Laboratory Report

Date: 11 Jan 2001

Introduction

This report is to document the details of an experiment conducted on this date at the Colloidal Science Laboratory, Westampton, NJ by Francis Key, principal scientist. The experiment involves the determination of whether nanometer size particles of metallic silver (nanoparticles) contained in a colloidal silver solution can be absorbed through the lining of the GI tract into the bloodstream after being ingested. In attendance to draw blood from the subject and witness the experiment was Diane Farrington, RN.

Background

Colloidal silver solutions have been available for several years over the counter and via the Internet. It has been estimated that worldwide, the number of people using colloidal silver as a dietary supplement on a daily basis is measured in the millions. Colloidal silver solutions typically contain silver in two forms, ions and nanoparticles. There has been conjecture regarding how the silver entered the human body and in what form the silver would circulate in the bloodstream when colloidal silver solutions were ingested. It was uncertain whether the nanoparticles could pass through the lining of the GI tract and enter the bloodstream.

Purpose

This experiment is designed to test the theory that silver nanoparticles contained in an ingested colloidal solution can enter the bloodstream by absorption through the lining of the GI tract. The colloidal silver solution chosen for this test contained only silver nanoparticles; no ionic silver was present in the solution. The silver nanoparticles have a mean diameter of 19 nm and are described in the accompanying particle size distribution report performed using a Malvern Zetasizer 3000HS Photon Correlation Spectrometer.

Test subject

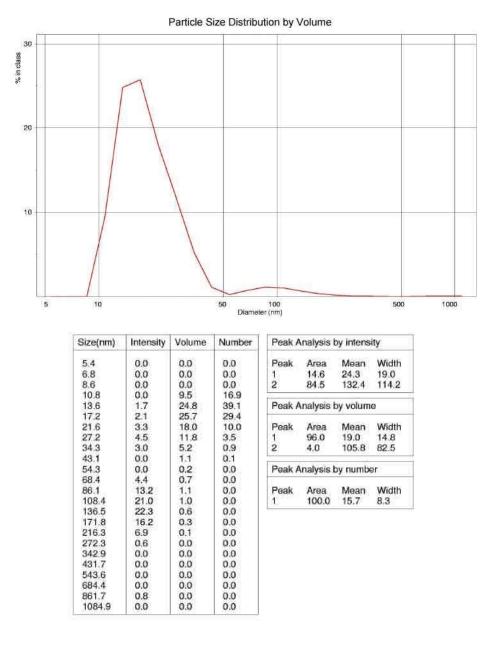
The test subject was an adult male Caucasian, 71 inches (180.3 cm) tall and weighing 215 lbs (97.5 kg) The subject is physically active and in good health. The subject consumes colloidal silver on a daily basis and also uses it topically on a regular basis. The subject's prior use of colloidal silver contributed to the high baseline reading of serum silver level. The subject did not ingest or use topical application of colloidal silver for 24 hours prior to the experiment. Approximately 2.5 hours before the experiment began, the subject ate a light breakfast. The stomach contents at the time the experiment commenced were not considered to significantly affect the results.

Colloidal silver solution

The colloidal silver solution used in this experiment was made by a process developed by the Colloidal Science Laboratory that produces a solution containing 100% silver nanoparticles and no ionic silver. The size distribution data indicates that the mean particle diameter is 19 nm and the width of the peak is 14.8 nm. Such particles contain 96% of the silver by volume. Additional information that describes the colloidal solution:

Concentration ¹ -	2.500-ppm measured with no nitric acid,		
	2.922-ppm measured with 25 uL of HNO ₃ per 30 mL of sample.		
Particle size -	Mean diameter is 19 nm. See distribution plot.		
Conductivity -	1.50 uS/cm		
рН -	7.0		
Percent particles -	100%		
Percent ionic -	0%		
Turbidity -	106 NTU		
Consumed by subject -	250 mL		

¹ The concentration reference was the 2.500-ppm value as measured without nitric acid. This value was used as the reference value since all the blood measurements would be made without using nitric acid. The technically correct concentration is the value measured using nitric acid, which is 2.922 ppm.



Blood samples

Blood was drawn from the subject using an 18 gauge Vacutainer needle taken from the antecubital space (inside of elbow) into 10 mL vacuum vials. The Vacutainer vials contained sodium heparin as an anticoagulant. The 10 mL of blood was diluted by addition of 40 mL of DI water to produce a 20% dilution. Dilution was necessary so that the blood could be drawn through the aspirator tubing into the nebulizer of the AAS for analysis.

Blood was drawn prior to ingestion to establish the baseline reading. Thereafter, blood was drawn at 30, 60, and 180 minutes after ingestion of the colloidal silver solution.

Measuring instrument settings

All the measurements of silver concentration were performed using a Perkin-Elmer 3030B Flame Atomic Absorption Spectrophotometer (AAS). The instrument settings were:

Slit width	0.7 nm		
Wavelength	338.3 nm		
Nebulizer	Glass impact bead		
Mode	AA, no background correction		
Flame	Air-acetylene, blue oxidizing		
Burner	10 cm		
Measurement time	3 second periods, 5 period average (15 seconds total per reading)		
Measured values	Average of 10 readings (150 seconds total)		

Calibration of AAS

The instrument was calibrated using a certified silver calibration solution of 1000 ± 3 ppm. The standard was diluted to produce diluted standards of 1 ppm and 5 ppm. The dilutions were prepared by weight, corrected for the density of the standard solution using an analytical balance that reads to 0.00001 grams. The accuracy of the diluted standards is $\pm 0.1\%$ relative to the 1000 ppm standard, which is accurate to within $\pm 0.3\%$

The diluted standards are pH adjusted using 25 uL of 70% HNO₃ per 30 mL of diluted standard. Normally, the blank DI water standard of 0 ppm would also be pH adjusted, but since the sample being measured would not have the nitric acid added, the blank was left without acid. This slight inconsistency was judged to be of little significance in the overall outcome of the measurements.

Measured value of blood silver

The baseline value was established by measurement prior to ingestion of the colloidal silver solution. Thereafter, blood samples were drawn at 30, 60 and 180 minutes after ingestion and silver measurements were made by the AAS on 20% dilutions of the drawn blood. The dilutions were made by adding 10 mL of blood to 40 mL of DI water.

The high sodium content and other chemicals present in the blood samples cause a very bright orange flame when the samples are vaporized. The altered nature of the flame ² can cause the measured values of silver to read high on this instrument if deuterium background correction is not used. This instrument is not fitted with the background correction hardware, so the absolute values of the measured blood serum are suspected of being higher than they actually are. While this causes anomalies in the absolute values reported, it does not affect the relative values of the samples when normalized to the baseline value since all the blood sample measurements are affected in a uniform fashion. The relative relationship of the measured values provides a clear indication of increasing silver levels in the blood samples after ingestion. Therefore, the overall validity of the test and the conclusions that can be drawn from the data remain unchanged. Blood silver levels normalized to the baseline-measured value are shown in the last column of the table. These data accurately represents the effects of ingesting the colloidal silver solution on the blood silver levels.

Measured Silver and Normalized Values

Sample	Measured Value Of 20% Dilution	Normalized Relative To Baseline
Baseline	0.028 ppm	+0%
+ 30 min.	0.075 ppm	+268%
+ 60 min.	0.084 ppm	+300%
+ 180 min.	0.105 ppm	+375%

² Evidence suggests that the incineration of blood cells in the flame produces an ash like residue that absorbs light passing through the flame. It is speculated that the increased light absorption resulting from the ash is primarily responsible for the abnormally high silver reading when aspirating whole blood.

Lessons learned

This is the first time the laboratory attempted to measure silver content in whole blood. It was not known whether the blood could be aspirated by the AAS. The inside diameter of the aspirator tube is only a few thousandths of an inch so clogging was a concern. Dilution of the blood to 20% with DI water was essential. Also essential is the use of an anticoagulant, in this case sodium heparin was used. While this method worked, it is now clear that better methods should be devised for future experiments that require the measurement of silver levels in blood samples using an AAS. Introduction of whole blood into the AAS causes the burner to frequently clog with the residue of the blood cells. This required frequent cleaning of the burner head and recalibration after about 10 measurements. When the experiment was complete, the nebulizer, mixing chamber and burner head had to be dismantled for a complete cleaning.

What to do differently the next time – before attempting such measurements in the future, a method must be devised to oxidize the silver particles in the blood samples to convert the metallic silver into ionic silver. Thereafter, the blood cells can be removed by centrifugation or filtration leaving only the blood serum containing the silver in ionic form, which can be measured by the AAS without clogging the machine and requiring the extensive cleanup. Alternatively, an Ion Selective Electrode could be used to measure the silver content once it was converted to ionic form.

Future experiments - should be made to determine the point in time when the blood silver level peaks and then falls back to half the peak value. It is expected that could be up to 24 hours. Experiments could also be conducted to test the other means of induction of silver into the body, i.e. inhalation of a nebulized colloidal silver solution. Yet, another experiment could be conducted with 100% ionic silver content instead of nanoparticles to provide additional insight into the absorption mechanism. If ionic silver is ingested, the measurement instrument would more logically be an Ion Specific Electrode type measurement of blood level silver.

Conclusions

The purpose of the experiment was to determine if ingested colloidal silver nanometer size metallic particles could enter the bloodstream by absorption through the lining of the GI tract. This was conclusively proven by the data presented. The colloidal solution ingested contained only silver particles and no ions. Because there is nothing in the gastric juices in the stomach or lower GI tract that could oxidize the silver particles thus converting them to ionic silver, it is speculated that any silver found in the blood samples would be in the form of the nanoparticles. The presence of silver in the blood samples suggests that the particles were being absorbed through the lining of the GI tract. Three hours after ingesting the colloidal silver solution the measured level of silver in the blood was still rising. This would seem to indicate that most of the absorption of particles took place in the small intestines. It is clear that longer-term measurements are required to determine the full extent of the time intervals involved.

About the author

Francis S. Key is the principle scientist and founder of the Colloidal Science Laboratory which conducts research on metal colloids. Mr. Key was educated at Columbia University and Newark College of Engineering. He has an extensive background in the fields of electrical engineering, computer science, and various branches of physics and engineering. Mr. Key's experience in scientific research and engineering spans a period over 35 years, beginning with his contributions to the design of space flight hardware used on the Apollo and Viking space missions. Mr. Key is known for meticulous attention to details, and relentless investigative procedures, both of which have led to innovative solutions to problems in defense, aerospace and private industry.

Mr. Key has carefully researched the production of colloidal silver, and has built a laboratory facility for colloidal research which is state of the art for the field. He has also instituted analytical methods and developed manufacturing processes which insure the highest purity and consistency possible for a colloidal product. To contact Mr. Key e-mail to: <u>frank@silver-colloids.com</u>