human body, and minimal risk is expected due to clinical exposure by inhalation, ingestion, dermal application or through the urological or haematogenous route” In addition, Bolton<sup>7</sup> has reviewed ten clinically controlled studies ranging from acute wounds to pressure ulcers, and concluded that “evidence...supports a conclusion of consistently positive effects on aspects of chronic wound healing such as autolytic debridement, depth reduction and bacterial burden.” In another relating to the development of a hydrocolloidal dressing, Bolton demonstrates that “the clinical literature reviewed supports the conclusion that silver dressings in general are safe on chronic and acute wounds, including minor OTC wounds treated by consumers, and may confer significant wound healing benefits.”

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## **Addition Discussion**

### **Simon Silver, Phd. Professor of Microbiology, University of Illinois**

1) *Silver-containing bandages such as Silverlon contain a large reservoir of immobilized silver, which is slowly and continuously released over time. The silver is not "dumped" quickly into the wound, as that would be ineffective. A small (almost un-measurable at any moment in time) fraction of the silver is released initially as nano-particles that can move from the bandage surface to the site on the wound tissue where bacterial infection would be harmful. Here the silver is in a preformed "delivery package". At the bacterial cell surface, silver is oxidized to cationic Ag<sup>+</sup>, which is quite generally agreed to be the bactericidal form of silver.*

The Ag<sup>+</sup> is, however, present at a very low level and only for a short time. As silver inhibits (or more accurately kills) the bacterial cell, it is re-reduced to Ag<sup>0</sup>, initially on the cell surface but sometimes later additional amounts of silver accumulates in the bacterial cell interior. Therefore the slow release of silver by Silverlon bandages is a positive feature that makes it much more effective, silver atom by silver atom, than would be silver nitrate (with initially all cationic silver and short availability). Slow release and longer availability of a small fraction as antimicrobial was a major benefit of silver sulfadiazine, which was introduced more than 30 years ago and has a long record of effective use.

The silver-containing bandages are one large step further with more controlled and slower release in a mobile delivery package. As has been demonstrated in extensive clinical use, both silver sulfadiazine and the newer silver-containing bandages have no inherent safety problems, and are remarkably harmless to the human tissue, initially in the area around the wound and later elsewhere in the body. *There have been occasional publications indicating specific cell toxicities*

*and long term “blue skin” problems with large amounts of orally-ingested silver, but these reports seem one-by-one flawed, very rare, and not a clinical concern.*

*2) It is highly unlikely that the Silverlon silver nylon delivery mechanism would lead to human cell toxicity and there is no evidence during its wide use to date of any such problem.* The same can be said as well for other silver-containing wound bandages and other silver-containing products such as silver-sulfadiazine. One must take nothing as assured and remain on alert, especially as nanocrystalline delivery mechanisms such as nanosilver have unusual properties associated with size rather than cation by cation antimicrobial activity.

The occasional reports of problems have each been unsupported by future work and usually are clearly flawed on first reading. One cannot be certain that a Haiti-size earthquake will not hit Amsterdam or Brussels next week. However, it makes no sense to begin with an assumption that such might happen.

*3) The overwhelming available evidence supports a high level of product safety for Silverlon and other silver-containing bandage products.* Nevertheless, the published literature on silver-containing bandages has been remarkably unsatisfactory. It is difficult to accept that most is “peer reviewed” when companies producing the products often provide financial support for the journals and special journal issues, with enclosed advertisements as well. So the problem here goes beyond that of company support for academic researchers and peer review of publications. This is unusual to the silver-antimicrobial field, and thus Silverlon finds itself in a situation where it can not analyze in depth the faults of reports of other products without seeming to be “part of the problem and not the solution.” By acknowledging this unusual level of difficulty and showing a willingness to work with independent scientists as well as government regulatory agencies, Argentum with Silverlon will try to distinguish itself – although there is no reason to think that its product Silverlon will be any safer or less safe than alternative silver-containing bandages. *From the available evidence (mostly extensive clinical use but also more limited laboratory mammalian cell culture studies), there seems no safety problem.* However, we recognize this issue should never be totally removed from consideration.

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