BACTERIAL ADHESION TO SOLID SURFACES AND ITS PREVENTION BY THE APPLICATION OF SILVER

by

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Submitted to the Institute of Biomedical Engineering
in partial fulfillment of the requirements
for the degree of
Master of Science
in
Biomedical Engineering

Boğaziçi University
September, 2001

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DATE OF APPROVAL: September 18, 2001.

To My Father,

Mother,

and Brothers

ACKNOWLEDGEMENT

I would like first to thank Prof. Dr. Sabri Altıntaş for suggesting to me this interesting topic for my thesis. I am also grateful to him for his help, guidance and orientation during my work.

I would like also to thank Prof. Dr. Yekta Ülgen and Assoc. Prof. Dr. Halil Özcan Gülçür for revising my thesis and providing me with valuable remarks and recommandations

I am also grateful to Dr. Ayşen Erdinçler and Mrs. Ayşe Tomruk from the Institut of Environmental Science and Technlogy-Boğaziçi University for allowing me to perform a part of my work in the laboratory of the Institute.

Special thanks go to the Department of Biology in Fatih University for allowing me to perform a major part of my experimental work in their Laboratories.

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ABSTRACT

Silver, under different forms, has been proved effective for the elimination of bacteria and other microorganisms from liquid mediums, solutions and solid materials used in industrial, sanitary, and biomedical fields, such as reactors, pipes, filters, medical devices, biomaterials, etc. The effectiveness of different forms of silver antimicrobial application depends on the nature of these forms, the methods of application, and the field of application.

The main objective of the thesis is to present a theoretical and experimental studies involving bacterial growth in liquid suspensions and solid surfaces and its prevention (disinfection) by different application of different forms of antibacterial silver: electro-colloidal silver, silver nitrate, silver sulfadiazine, and antimicrobial ceramics impregnated with silver ions. Bacterial growth is detected by viable bacterial agar plate counting and by observation of turbidity in suspensions. The experimental results showed superiority of the antimicrobial effect electro-colloidal silver over other used forms of silver used to disinfect liquid suspension. It was also shown that the antibacterial properties of the hygienic ceramics impregnated with silver ions were strong and durable.

Keywords: Bacterial adhesion, bacterial counting, disinfection, colloidal silver, hygienic ceramic.

BAKTERİLERİN KATI YÜZEYLERE TUTNMASI VE GÜMÜŞ UYGULANMASI İLE ÖNLENMESİ

ÖZET

Gümüşün, farklı biçimler altında, reaktörler, borular, filtreler, tibbi cihazlar, biyomedikal malzemeler, endüstiyel, sıhhi tesisat ve biyomedikal alanlarda kullanılan akışkan ortam, karışım ve katı malzemelerden bakteri ve diğer mikroorganizmalar artılmasında etkin bir rol oynadığı ispatlamıştır. Gümüş farklı biçimlerinin antimikrobik uygulamalarının etkinliği, bu biçimlerin doğasına, bu uygulama yöntemlerine ve uygulama alanına bağlıdır.

Bu tezin başlıca amacı, elektro-kolloidal gümüş, gümüş nitrat, gümüş sulfadiyazin, ve gümüş iyonlarıyla doyurulmuş antimikrobik seramik gibi değişik biçimlerdeki antibakteriyel gümüş vasıtasıyla, katı yüzey ve akışkan asılılarda bakteriyel büyümeyi önleyen uygulamaları içerer deneysel çalışma sunmaktır. Bakteriyel büyüme, yaşayabilen bakteriyel agar levhası, sayımı ve asılılardaki bulanıklığın gözlemelenmesiyle algılanır. Deneysel sonuçlar, akışkan asılıların dezenfektesinde elektro-kolloidal antimikrobik etkisin kullanılmış diğer gümüş türlerine nazaran üstünlüğünü göstermiştir. Bu çalışmada, gümüş iyonlarıyla doyurulmuş hijyenik seramiklerin antibakteriyel özelliklerinin kuvvetli ve kalıcı olduğu da gösterilmiştir.

Anahtar kelimeler: Bakteriyel yapışma, bakteriyel sayma, dezenfeksiyon, kolloida gümüş, hijyenik ceramik.

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LIST OF ABBREVIATION

A	absorbance
ASS	atomic absorption spectroscopy
ATP	Adenosine Triphosphate
Avg	average
c	velocity of light
CFU	colony forming unit
CS	colloidal silver
DNA	Deoxyribonucleic Acid
E	energy
h	Plank's constant
HA	Hydroxylapatite
λ	wavelength
MBC	minimum bactericidal concentration
MIC	minimum inhibitory concentration
MKC	minimum killing concentration
υ	frequency
ppb	part per billion
ppm	part per million
Std Dev	standard deviation
UV	Ultra-violet

1. INTRODUCTION

Bacterial adhesion to biomaterial surfaces is the major barrier to the extensive use of prosthetics, since it causes biomaterial-centered infection and the lack of successful tissue integrity or compatibility with biomaterial surface [1]. This complication may lead to complete failure of the implant, possible amputation, prolonged hospitalization, and even death [2]. In addition, industrial and sanitary application of materials is also faced by the problem of adhesion of microorganisms on solid material surfaces, which is sometimes called biofouling. Therefore, antimicrobial substances are being developed for application with materials in the medical and industrial fields.

Antimicrobial agents used on biomaterials are classified into two groups: the synthetic or natural inorganic groups, and the synthetic or natural organic groups. Inorganic or metallic antimicrobials, which depend on the metal ions, have defects related to toxicity and coloring. Organic antimicrobials also have defects related such as high volatility, low heat resistance, coloring and high solubility for water and organic solvents [3]. There is a new tendency merging towards the use of metallic antimicrobials in different methods for different biomedical and industrial applications. These new methods try to avoid the known disadvantages of the use of metallic antimicrobials.

The antimicrobial effects of metallic ions such as Ag, Au, Pt, Pd, Ir (i.e. noble metals), and Cu, Sn, Sb, Zn is known [4]. The metallic salts of the above mentioned metals, whether organic or inorganic, have been known to have especially superior disinfectant property for bacteria such as *E. coli, S. aureus, Bacilli, Vibrio, salmonella,* and other microorganisms such as molds or round worms [5].

It is considered that the antimicrobial metals exhibit antimicrobial activity because when metal ions are absorbed into microbial cells, they inhibit respiration and basal metabolism of electron transfer and the transport of substrate through cell membrane [6]. Also, silver ions are able to complex with cellular DNA. This complex

formation interrupts the normal role of DNA and thus prevents the multiplication of bacterial cells [7].

Of the metallic ions with antimicrobial properties, silver is perhaps the best known due its unusually good bioactivity at low concentration. This phenomenon is termed as oligodynamic action [4]. Silver in its metallic state is safe because it is little absorbed in the human body [7]. When the National Aeronautic and Space Administration (NASA) was searching for a mean to purify the recycled water for the Apollo astronauts, they selected silver because of its high potency disinfecting capabilities.

Silver that is used for antimicrobial function is of different types:
Electro-colloidal silver (electrically sintered into pure water).
Mild silver proteins (MSP): This process chemically infuses or binds the silver to a protein. The part per million vary from 20 PPM to 200 PPM and concentrated as high as 5000 PPM.
Powdered silver: A very high voltage is used to disintegrate the silver, also known as water-soluble silver.
Ground silver: silver is pulverized and ground into a fine powder.
Silver oxide: Once used for chores and epilepsy
Silver salts: These silver compounds cannot be truly considered as colloidal silver. They are produced chemically or electrochemically. They may possess a positive charge but are usually considered as silver compounds. Below are examples of these silver compounds and their uses.
Silver sulfadiazine: Antibacterial and antifungal use in burn cases.
Silver nitrate: Disinfectant uses. Prodiatry, prevent blindness in infant's eye (used at birth), ulcer treatment.
Silver iodide: Disinfectant qualities.
Silver chloride: Disinfectant qualities.
Silver lactate: Astringent and antiseptic.
Silver picrate: Used for monilliasis and trichomoniasis [8].

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The objective of this thesis is to study the antimicrobial efficacy of different forms of silver: electro-colloidal silver, silver nitrate, and silver sulfadiazine. These compounds are used with bacterial suspensions of *S. epidermidis*. In addition, these compound are to be applied on solid surfaces of ceramics and their antimicrobial effects are compared to that of a manufactured antimicrobial ceramic where silver ions were impregnated.

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In this first section, an introduction to the thesis was given. In the second section, a study about bacterial structure and growth is presented. In the third section, a literature study about bacterial adhesion to solid surfaces and the factor governing it is presented. The fourth section includes a review about physical and chemical control of the growth of microorganisms. The fifth section includes a literature survey about the biomedical and industrial application of silver and silver compounds as antimicrobial agent. The sixth section includes a study of experimental techniques for producing electro-colloidal silver solutions and their analysis for silver content and the results of the application of these techniques. It also includes an introductory study of bacterial culturing, microscopic observation, and enumeration. Afterwards, a description of an experimental work involving culturing the bacterium *Staphylococcus epidermidis*, its enumeration, and bacterial application of silver under different forms against this bacterium in suspension and solid surfaces. Section 7 includes a conclusion for the thesis.

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instance, formaldehyde at high concentrations (20%) is sterilant, at intermediate concentration, it could be a disinfectant, and at very low concentration it could be used as an antiseptic. Phedisinfectant but, at 0.05%, isat 0.05% can be used as an antiseptic [10].

Antiseptics are chemicals that kill microorganisms but are relatively safe to use externally on humans and animals. Antiseptics include such chemicals as 70% isopropyl alcohol, 3% hydrogen peroxide, 1% tincture of iodine, 1% silver nitrate, and 1% mercurochrome (a mercury-containing compound) [10].

Disinfectants, chemicals that are usually more toxic than antiseptics, are used to kill microorganisms on floor, toilets, showers, bench tops and equipment. Disinfectants include chemicals such as 0.5% chlorine bleach (chlorox), 3% phenol (carbolic acid), 5% quaternary ammonium compounds (Roccal a% ethyl alcohol and 80% isopropyl alcohol. Alcohols are considered as disinfectants rather than antiseptics because at these concentrations they are not safe to use on mucous membranes. Many disinfectant are composed of a mixture of chemicals: Lysol disinfectant consists of 0.1% o-phenylphenol and 79% ethyl alcohol while Lysol cleaner is made up of 2.7% alkyldimethylbenzyl ammonium chloride, 0.13% tetrasodium ethyldiamine tetraacetate, and 0.34% ethyl alcohol [10].

Chemical sterilants are compounds that are very toxic to life and must be used with great caution. Chemical sterilants such as the gases ethylene oxide (12%) and propiolactone are used to kill microorganisms in heat labile materials such as petri dishes plastic pipettes, plastic filtering systems, etc., and surgical implants. Liquid glutaraldehyde (20%) has been used to sterilize anesthesia tubing and surgical instruments since it rinses off easily with water [10].

Pesticides are chemicals that are used against insects, arachnids, nematodes, algae, fungi, and bacteria. For example, solutions of copper sulfate (Bordeaux mixtures) kill algae and fungi, 1-% copper-8-hydroxyquinolate kills fungi and bacteria, and

400g/ml-ethylene bromide kill insects and arachnids in grains and on fruits as well as nematodes in soils [10].

Food preservatives are chemicals that are added to food in order to inhibit the growth of microorganisms. Some of the preservatives are natural compounds such as vitamin C and propionic acid and probably not harmful to the consumer at the added concentration. Many of the preservatives such as sodium nitrite, ethyl formate and polymyxin B can be dangerous if consumed in large counts. Common food preservatives include sodium chloride, sugars, and acetic (vinegar), ascorbic (vitamin C), benzoic, lactic, propionic, sorbic, and sulfurous acids. Fungi are inhibited by preservatives like benzoic acid, sorbic acid, propionic acid, sulfur dioxide, sodium diacetate, and ethyl formate, while bacteria are commonly inhibited by vitamin C, sodium nitrite, sodium chloride, sugars, and polymyxin B [10].

Chemotherapeutic agents are chemical substances that have a high degree of antimicrobial activity and can be used internally through ingestion or intravenous injection. Chemotherapeutic agents that are produced by microorganisms are called antibiotics. The best known antibiotics are penicillin (produced by a fungus) and chloramphenicol (produced by a bacterium). The chemotherapeutic agents that are produced by scientists are called synthetic drugs [10].

Factors that influence the effectiveness of chemical control agents include: The size of microbial population; the organisms' susceptibility to the antimicrobial; the volume of material to be sterilized; the length of time the antimicrobial is used on the microorganisms; the concentration of the antimicrobial; the temperature; the water activity of the substance to be sterilized; and the amount of organic matter [10].

The microbiocidial efficiency of a chemical is often determined with respect to phenol, a commonly used disinfectant. The efficiency of a chemical with respect to phenol is called phenol coefficient (PC). The phenol coefficient is calculated by dividing the highest dilution of the antimicrobial of interest that kills microorganism after

incubation for 10 minutes but not after incubation for 5 minutes by the highest dilution of phenol that has the same characteristics. Chemicals that have phenol coefficients greater than 1 are more effective than phenol [10].

The best chemotherapeutic agent s are those that kill microorganisms rather than inhibit their growth, but lysis or cell rupture do not occur. Agents that kill are called microbiocidial, while those that inhibit are called microbiostatic. An antimicrobial agent may be microbiostatic at low concentration and microbiocidial at high concentration [10]. Microbiolytic agents are those agents that kill by cell lysis, which is observed as a decrease in cell number or in turbidity after the agent is added [29]. A chemotherapeutic agent is supposed to kill the microorganisms at risk, with little or no effect on host. This is called selective toxicity [10].

Some antimicrobials do not kill organisms but merely inhibit their growth and reproduction. Other antimicrobials are inhibitory if used at low concentrations but kill if used in high concentration. Because of this, it is important to know the minimum inhibitory concentration (MIC) and the minimum killing concentration (MKC) for an antimicrobial with organism that may be present [10].

Some antimicrobials affect a large number of different organisms and are known as broad-spectrum antimicrobials. Others affective against a small group of organisms and consequently they are known as narrow spectrum antimicrobials. The best antimicrobials are those that kills undesirable microorganisms at low concentrations but have no toxic effect on the host (plant, animal and human) [10].

Here, some types of germicides and the way they kill are briefly described.

Phenol and Phenolics: Phenol consists of a hydroxyl group attached to benzene ring. Phenolics have this structure and additional components. Both act by denaturing proteins. Phenolics also act on lipids. Hexachlorophene is a phenolic ounce used as antiseptic but now replaced by chlorhexidine, which is less toxic to humans [9].

Alcohols: Alcohols are compounds with a hydroxyl group. They kill microorganisms by denaturing protein and disrupting lipids in the plasma membrane. They do not kill endospores. Ethanol and isopropanol are commonly used clinical disinfectants [9].

Halogens and hydrogen peroxide: Halogens are oxidizing agents. They inactivate enzymes by oxidizing certain functional groups. Iodine is antiseptic and chloride is a disinfectant. Hydrogen peroxide is not a halogenbut acts in the same way. It is used as a weak antiseptic for cleaning wounds and disinfecting fragile medical instrument and contact lenses [9].

Surfactants: Surfactants are compounds with hydrophilic and hydrophobic parts that penetrate oily substances in water and form an emulsion. Soaps and detergents are surfactants. They do not kill microorganisms but control them by washing them away. Quaternary ammonium salts are powerful surfactant germicides that are cationic. They kill all classes of microorganisms and viruses that have membranes, though higher concentrations are required for Gram negative bacteria. Anionic surfactants are mild disinfectants that act like quaternary ammonium salts but less effectively. They are used to disinfect skin and other surfaces, such as bench top in microbiological laboratories [9].

Alkylating Agents: Alkylating agents attach short chains of carbon atoms to enzymes, which inactivate them and kills the cell. Formaldehyde, formaline, glutaraldehyde ethylene oxide are examples. Ethylene oxide is a gas and a sterilizing agent used for heat sensitive materials and unwieldy objects. However, it is very toxic to humans [9].

Heavy Metals: Salts of heavy metals react with sulfhydryl (-SH) groups of proteins, "poison" enzymes and thereby kill microbial cells. Salts of mercury and silver have been used in medicine for years. The mercury salt mercury chloride have been used was once widely used as an antiseptic but is no more used today because it is highly toxic. Instead, organic compounds that contain mercury, including Merthiolate and Mercurochrome,

which are less toxic are used. Both are basic home medicine chest and first aid kit supply for disinfecting skin and mucous membranes [9].

Silver salts and colloidal silver (finely dispersed silver particles) were once widely used antiseptics. State law uniformly required that a solution of silver nitrate be applied to the eyes of newborns to avoid ophthalmic gonorrhea and the blindness that results. Today, however, the trend is away form using silver nitrate and toward using antibiotics. In general, silver salts have limited use today because they can irritate skin; though they continue to be helpful in preventing infections of burned skin [9]. Silver antimicrobials are discussed in more details in coming sections.

4.2.3 Aseptic Techniques

Aseptic techniques are the procedure used to prevent contamination of previously uncontaminated and to obtain and perpetuate pure cultures of microorganisms. A surgical team, performing even the most insignificant of procedures, employs the aseptic technique in order to prevent the contamination of surgical incisions or internal organs with microorganisms. Rudimentary aseptic techniques would includes personnel washing their hands with antiseptic and mild disinfectants, treating bench tops with disinfectants, sterilizing all materials and equipment used in operation (including instruments, gloves, surgical drapes, masks and gowns), and maintain their sterility by careful wrapping and handling [9].

5. ANTIMICROBIAL SILVER MATERIALS

As mentioned before, antimicrobial agents used on biomaterials are classified into two groups: the synthetic or natural inorganic groups, and the synthetic or natural organic groups. There is a new tendency merging towards the use of metallic antimicrobials in different methods for different biomedical and industrial applications. These new methods avoid the known disadvantages of the use of the metallic antimicrobials [3].

5.1 Methods of Antimicrobial Application of Silver

In modern medical practice, both inorganic and organic soluble silver salts are used to prevent and treat microbial infection. While these compounds are effective as soluble salts, they do not provide prolonged protection due to loss through removal or complexation of free silver ions. They must be reapplied at frequent intervals to overcome this problem. Reapplication is not always practical, especially, where an indwelling or implanted medical device is involved. Attempts were made to slow the release of silver ions during treatment by creating silver-containing complexes that lower the level of solubility-like colloidal silver proteins. Such compounds are formulated as creams. [30]. The following is a presentation of new method for biomedical and hygienic application of antimicrobial silver in different forms.

5.1.1 Antimicrobial Silver with Surgical Bioinert Implants

Surgical implants, especially endoprosthetic orthopedic implants and sutures, are rendered antimicrobial by the presence of a bioerodible metallic silver component. This component, especially as a surface coating, provides in vivo a sustained release of silver ions in a concentration sufficient to provide a localized antimicrobial effect insufficient to cause harm to tissue [31].

In early years of implant surgery, silver was employed in the manufacture of the endoprosthetic implants. However, the use of silver and silver plated implants has

generally ceased by about 1935 in the ever continuing search for greater bioinertness for implant materials. In the particular case of orthopedic implants, silver is generally considered unacceptable as an implant material because of poor mechanical properties, connective tissue reaction and excessive subperiosteal bone growth [31].

It has been proposed that inorganic silver compounds should be incorporated in bone cement to reduce the risk of postoperative infection following insertion of endoprosthetic orthopedic implant. In particular, it was proposed that low concentrations of inorganic silver compounds should be incorporated in polymethyl methacrylate bone cement for this purpose. The compounds that they evaluated for this purpose were silver chloride (AgCl), silver oxide (Ag_2O), silver sulfate (Ag_2SO_4), silver phosphate (Ag_3PO_4), and chloridal silver (Ag-AgCl). The least effective of the compounds evaluated was chloridal silver. It has also been reported that the electrically generated silver ions constitute a potent broad-spectrum antibacterial agent of use as adjunctive treatment of osteomyelitis [31].

The clinical use of metallic silver is limited and, in particular, that diffusion of silver ions from a metallic surface such as silver foil is negligible. The use of metallic silver in endoprosthetic implant is counter-indicated. The failure of metallic silver coating to generate the required antimicrobial efficacy is due to the fact that silver metal surface does not release antimicrobial amount of silver ion [30]. Further, in the field of orthopedic surgery the usage of silver salts and electrically generated silver ions for the prophylactic or adjunctive treatment of postoperative infection following implant surgery is recommended [31].

Metallic silver should be activated in the sense that it erodes in vivo to provide a sustained release of silver ion at a concentration sufficient to produce a localized antimicrobial effect but insufficient to cause significant damage to connective tissues. The other criterion is that the structural material of the implant should be a substantial bioinert material so that the mechanical integrity of the implant is retained despite the erosion of the metallic silver [31].

A method was designed to render antimicrobial an endoprosthetic implant, comprising a permanent implant structure formed of a substantially bioinert structural material providing permanent implant structure having permanent mechanical integrity. Also, the implant is treated so that the antimicrobial component latently bioerodible to provide in vivo a sustained release of silver ions in a concentration sufficient to provide a localized antimicrobial effect but insufficient to provide alcoholized antimicrobial effect but insufficient to cause significant damage to connective tissues. This method could be used for prophylactic treatment of postoperative infection following endoprosthetic surgery [31].

The implant can be made of any structural material which is substantially bioinert but usually will be made of titanium alloy, or cobalt-chrome-molybdenum alloy, or ceramic material, or any combination of these materials [31].

The metallic silver component can be made of commercially pure (i.e. 99.99% pure) silver metal or of a silver alloy, for example dental amalgam or silver solder. In order to promote galvanic action producing silver ions, there can be used an alloy of silver with a nobler metal such as gold or platinum. The silver component will be deposited in or on the permanent implant structure [31].

The quantity of silver in the composite implant and the rate of erosion in vivo are such as to provide a sustained release of silver ions in sufficient concentration to produce a localized antimicrobial effect but insufficient to damage connective tissues. The required concentration balance readily can be achieved because antimicrobial activity is provided at a concentration of the order of nanograms/ml, whereas connective tissue damage results of six order higher concentrations, i.e. mg/ml [31].

5.1.2 Antimicrobial Silver with Hydroxyapatite

A method for making antimicrobial hydroxyapatite powders consists of including hydroxyapatite which has good affinity for living body with antimicrobial ions such as silver, copper or zinc, or organic antimicrobials such as honokitiol, tanine, lyzozyme, protamine, or sorbic acid. These powders widely used in the medical dental and hygienic field such as artificial bones, artificial root of a tooth, dentifrice, dental cement, and supplement for bone for bone deficit and cosmetic materials [3].

As mentioned before, hydroxyapatite has a good affinity for living tissues such as bones and teeth, and hence a good affinity for biomolecules and microorganisms. Therefore bacteria are absorbed and breed in hydroxyapatite [3]. It is even used as a substance to concentrate bacteria from foods and other stuffs [32]. Accordingly, when hydroxyapatite is used as a living body material, it is necessary to consider its contamination with bacteria. To solve this problem, hydroxyapatite is to be used with antimicrobials [3].

Antimicrobial hydroxyapatite containing antimicrobial metal ions could be producing by having antimicrobial metal salts present with hydroxyapatite when being manufactured, or by reacting hydroxyapatite with these salts. For example, a calcium chloride solution can be dripped into or otherwise added to a solution containing disodium monohydrogen phosphate and antimicrobial metal salt selected from silver, copper, and zinc salts. The HA is produced from the solution, then filtered, washed with distilled water, dried and crushed. Alternatively, hydroxyapatite produced by the usual method can be suspended in water, and water-soluble antimicrobial salts added to the suspension. Then the precipitates are washed with distilled water, dried and crushed. The acid radicals, metal salts and calcium salts produced by changing the calcium ions of hydroxylapatite to one of the three metal ions, coexist with the antimicrobial hydroxyapatites. Thus, it is necessary to remove these contaminants by washing fully antimicrobial hydroxyapatite with water. The antimicrobial hydroxyapatites produced retain their antimicrobial activity for a long period, and the amounts of metal soluble in

water are ppm or less. Thus, the product can be used safely and their antimicrobial properties taken advantage of by adding the hydroxyapatite in amount of 10% or less preferably 0.5-5% to other materials [3].

The antimicrobial hydroxyapatite prepared following this method are useful because they are safe and have good affinity for a living body in the fields of foods, living body materials, cosmetics, fibers, cellulose, plastics, filters, and water absorption, polymers where antimicrobial properties are needed. When used in a dentifrice, an anticaries effect is obtained by the protein removal action and by the fine filling effect of hydroxyapatite. Pharmaceutical agents stay on the surface of a tooth with hydroxyapatite for a long period and the highly preventive agents of dental carries are developed by this method [3].

5.1.3 Antimicrobial Silver with Other Ceramics

An light antimicrobial ceramic contains an antibacterial material is produced by loading an antibacterial metal such as silver on a calcium ceramic carrier, an aggregate such as cordite and has a bulk density of 0.6-1.2 g/ml [33].

There has been conventionally been antibacterial metal such as silver, copper, and zinc loaded on a ceramic. Such antibacterial ceramics are used as soil conditioners due to their antibacterial property. An antibacterial ceramic having the shape of a pellet with enlarged specific surface area or the shape of Rasching ring has been used for treating water to improve its quality. Incidentally, the aforementioned antibacterial ceramic is formed to be relatively porous to enhance its antimicrobial property. However, many of such conventional antimicrobial ceramics are dense and heavy [33].

Firing a mixture of an antimicrobial metal and a specific material, and controlling the bulk density and porosity of the sintered body leads to the completion of this invention [33].

The method for producing a light weight antimicrobial ceramic of the present invention include steps of: preparing an antimicrobial ceramic material by loading an antimicrobial metal or metallic ion on a carrier; mixing the obtained antimicrobial ceramic material of 0.01-20 (%wt) with an oxide inorganic material and /or non-oxide material of 80-99.99 (%wt) to obtain a first mixture; mixing a combustible of 5-50 weight parts to obtain with the mixture of 100 parts weight with the mixture of 100 weight part to obtain a second mixture; and firing the second mixture with at a temperature 500-1300°C [33].

The antimicrobial metal or metallic ion could be silver, copper, zinc, platinum, chromium, tin, arsenic, lead, cadmium, or many mixtures of these components. The aforementioned carrier may be phosphate silica, zeolite, or many mixtures of these components. Phosphate for the carrier may be calcium phosphate, zinc phosphate zirconium phosphate, aluminum phosphate, or any mixture of these components. Calcium phosphate .for the carrier may be tricalcium phosphate, calcium phosphate, calcium metaphosphate, hydroxyapatite, apatite halides or any mixture of these components [33].

The methods for loading the antimicrobial metals or metallic ions on the aforementioned carriers are not limited. Suitable methods include, for example, ion exchange, or firing at about 800°C or higher after ion exchange, or firirng at about 80^0C or higher an aqueous solution containing a water-soluble salt having antimicrobial component and then collecting the precipitate. Among these methods, the preferred is a one in which a water-soluble salt having an antimicrobial component is used because this method prevents elusion of the antibacterial metallic component and reduces the production cost [33].

Another type of disinfectant bioceramic material which can be easily manufactured, has a stable disinfectant property, catches heavy metals which are harmful to human body, and radiates far infra red rays with a positive influence on living things. Examples of the base materials use are compounds containing alkalineaining alkaline

earth metals and phosphorous such as granulated bone, keel (fossil of vertebra), artificial apatite, phosphate calcium ore, tricalcium phosphate, $Mg_3(PO4)_2.5H_2O$, $MgO(SiO)_3.xH_2O$ (x is a coefficient indicating the number of water molecules) in which compounds containing calcium and phosphorous are not preferable, with these base materials, by reacting metals with disinfectant and harmless property such as silver, copper, or zinc, a disinfectant bioceramic material is obtained. A polymer or wallpaper is manufactured and a proper amount of the above bioceramic composition is added [5].

The above bioceramic composition with its useful properties, could be made from the burning of the bone of vertebrate animals such as cows, pigs, etc. It could be also made of a compound containing phosphorous and alkaline earth metals such as artificial apatite, as a base material with metals of disinfectant and harmless properties such as silver, copper or zinc. Compounds containing calcium and phosphorous are most preferred in that they have disinfectant properties against harmful bacteria by themselves. Moreover, these compounds activate beneficial bacteria for the human body. Using the base materials manufactured as above by reacting various kinds of metals with disinfectant and harm free properties such as, silver, copper or zinc, a disinfectant ceramic material of the present invention could be obtained. Here, the ionic tendencies of the above-mentioned metals (silver, copper, and zinc) are lower than those of alkaline earth metals, and hence a substitution reaction is expected. In the case where disinfectant metals have a substituting reaction with the base materials in accordance with the above-mentioned ratio, the ceramic materials thus made are capable of having a disinfectant property due to the disinfectant metals substituted. At the same time, alkaline earth metals remaining non-substituted can catch harmful ions of heavy me^{ta}ls s^{uc}h as $Hg2^{+}$, Pb2+, and Cd2+ by substitution and furthe get rid of harmful anions such as Cl^{-}, and F^{-}. The examples of the base materials which are most preferable for using as containers or wrappers for food are compounds containing calcium and phosphorous such as TCP, artificial hydroxylapatite, or $Ca_{10}(PO_4)_6(OH)_2$ (which is a primary composition of granulated bone of vertebrate [5].

However, the artificial apatite is too expensive, and TCP has an inferior affinity with the human body compared to the granulated bone. For base materials other than the food storing container or other kitchen goods, TCP or phosphate calcium ore can be used as base materials [5].

When bone of a vertebrate animal is burnt at a temperature of 1000° C, most of the organic and inorganic substances evaporate, and the remains after the evaporation become porous materials which are able to absorb impurities and heavy metals with ease. Thus, they can promote the effect of catching heavy metals. When granulated into very fine particles having an average particle diameter less than 0.2 μm, the porous materials comes to have a specific surface area of roughly 200 m^2 /g. Since, these granulated particles have a property of radiating far infra-red rays, they can also be used as the materials for making a far infra-rg ceramic material [5].

Also, in the case of using the base materials as an artificial bone or tooth for man, by adding a proper amount of the above disinfectant metals, the occurrence of inflammation or carious tooth after an operation can be remarkably reduced. Additionally, a container for food made of the base materials containing calcium salt, such as the above granulated bone, may help water or wine to become more tasteful by eluting small quantities of calcium, which may supply a human body with calcium ions, which are likely to be in short supply [5].

5.1.4 Silver with Biomedical Polymer-Based Antimicrobial Devices

5.1.4.1 Antimicrobial Silver Complex Salt with Silica Gel

A new antimicrobial composition of silver thiosulfate complex salt is carried on silica gel particles having an equilibrium water content of 10% or smaller. At least a part of the surface of the silica gel particles carrying silver thiosulfate complex salt is covered with a coating layer composed of silicon dioxide formed by means of hydrolysis of alkoxysilane [34].

The silver salt antimicrobial agents have a disadvantage that silver ions of the agent would be allowed to react with chloride ions in tap water to form insoluble silver chloride, resulting in a loss of their antimicrobial effect. Further, the silver salts, since they have a high photochemical reactivity, suffer another disadvantage that they become black and deteriorate their antimicrobial performance by being transferred into metallic silver or silver oxide, when exposed to light. In addition, the silver salt antimicrobial agent have a further disadvantage that in case of incorporating them into the resin by kneading or compounding and the resin is molded at a temperature of 200°C or above, it is difficult to obtain a white or transparent resin molded product because the antimicrobial agent may sometimes discolor or make a dull color at the heating temperature during the heating process [34].

An antimicrobial composition that avoid the above disadvantages and deficiencies inherent to the prior art silver salt antimicrobial agents and composition. The presented antibacterial composition in accordance with the present invention hardly produces white turbidity when contacted with tap water, showing no formation of AgCl. Thus a stable antimicrobial effect. Also, this composition will not discolor at the molding temperature of resin and can give a white or transparent resin molded product [34].

This new composition consists of a silver thiosulfate complex salt and silica gel particles having an equilibrium water content of 10% per weight or smaller at room temperature and ordinary humidity (at 25°C, relative humidity is 70%), and carrying said silver thiosulfate complex salt [34].

Silica gel particles as the carrier having two hydroxyl groups (-OH) or less for nm^2 of their surface are particularly preferred in point of view of the thermal stability of the carried silver thiosulfate complex salt. In the above mentioned antibacterial composition of this method, at least part of the surface of the silica gel particulate carrier is preferably covered with a coating layer. In the compliance with a covering area, thickness, porosity, and the like of the layer of the coating material, the degree and the

rate of releasing of the silver thiosulfate complex salt carried on the carrier are properly controlled [34].

The present composition comprises potassium silver thiosulfate salt carried on silica gel particles. Although the silver thiosulfate complex salt is difficult to obtain as a single substance in a solid state, it is possible to immobilize the salt in a state of being carried on a carrier, by first preparing as a water soluble compound, then impregnating a silica gel carrier with an aqueous solution of the compound, and drying the impregnated carrier [34].

The silver thiosulfate complex salt can be obtained by adding at least one salt selected from the group consisting of a sulfite salt and a hydrogen sulfite salt to a water soluble silver salt, and then adding a thiosulfate salt. Each of said sulfite salt, hydrogen sulfite salt and thiosulfate salt is preferably a sodium salt, and more preferably a potassium salt [34].

This method further provides an antibacterial composition comprising a potassium salt of silver thiosulfate complex carried on silica gel particles [34].

The silver thiosulfate complex salts used in the present invention form radicals near them by an action of oxygen in the air, and the radicals exert an influence on the surface of a membrane of the microorganism which accesses to the radicals and thus the silver salts demonstrate the antibacterial activities. Such an influence is called oligodynamic reaction. That is, it is presumed that the silver thiosulfate complex salts convert the oxygen near the salts into active oxygen by their catalytic action. The active oxygen gives a damage on a cell membrane covering the surface of the microorganism and demonstrates their antibacterial actions on the microorganisms by giving a defect on a genetic information, thereby suppressing the growth of microorganisms [34].

5.1.4.2 Antimicrobial Composition of Silver Nitrate and Gentian Violet

Indwelling devices such as catheters are made silastic and polymer-based with improved antimicrobial properties. They would include low amount of gentian violet or a combination of gentian violet and silver nitrate. These devices are made of polyurethane, silicon, or other similarly absorptive materials [35].

Catheters for patient care that are treated to include metal antimicrobial compounds, such as silver and silver oxide, gold thioglycolate and copper oxide. Silver nitrate pretreatment of silastic catheters (using a silver nitrate in alcohol technique) has previously been reported by the present inventor to impart *E. coli* resistance to a treated surface. Silver nitrate suspended in alcohol has also been observed to provide a more effective and rapid impregnation of silastic rather than silver nitrate in water. Silver sulfadiazine with chlorhexidine and other sulfa drugs have also been used with commercial catheters to enhance infection resistance [35].

This method demonstrates that low amounts of gentian violet, or a combination of gentian violet and silver nitrate, provides for an improved and microbially resistant device using anti-microbial agents that do not give rise to antibiotic resistant microbial strains. The present invention, in a general and overall sense, concerns medical devices of silicone or polyurethane, and like materials, that includes relatively low concentrations of gentian violet or mixtures of gentian violet and silver nitrate. These devices are found by the present inventors to have extraordinarily effective antibacterial and antifungal activity. These elegantly simple devices provide amazingly effective antimicrobial activity at concentrations several folds lower than gentian violet concentrations previously contemplated in the literature. Potential undesirable characteristics associated with "tattooing" or discoloration of tissues are thus reduced, without loss of bacteriostatic activity. Other advantages of the invention include improved, longer-term protection against infection of both bacterial and fungal origin. Such is accomplished without antibiotics, thus reducing the incidence of resistant bacterial infection [35].

5.1.4.3 Antimicrobial Synergistic Composition of a Silver Salt and Chlorhexidine

A method of preparing an infection-resistant medical device comprising one or more matrix-forming polymers selected from the group consisting of biomedical polyurethane, biomedical silicones and biodegradable polymers, and antimicrobial agents, especially a synergistic combination of a silver salt and chlorhexidine (or its salts); also disclosed are medical devices having the synergistic composition therein or compositions thereon [36].

The charge that the polyurethane polymer carries influences the affinity for the antimicrobial agent. When the antimicrobial is silver chlorhexidine acetate (CHA), the polymer of choice would be a polyether polyurethane, specifically Pellathane [36].

The biomedical Silicones that can be used in this composition include the silicon rubbers or elastomers. The selection of specific polymeric coating agent to form a coating agent to form a coating matrix will depend upon the nature of the surface to which the coating will be applied. It is preferred that biomedical polyurethane be applied to polyurethane surface to assure good adherence. A biomedical silicone such as a mixture of Silastic type A Medical Adhesin and MDX4-4159, is suitable to coat a device that is fabricated of silicone, polyurethane or of latex [36].

It has further been found that use of a biodegradable polymer in the coating composition of this invention, either alone or in combination with one or more of the other biomedical polymers, enhances the character of the polymer matrix [36].

The use of degradable polymer modulates the release of antimicrobial agents. The initial burst of agent which during the first few days after implantation is mere of less eliminated since the agent is bound in the biodegradable polymer and will be released only when degradation of the polymer occurs. Inclusion of a biodegradable polymer such as polylactic acid (PLA), in the matrix gives prolonged biocidial activity [36].

An additional advantage of using a biodegradable polymer such as PLA in a polyurethane matrix is to allow improved tissue ingrowth simultaneously with a prolonged antimicrobial effect as the biodegradable polymer degrades. Thus, this embodiment of the invention is particularly important in orthopedic applications as well as in such devices as arterial grafts where there is a need for formation of the pseudo intima or the growth of tissue into the interstices of orthopedic implants and arterial grafts, as well as cuffs which anchor catheters in place [36].

Antimicrobial agents useful according to this first embodiment of the invention include the biguanides, especially chlorhexidine and its salts, including chlorhexidine acetate, chlorhexidine gluconate, chlorhexidine hydrochloride, and chlorhexidine sulfate, silver and its salts, including silver acetate, silver benzoate, silver carbonate, silver iodate, silver iodide, silver lactate, silver laureate, silver nitrate, silver oxide, silver palmitate, silver protein, and silver sulfadiazine, polymyxin, tetracycline, aminoglycosides, such as tobramycin and gentamicin, rifampicin, bacitracin, neomycin, chloramphenicol, miconazole, quinolones such as oxolinic acid, norfloxacin, nalidixic acid, pefloxacin, enoxacin and ciprofloxacin, penicillins such as oxacillin and pipracil, nonoxynol 9, fusidic acid, cephalosporins, ephalosporins, and combinations thereof

From the above list, unexpectedly, some special combinations have been found. The combination of the biguanides, especially chlorhexidine and its salts with silver salts cause a special synergistic sustaining of antimicrobial action, as described in the second embodiment of the invention below. The biguanides are also synergistically effective with nalidixic acid and its derivatives. Another effective combination is chlorhexidn is chlorhexidine acetate and where the antimicrobial agent used is insoluble in the coating vehicle, as is the case with most of the silver salts and the water insoluble chlorhexidine, it is preferred that the agent be very finely subdivided, as by grinding with a mortar and pestle. A preferred product is micronized, e.g., a product wherein all particles are of a size of 5μm or less. In the case of the preferred silv preferred silver sulfadiazine,a micronized product may be used. The only way to use chlorhexidine internally is in the

time release matrix system described above that allows for a dose that is non-toxic to the patient but effective against microorganisms [36].

5.1.4.4 Silver with heart prosthetics

Another form of silver application includes an antimicrobial metallic coating on portions of the prosthesis, usually fabric, to enhance the overall acceptability of the implantable device. The preferred metal is silver, which is applied to surfaces that are exposed to the heart tissue. Mechanical and bioprosthetic heart valves benefit from this coating and it may also be applied to annuloplasty rings, composite valved grafts, sutures, pledges, a heart girdle or other implantable devices. The silver-treated portions of these devices inhibit or greatly reduce colonization of endocarditis-causing bacteria without affecting the overall biocompatibility of the device [37].

There are several ways to provide the silver coating to the devices. First, the fabric used in the construction of the devices may be coated after the fabric is formed. Second, the yarn or fiber that makes up the fabric can be coated before the fabric is formed. Third, after the fabric portion is constructed, the fabric portion itself may be coated with the silver. In addition, the silver coating may be applied directly to a device. In one embodiment, the amount of silver required is quite small, generally in the range of 1-100 mg of silver per gram of fabric, preferably 20-50 mg of silver per gram of fabric. Coating the fiber may be advantageous because this technique may produce a more optimal distribution of the silver in the completed product. For all the coating methods, the porosity of the textile must be preserved so that the tissue ingrowth properties are adequate [37].

For stented bioprosthetic heart valves, the fabric used for the sewing cuff could be coated in the same manner as described above. It is also contemplated that the stent for these valves may be coated with silver to further enhance the infection resistance of the device. In stentless bioprosthetic heart valves, the outer fabric wraps may also be treated with the silver coating [37].

The thin film silver coating may be deposited or carried in the fabric member, fibers, or sewing cuff using methods known to the art. Through action the term "coating" or "coated" may be used to mean that the silver may be coated on the surface of the device or component or may be implanted within or into the device or component as well as on the surface of the device or component, depending on how the silver was applied. For instance, the silver coatings may be applied by vapor-deposition or by sputtering. Although any of a number of techniques can be used to create the silver coating, it is important that the coating be extremely adherent to the fabric or other materials to prevent excessive circulation of cytotoxic silver materials throughout the body, while retaining porosity necessary for tissue ingrowth [37].

As is known in this art, other metals may be adhered to the fiber before the coating of silver is applied. Similar methods may be employed for coating the fabric or the assembled sewing cuff by utilizing fixtures, such as mandrel, to rotate or position the fabric or cuff to provide a uniform coating of silver [37].

To achieve uniform and tightly adherent silver coating, vapor deposition techniques, such as ion beam implantation and ion beam-assisted deposition (IBAD) are contemplated within this disclosure [37].

5.1.4.5 Silver with Antimicrobial Resin for Ophthalmic Solution

A method for improving the antibacterial efficacy of an ophthalmic solution, particularly a solution for the care of contact lenses, comprising providing an article molded from plastic resin including an inorganic carrier retaining antibacterial metal ions, and placing the ophthalmic solution in contact with the plastic resin [38].

It is understood that the term "improve the antibacterial efficacy" includes imparting antibacterial properties to a solution, such as solutions which contain no preservatives or other antibacterial agents, as well as enhancing antibacterial properties of a solution that already contains an antibacterial component [38].

The antibacterial efficacy of the solution is improved by contacting the solution with the article composed of a plastic resin having incorporated therein antibacterial metal ions retained in an inorganic carrier [38].

The method is particularly useful for contact lens cases since the prevention of bacterial contamination is more dependent on the user following care regimen instructions. For example, a user may fail to properly clean the lens case between uses, and if the case becomes contaminated, a biofilm can then form on the surfaces of the case from colonization of the contaminating microorganisms. Such a biofilm provides means for bacteria to survive even when later subjected to a preserved solution since the biofilm "protects" microorganisms from contacting the solution. The invention provides a method of inhibiting the formation of such a biofilm on the surfaces of the contact lens case to further enhance the efficacy of an ophthalmic solution when placed in contact therewith [38].

The plastic resin is preferably a thermoplastic or thermosetting resin suitable for molding, such as injection molding or blow molding. Additionally, the resin is selected such that it is compatible with the inorganic carrier retaining the antibacterial metal ions. One class of resins are thermoplastic polyolefin resins, including high-density polyethylene, low density polyethylene and polypropylene. Other resins include polycarbonates, polyvinyl chlorides, polystyrene, as well as other known plastic resins [38].

The inorganic carrier must be one that retains the antibacterial metal ions and which is suitable for molding processes when combined with the plastic resin. Particularly, it is desirable that when the ophthalmic solution is in contact with the plastic resin, only the metal ions leach in the solution while the inorganic carrier remains in the resin [38].

A preferred inorganic carrier is a zeolite. Zeolites may be defined as a silicate of aluminum and a metal oxide having a three-dimensional skeletal structure, and represented by the formula $xM_{1/2}O.Al_2O_3.ySiO_2.zH_2O$, wherein M is a univalent or bivalent metal ion such as sodium or calcium, n is the valence of this metal ion and x is a coefficient of this metal ion, y is a coefficient of the silica, and z is the number of water crystallized. For the zeolite employed in the method of this invention, the metal ion M is exchangeable with the antibacterial metal ions, such as silver ions, copper ions or zinc ions, whereby the antibacterial metal ions are retained in the zeolite. Silver metal ions may be incorporated in the zeolite at 0.001 to 10% by weight (based on weight of anhydrous zeolite), more preferably 0.001 to 5%; zinc or copper ions may be incorporated at 0.01 to 25% by weight, more preferably 0.01 to 15% by weight. Another inorganic carrier that can retain antibacterial metal ions is an amorphous aluminosilicate. Such materials have an amorphous three-dimensional skeleton and may be represented by the formula $xM_{1/2}O.Al_2O_3.ySiO_2$ wherein the variables are defined as above for the zeolite. Silver metal ions may be incorporated in the carrier at 0.001 to 20% by weight (based on a dry weight of the carrier), more preferably 0.001 to 12%; zinc or copper ions are incorporated at 0.01 to 15% by weight, more preferably 0.01 to 10% by weight [38].

5.1.4.6 A Crystalline Silver Antimicrobial Composition with Polymers

The method below relates to a novel antimicrobial polymer composition comprising a novel crystalline antimicrobial composition. More particularly, the present invention relates to an antimicrobial polymer composition comprising a crystalline antimicrobial composition and a polymer, wherein said crystalline antimicrobial composition comprises a crystalline silicon dioxide containing silver ions and one or two optional metal ions selected from the group consisting of zinc and copper [39].

This method provides an antimicrobial polymer composition comprising a crystalline antimicrobial composition and a polymer, wherein said crystalline antimicrobial composition comprises a crystalline silicon dioxide containing silver ions and one or two optional metal ions selected from the group consisting of zinc and copper.

The present invention also provides a process for preparing an antimicrobial composition of said antimicrobial polymer composition comprising steps of 1) preheating an antimicrobial composition having an antimicrobial coating of an aluminosilicate provided on the surface of silica gel, wherein said aluminosilicate contains silver ions and one or two optional metal ions selected from the group consisting of zinc and copper, to a temperature between 250° and 500° C. to substantially remove water, 2) sintering at a temperature from 800° to 1300° C., and 3) mixing the obtained crystalline antimicrobial composition with a polymer [39].

Both halogenated and non-halogenated organic polymers may be used in preparing the antimicrobial polymer composition of the present invention. Non-halogenated organic polymers may be synthetic or semi-synthetic and include the following: thermoplastic synthetic polymers such as polyethylene, polypropylene, polystyrene, polyamide, polyesters, polyvinyl alcohol, polycarbonates, polyacetals, ABS resins, acrylic resins, fluorine resins, polyurethane elastomers and polyester elastomers; thermosetting synthetic polymers such as phenolic resins, urea resins, melamine resins, unsaturated polyester resins, epoxy resins and urethane resins; and regenerated or semi-synthetic polymers such as rayon, cuprammonium rayon, cellulose monoacetate, cellulose diacetate and cellulose triacetate. If a strong antimicrobial and/or microbicidal effect is necessary, the polymer composition is preferably foamed or otherwise shaped into a net, a fiber, etc. Preferred from this viewpoint are organic or fiber-forming polymers such as synthetic polymers exemplified by nylon 6, nylon 66, polyvinyl alcohol, polyethylene terephthalate, polybutylene terephthalate, polyacrylonitrile, polyethylene, polypropylene and copolymers thereof, and regenerated or semi-synthetic polymers exemplified by rayon, cuprammonium rayon, cellulose monoacetate, cellulose diacetate and cellulose triacetate. Halogenated organic polymers that can be used in the present invention are also not limited to any particular kinds and may be examplified by polyvinyl chloride and polyvinylidine chloride [39].

5.1.5 Some Other Applications of Antimicrobial Silver

A method and a portable apparatus is provided which can be transported to the area of treatment, generate silver ions or ions of other selected elements such as copper, uniformly distribute the ions over both confined and extended areas of water and land, and destroy infectious bacteria and/or other undesirable microorganisms in such areas. Water under pressure is passed through a manifold containing silver electrodes (or other desired metal electrodes) which are energized to produce ions, (primarily silver and secondarily copper). Being the reference base for the International Ampere and Faraday's Law, it was well known to be readily ionized. Accordingly, N.A.S.A. developed a simple electrolytic system to generate silver ions to be circulated in the water to be treated. A control unit generates a direct current which is applied across electrodes of specially formulated silver alloy immersed in the water being treated. The dose rate at which these ions are generated and liberated into the water is set and maintained by solid-state microprocessor circuitry in the control unit. This same system, with the addition of copper to the silver and with other minor adaptations, is now being used in the treatment of water in many commercial and industrial applications such as swimming pools, spas, air conditioning cooling towers, food processing, etc. At the same time, several patents have been issued in recent years which cover unique ways of using silver in water treatment and disinfecting [40].

A method was provided for preventing a microorganism from growing on surfaces such as the skin, walls, floors, countertops, and food preparation surfaces as well as in absorbent materials such as diapers, clothing, bedding, bedpads surgical apparel, surgical masks and the like. This method includes incorporating an effective amount of zeolite onto the surface or into the absorbent material to inhibit such microorganisms from growing [41].

According to the present invention, zeolites prevent microbial growth thereby inhibiting urea from converting to ammonia and carbon dioxide. Therefore, zeolites have two beneficial effects: inhibition of bacterial growth and inhibition of ammonia and

carbon dioxide production. Zeolites can be chemically synthesized and occur naturally in volcanic rocks, altered basalts, ores and clay deposits. Zeolites include crystalline, hydrated alkali-aluminum silicates of the general formula: $M_2/n\ O.(Al_2O_3).[y(SiO_2)].wH_2O$ wherein M is a cation of valence n, w is the number of water molecules, and y is 2 or more. The cation is mobile and can undergo ion exchange [41].

5.1.6 Depositing Antimicrobial Silver on Surfaces

Metallic compounds may be applied to a surface by metal surface deposition, electroless plating, chemical derivatization, and coating [42].

Metallic silver is deposited upon then surface of a non-conductive substrate using a multi-step-wet deposition. The surface is cleaned, and then activated in an aqueous solution containing stannous tin. The silver is deposited as a colloidal material from a solution containing a silver salt and a reducing agent that reduces the salt to form metallic silver by nucleating throughout the solution. After the substrate is coated, the coating is stabilized in an aqueous solution of a salt of a noble metal like gold or platinum, dissolved in a dilute, hydrophobic acid. This process is particularly effective for depositing uniform film of 2 to 2000 Angstrom thickness, which strongly adhere to the substrate. A thin film can reduce the incidence of infection caused by a device introduced into the human body [43].

In one method, metallic silver coating is accomplished by contacting the surface with a carbonyl compound, e.g. an aldehyde such as glutaraldehyde, a sugar such as glucose, or an aldehyde functionally generating compound. Then, the substrate is dried and contacted with a metal salt, e.g. silver nitrate, or metal carboxylate solution, e.g. a dichloride s solution to deposit the metal on the surface. In another embodiment, the substrate is contacted with an activator, e.g. a dichloride solution, dried, and then contacted with a metal salt or metal carboxylate salt solution, either with or without an amine containing compound solution, so as to deposit the metal on the surface of the substrate [44].

Some antimicrobial materials are molecularly designed to enable a matrix bound biocide retain high activity without elution of any compound into contacting solutions, carriers or other materials. Their activity stems from the cooperative biocidial action of its components. Selective transfer of one component form with the matrix directly to the microorganism upon contacting is achieved via a "hand-off" mechanism upon engagement and penetration of the microorganism's cell membrane [44].

The antimicrobial material of this type comprises a combination of an organic material which forms a matrix, and a broad spectrum biocide intercalated in the matrix that interact sufficiently strongly with the organic material that the biocide does not leach from the matrix. The organic material must possess two important properties: it must be able to bind or complex with the biocid with the biocie, and must be capable of dissolving into or adhering to the cell membrane of the organism. The organic material preferably is capable of being immobilized on a surface and which binds the biocide in such a manner as to permit release of the biocide into the organism but not the surrounding environment. The biocide preferably is a low-molecular-weight metallic material that is toxic to the microorganism and is capable of complexing or reversibly binding to the organic matrix material, but which preferentially binds to cellular proteins of the microorganism [44].

5.2 Colloidal Silver

5.2.1 Structure, Concentration, and Antimicrobial Activity of Colloidal Silver

At least four different products in the market are being called colloidal silver. The first one is the original kind called also electro-colloidal silver. The second is mild silver protein, which may appear as transparent clear or amber. The third is the ground or powder silver, with a silver content of 100-500 ppm. It is made when a silver wire is rapidly disintegrated by a high voltage electric discharge. The microscopic dust is collected and then dissolved in water or added to salves or cream for topical use. The fourth one is a group of different ionic silver salt solutions [45].

Colloidal silver is pure elemental silver that is evenly distributed into a second substance, i.e. water. The silver is not dissolved but suspended evenly throughout the solution as a result of the positive electric charge that is attached to each particle of silver. The charged silver particle, i.e. silver ions, form into aggregate of atom microcluster of silver that interact with each other and create a synergistic effect, each electrically charged particle of the microcluster has a magnetic field. Together the particles create a phenomenon called Brownian movement, which is a sort of random oscillating motion. In other words, the microclusters also have a positive charge and repel each other, that is how they stay suspended and evenly distributed in the water. This colloidal condition is most easily detected by what is called Tyndal effect where a narrow beam of light is shined through the liquid to produce a cone shaped dispersion of light. The particles so illuminated exhibit a random, zigzag motion called Brownian motion when observed under a microscope. When something is completely dissolved, both the Brownian and Tyndal effect disappear [8, 45].

Electro-colloidal silver is both ionic and colloidal. It is considered colloidal because of the particle size and ionic because of the particle size. In fact, most of the biological studies suggest that the ionic characteristics of colloidal silver are what make such a good germicide [45].

In order to produce electro-colloidal silver, at least .999% pure silver is placed in water, an electrical charge is induced into the silver, and as a result particles of silver are electrically sintered off of the metallic silver rods or wire into water. In the water, the silver forms submicroscopic clusters (atomic microclusters) optimally 10 to 100 Angstrom. Parts per million is a way to express the concentration of colloidal silver [8]. It is nearly impossible to standardize Electro colloidal silver. Even when the voltage, the water and the water temperature are identical, different batches will proceed at different rates in different days. The rate of the reaction can vary by over 100% depending on the day [45].

The total allowable impurities in .999 pure silver are 1000 ppm. These impurities and their maximums are copper (800 ppm), lead (250 ppm), iron (200 ppm), and bismuth (10 ppm). When this product is used to make electro-colloidal silver at a concentration of 5 ppm, the total impurity concentration drops to 4-ppb (parts per billion) copper, 1.25 ppb lead, 1 ppb iron, 0.05 ppb bismuth. In fact when analyzing samples of colloidal silver, the concentration of impurities is much more greater than the above figures and different elements such as Ca, Mg, K, and Mn are detected, showing that most of the impurities usually come from the used water [45].

In a previous study, various forms of silver were tested form their antimicrobial activities. Electro-colloidal silver out performed silver nitrate, silver chloride and silver sulfadiazine as a broad-spectrum germicide. For all classes of bacteria, fungi, and molds, colloidal silver performed better and at lower concentration. A conclusion was drawn that any additives reduce the effectiveness of silver ion, as silver salts being 100 times less effective than colloidal silver [45].

The positive effects of colloidal silver are not yet fully understood; therefore, the following explanation is hypothetical. The obsercal. The observed antimicrobial activity of colloidal silver appears to the result of silver microcluster' s frequency, motion, and magnetic field that the particle generate [8].

The Brownian movement and collective charge of each colloidal silver microclusters have several effects. These microclusters of silver have a frequency that is apparently disruptive to pathogens the oscillating particles seemingly gyrate in the fluids of the body interacting with cells including pathogens. Then acting as catalysts, the silver disables the oxygen-metabolizing enzyme, destroying these pathogenic particles, not by attack, but by creating an environment hostile to its existence [8].

The colloidal silver microclusters frequency appears to be disruptive to the pathogenic cells' ability to manufacture the enzyme used to process oxygen. Aerobic pathogenic cells need to quickly replace their spent oxygen used to survive, without the required enzyme pathogenic cells need to metabolize oxygen they are in effect suffocated within 6 minutes of initial contact with the silver [8].

Furthermore, researchers have also noticed that the Hexhermer effect, also referred to as a healing crisis or healing response caused by a reaction in the body to the toxins by the dead pathogenic cells as they "die off" and decompose, is lessened or not as apparent with the use of colloidal silver [8].

Some researches indicate that colloidal silver appears to concentrate in the Kuppfer cells of the liver. Colloidal silver localized in the Kuppfer cells of the liver may be allowing the body to grab and filter out of the pathogen with the help of silver that is localized in the liver Kuppfer cells and sweep them before the pathogen is lysed (decomposed/dissolved). What appears to happen is that the dead pathogens are immediately swept from the human body before there is a substantial toxic build-up [8].

5.2.2 Some Applications of Colloidal Silver

Colloidal silver could be used as a disinfectant for a hemodialysis system. Colloidal silver is introduced to the blood and dialysate sides of a hemodialysis session and is not flushed from the system until another dialysis is to be performed. In the past, formaldehyde and renaline (a hydrogen peroxide-peracetic acid based product) were

used. Being extremely toxic if introduced in a patient, formaldehyde was carefully applied in a way to avoid its presence in the blood, tubing, and blood side of dialyzer before reuse. Therefore, there was a need for a disinfectant that is chemically pure, not toxic, and easily removable from the dialyzer. The need can be filled by properly using colloidal silver as disinfectant, preferably at a concentration of 3 ppm. This disinfectant is circulated to fill the dialyzer and associated set of dialysate and blood tubing and related apparatus after they have been rinsed followtreatment. The preferred concentration of the used colloidal silver is 3 ppm (±10%). Greater concentrations could be used but there is no significant increase in disinfecting effectiveness, and there is the disadvantage of being more costly. In fact, a concentration above 5 ppm does not practically give as good results as a concentration about 3 ppm [46].

A colloidal silver solution can be produced in a batch procedure by suspending two substantially pure silver electrodes, as anode and cathode, in distilled water and subjecting the electrodes to a relatively high alternating current voltage as, for example, 440 V. This procedure is continued until sufficient silver particle has sloughed into then water to achieve the desired concentration of 3 ppm. These silver particles are very minute, typically in the range of about 0.005 to 0.015 μm and normally have the same polarity. Hence, these particles remain suspended in the water in a colloidal state [46].

A composition of matter comprising a therapeutic active component with antiseptic and osmotic characteristics for treatment of burns and open wounds experienced by animals and man. The compound is in solution form and composed of colloidal silver, Helichrysum angustifolium or Helichrysum italicum oil and raw honey emulsified with water lecithin by agitation [47].

Silver complex compounds and colloidal silver are known are known in the prior art for use as therapeutic and antimicrobial treatments of wound and thermal injuries experienced by humans [47]. Also, colloidal silver has been used topically for conjunctivitis, urethritis, and vaginitis [7]. Colloidal silver, earlier used as an antibacterial therapy, has declined in use as a result of the development of modern

antibiotics, the cost of the colloidal silver production (which has become much lower nowadays) and the possible adverse reaction of skin discoloration. The present compound is of low viscosity colloidal silver based compound which has eliminated the risk of skin discoloration and which is produced at little cost. Helichrysum is distilled oil product, flowering heads of Helichrysum angustifolium or italicum. Raw honey provides antiseptic qualities and retards the loss of fluids from the trauma site. Water-soluble lecithin is used as an emulsifier. Colloidal silver effectively eliminates bacteria, virus, and fungi precluding mutation and thus resistance to future uses [47].

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6.2.1 Introduction

Our obtained electro-colloidal silver solutions were of 3.32 and 12.72 ppm. These concentrations were determined by Atomic Absorption Spectroscopy (AAS) (see figure 6.2). This technique involves valence electron transition yielding radiation with wavelength in the ultraviolet-visible region of the spectrum [48].

Atomic Spectra

Electron orbits in an atom are characterized by the major (n) and azimuthal (l) quantum numbers. When an electron undergoes a transition from the high energy level (E_{nl}) to a lower enregy level (E_{n1l1}), light of frequency

$$\upsilon = (E_{nl} - E_{n1l1})/h = \Delta E/h \qquad (6.1)$$

is given off. In terms of wavelength

$$\lambda = c/v = hc/\Delta E. \qquad (6.2)$$

The constant h and c in these equations are Plank's constant and the velocity of light, respectively. The parameters ΔE, υ and λ are unique for a given electronic transition. An element can undergo many electronic transitions. This results in a series of sharp lines-a spectrum-which is uniquely characteristic of each element [48].

Emission and absorption can be related as shown in figure 6.3. An electron of an atom in its lowest energy state, the ground state, can absorb a quantum of energy ($+\Delta E$) and undergo transition to a low-lying excited state. Emission occurs when this quantum of energy is released ($-\Delta E$) and the electron returns to the ground state. A transition to and from the ground state, is called a resonance transition. Resonance lines are the most useful analytical lines for atomic absorption spectroscopy [48].

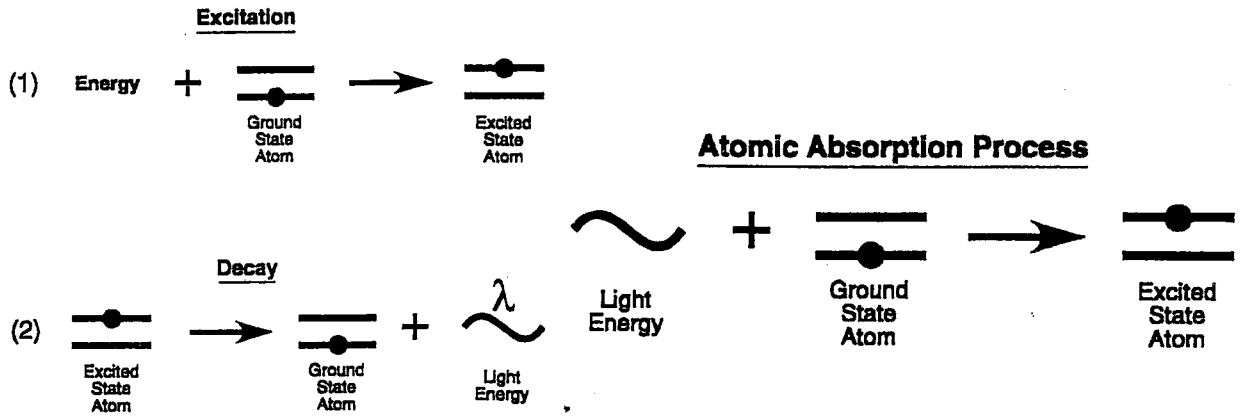

Figure 6.3 Excitation, Decay, and Atomic Absorption Process [48].

Absorption Expression

Absorption of monochromatic radiation is governed by Lambert and Beer's laws. Lambert's law states that light absorbed in a transparent absorption cell is independent of incident light intensity. An equal fraction of the light is absorbed by each successive layer of absorbing medium. Beer's law states that absorption of light is likewise exponentially proportional to the number of absorbing species in the path of the light beam [48].

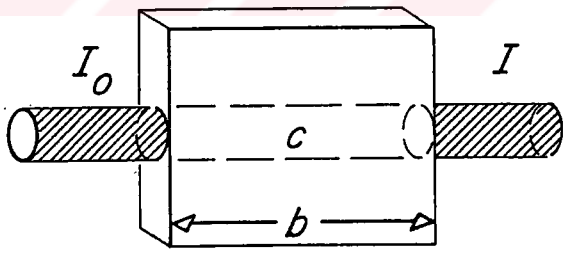

Figure 6.4 Atomic Absorption of Length b [48].

Referring to Figure 6.4, the incident beam of monochromatic radiation I_0 falls on an absorption cell of length b. The transmittance is given by

$$T = e^{-abc}. \quad (6.3)$$

Then, since

$$\log_{10}(1/T) = \log_{10}(I_0/I) = abc$$

and

$$\log_{10}(I_0/I) = A,$$

where A is the absorbance , then

$$A = abc, \quad (6.4)$$

where a is a constant for a given system and c is the concentration of the analyte atoms in the flame. This expression, known as the Beer-Lambert law, predicts a linear relationship between absorbance and concentration, as long as a and b remain constant [48].

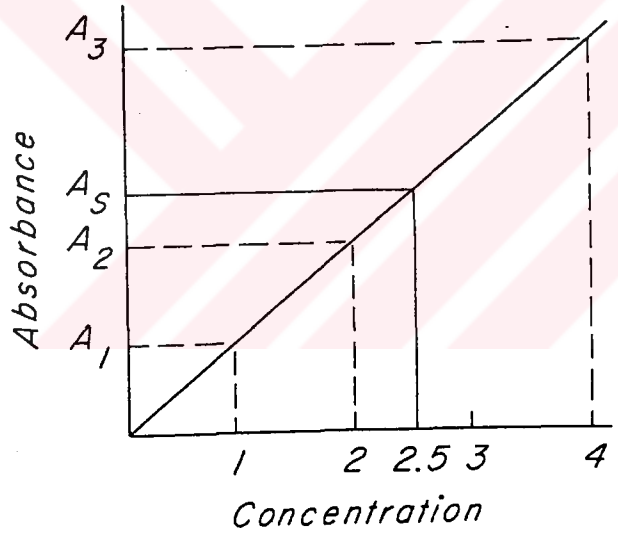

Figure 6.5 Linear Calibration Graph [48].

Figure 6.5 shows a linear calibration graph. Three solutions of known concentration 1, 2, and 4 ppb of an element is nebulized into an atomic absorption flame and the absorbances are found to be A_1, A_2, and A_3 respectively. As can be seen, this yields a straight line as predicted by the Beer-Lambert law. A sample to be analyzed is

then nebulized. The absorbance obtained is A_s. By interpolation on the calibration graph for the sample [48].

The concentration working range for an element is determined by atomic absorption spectroscopy is generally 4 to 5 orders of magnitude. At the upper end of the concentration range, it is common to have the graph bend toward the concentration axis (Figure 6.6, Curve B). Less frequently, the calibration curves throughout the whole working range (curve D) [48].

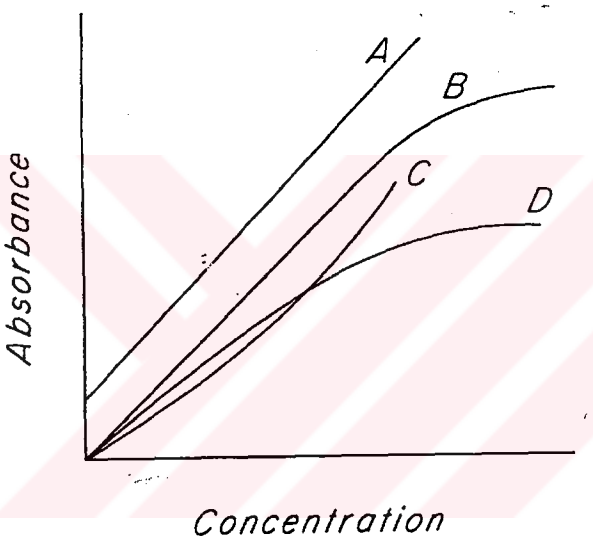

Figure 6.6 Calibration graphs showing departures from ideality [48].

Atomic Absorption Spectrophotometer

The essential components of an atomic absorption spectrometer are diagrammed in Figure 6.7. In most commercial instrumentation, A is a hollow cathode lamp, B a flame or electrothermal device, C a grating monochromator, and D a photomultiplier. The principle of AAS could be explained as follows [48].

The hollow-cathode lamp emits radiation characteristic of the cathode material, usually a single elements (analyte). This beam consisting largely of resonance radiation is electronically or mechanically pulsed. Analyte atoms are produced thermally in the atom reservoir. Ground-state atoms, which predominate under the experimental conditions, absorb resonance radiation from the lamp, reducing the intensity of the incident beam. The monochromator isolates the desired resonance line and allows this radiation to fall on the photomultiplier. An electrical signal is generated. The electronics of the unit are designed to respond selectively to the pulsed radiation emanating from the radiation source. Signal processing occurs, which results in electronic output proportional to the absorption by analyte atoms [48].

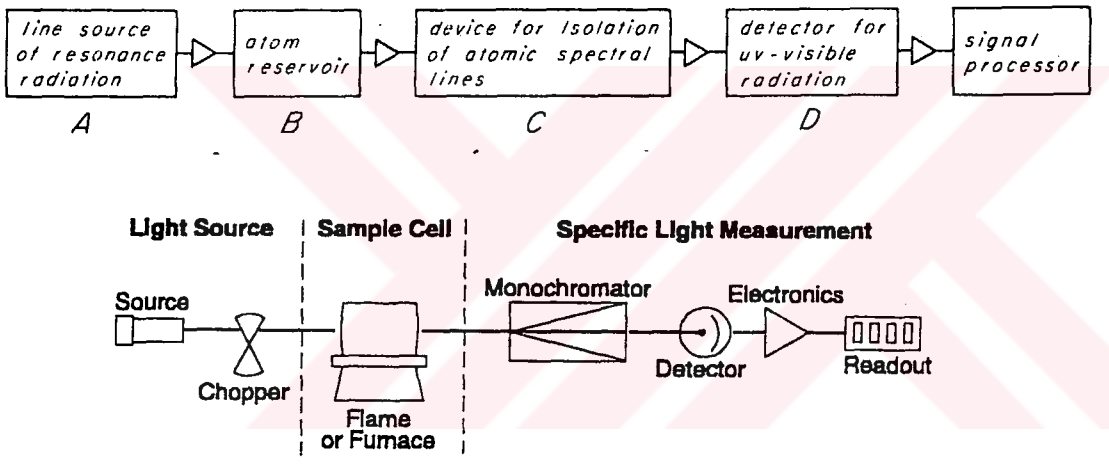

Figure 6.7 Atomic Absorption spectrometry. (a) A block diagram of an atomic absorption spectrometer. (b) A single-beam atomic absorption spectrometer [48].

Absorption spectra are relatively simple, consisting predominantly of resonance transitions. The monochromator need not be capable of especially high resolution, its function being to isolate the resonance line of interest and to diminish the light flux from non-absorbing lines in the source and emission from the flame does not produce noise. The very small fluorescence signal from analyte atoms is emitted isotropically and will be insignificant component of the signal falling on the photo-multiplier [48].

Instrument offerings to date are for single- or two-element analysis. Development of simultaneous multi-element atomic absorption equipment has been hindered by problems with optical alignment of components, need for different operating conditions for many elements, and the relatively short linear range of calibration curves [48].

6.2.2 Calibration Procedure

There are two commonly employed approaches to calibration in atomic absorption spectroscopy. These are the methods of direct comparison and standard addition. These are the methods of direct comparison and standard additions [48].

Direct comparison

This is the simplest approach, and when applicable can be used with many instruments to give a direct readout of the concentration of an element in an unknown sample [48].

A number of standards (usually three to five) in increasing concentrations, as well as a blank, are prepared to cover the concentration range. These solutions are run in absorbance to check for linearity of the calibration curve. If curvature is present, the fact is noted and, with most modern instruments, correction can be made to allow direct readout [48].

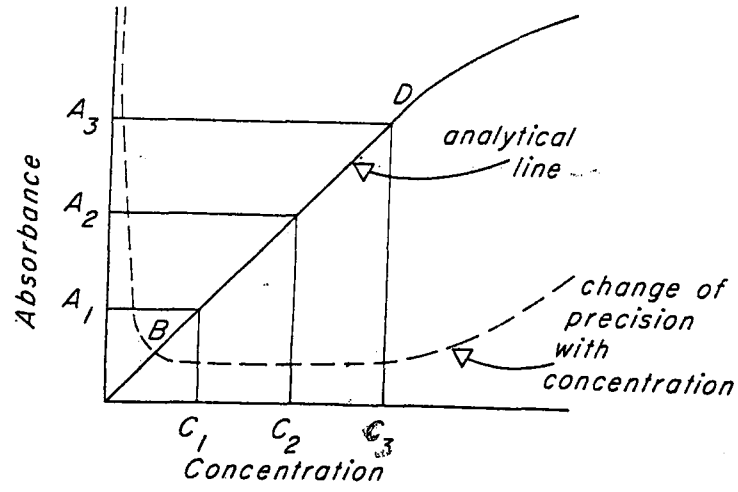

Figure 6.8 Calibration graph for method of direct comparison [48].

When absorbances have been obtained, these values (A_1, A_2, A_3 in figure 6.8) can be plotted against concentration (C_1, C_2, C_3) for a standard and best fit line drawn. The portion of the analytical line BD gives the best results, as shown by the dashed line. In many cases, the line thus produced will be straight. When curvature occurs, usually at higher concentrations, a French curve or other manual curve-fitting devices are used to draw the line. Much poorer results are obtained in the curved region of the calibration graph, and it is advisable, where possible, not to use the curved portion of the graph. Likewise, at low values of the absorbance, where the change of precision with concentration is high decision and hence poorer results are obtained. If the calibration graphs pass through zero absorbance and concentration, as in fig., then all is well. However, if the line intercepts on the absorbance axis after subtraction of the blank, an error due to non-specific absorption may be present [48].

Most new equipment has the capability for direct readout in concentration. It is very important to check calibration linearity. Any non-linearity is readily detectable and methods for curve correction can be devised. At least three standards should be used. The actual technique employed for direct readout and curve corrections depend on the particular equipment. Instruments with microprocessors can curve-correct readily [48].

It is not enough to read the standards only once during a run. For best accuracy, one of the standards is run at least three times over a set of 25 sample. This will allows a statistical analysis to be done. Means of standard readings are used and linear regression analysis is possible [48].

In both the direct readout and plotting methods, the importance of using blank cannot be over emphasized. In work at low concentration, the level of analyte in reagents is often an important consideration [48].

Output can be fed into a desktop or large-scale computers. This can be done through manual methods or with proper interfacing devices, automatically [48].

When an instrument is provided with a microprocessor, the calibration procedure is simplifies. Experimental parameters are entered by keyboard. Any integration time or scale expansion factors between fixed upper and lower limits can be chosen. Values for the standards are entered and the standards are subsequently nebulized. Linear regressions and fitting-curve programs are carried out automatically. Samples are then nebulized and direct readout of concentration is obtained. Some recent instruments do other statistical calculations on the data as well. Better precision and accuracy are obtained in the curved region compared to the manual methods. However, for better best precision and accuracy, the linear portion should be used [48].

Methods of Additions

When samples contain a low concentration of analyte in large amounts of varyoing matrix constituents, it is often difficult to prepare useful standard solutions. In tyhis case it is possible to add small amounts of conventional standard solutions, in increasing amounbt to aliquots of each sample. Graph such as that shown in Figure 6.9 can the be constructed. The method of stqandard addition is often used in work with electrothwermal atomizer. For this method to work the added analyte must behave

during the atomization process in an identical way to the analyte that was originally present in the sample, particularly using electrothermal techniques. The calibration curve must be linear wtihin the concentration range used. Such condition commonly exists with flame techniques. However, it is well known that working curves for electothermal methods, particularly in heavy matrix solutions, are often characterized by relatively short linear regions. The standard additions approach in this case can lead to serious errors [48].

The usual method for standard addition involves using three aliquots of the sample. The first aliquot is diluted to volume while the second and third are spiked with increasing amount of standard and then diluted to a same volume. It is best to spike samples such that the second volume has a concentration of approximately twice of the unknown and the third three times the unknown. The absorbances are obtained, A_1, A_2, A_3, respectively, and the graph constructed as shown in figure 6.9. The line is extrapolated to intersect the x-axis. While it may be possible with some equipment to obtain a direct readout in concentration, the procedure is time consuming and subject to limitations of the instrument in generating negative numbers. If no-specific absorption is a problem background correction must be employed [48].

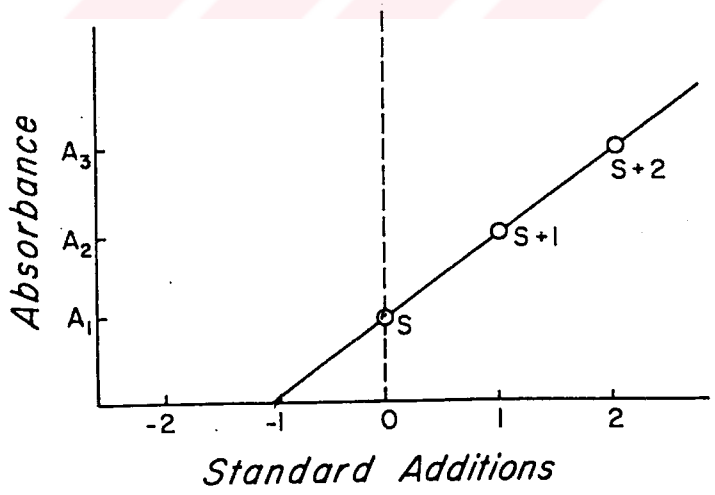

Figure 6.9 Calibration graph for method of standard additions [48].

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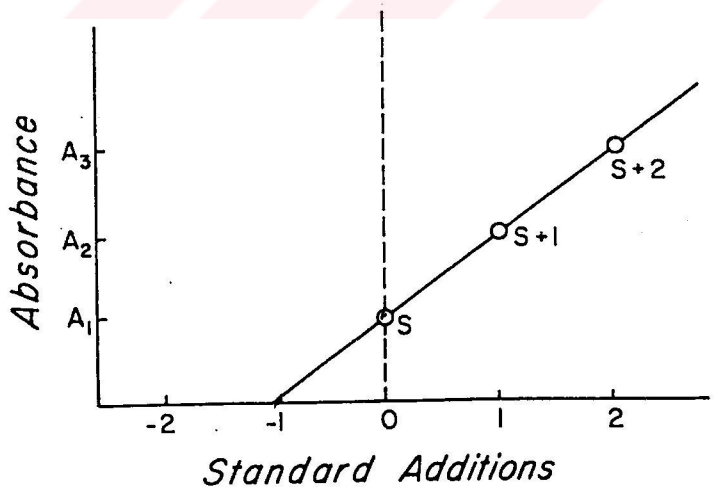

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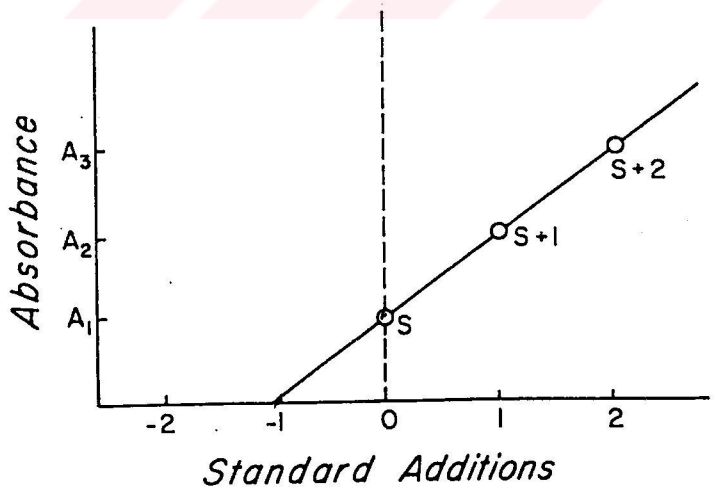


Figure 6.9 Calibration graph for method of standard additions [48].

structural components such as endospores and flagella as well as inclusions such as starch and phosphate granules. Stains that can be used to detect the presence or absence of specialized parts of a cell are known as structural stains. Differential stains, on the other hand, are procedures that enable one to distinguish between cell types. The Gram stain and the acid-fast stain are differential staining techniques that allow bacteria to be divide into major groups [10].

Dyes are often divided into two types: basic dyes and acidic dyes. Basic dyes are those in which the colored portion or chromophore is positively charged while acidic dyes are those in which the colored portion is negatively charged. Basic dyes are used most often because microorganism tend to have an excess of negatively charged groups associated with their wall and cytoplasm under the conditions used for staining. The positively charged dyes are strongly attracted to the many negatively charged groups in cells, resulting in efficient staining. The negatively charged acidic dyes frequently are used to stain the background rather than the microorganisms themselves. Acidic dyes frequently are used to stain the background rather than the microorganisms themselves. Acidic dyes generally do not stain cells because they repelled by the many negative charges associated with the cells. Nevertheless, acidic dyes occasionally are used to stain positively charged structures within cells. It is important to recognize that changing the pH of the environment can change the charge and binding properties of dyes and structure [10].

Whenever a staining technique results in the staining of the objects, the technique is described as a positive stain whether or not a basic (positive charge) or acidic (negative charge) has been used. Similarly, whenever the background is stained to create contrast with the unstained object, the stain technique is called a negative stain [10].

6.3.2.1 Positive and Negative Staining

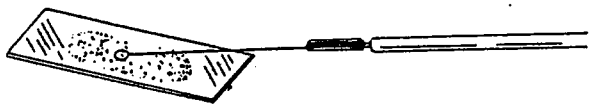
A simple stain is a procedure in which only one stain is used to create a contrast between the specimen and its background. Generally, simple stains involve the use of

basic dyes such as crystal violet, methylene blue, basic carbofuschin (red), safarine (red), or malachite green. Infrequently, simple stain involve the use of acidic dyes such as nigrosine and Congo red [10].

Since simple staining procedure are rapid and easy to carry out, they are often used when information about cell shape, size, and arrangement is described (Figure 6.12). A simple stain helps microscopists determine the dimension and shape of microorganisms [10].

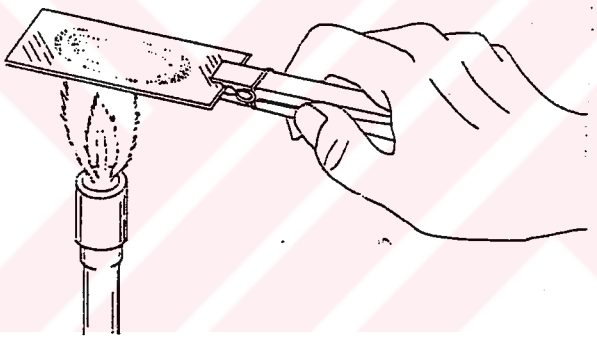
Usually, a simple stain involves the staining of dried preparation of cells on a glass slide (Figure 6.12). The dried preparation of cells is known as a smear. Smears can be prepared from cells in a liquid culture or from growth on an agar plate or slant. When using a liquid suspension, one to several loopfuls is smeared onto a glass slide and then allowed to air dry. The cells in the dry smear are attached or fixed to the slide by briefly heating the slide over a gas burner flame. This procedure is known as heat fixation. When using colonies or growth from a semisolid medium, a loopful of water is placed on the slide and a very small amount of material is mixed with the water to separate and suspend the cells. The suspension is then spread out, air-dried, and heat-fixed. In a good smear, individual organisms are visible microscopically and organisms are not piled on top of each other. In order to insure well-dispersed microorganisms, the drop used to make the smear should be only slightly turbid (cloudy) [10].

1. Place several large loopfuls of a liquid culture on a clean slide. If the culture is on an agar plate or slant, place several loopfuls of water on the slide and then mix in a very small amount of the solid growth (e.g. part of a colony).



2. Spread the liquid culture or mixture over the slide so as to create a thin film.

3. Air dry the smear. Use a warming plate if possible to speed up the drying, but do not use a gas burner.



4. Heat fix the dried smear by placing the bottom of the slide over the gas burner flame for three seconds. An alternate method is to pass the slide through the flame three times.

Figure 6.12 Procedure for positive staining [10].

6.3.2.2 Differential (Gram) Staining

Differential stains are very useful in microbiology because they can be used to distinguish between groups of bacteria. An important differential stain is the Gram stain. The gram stain is one of the most important steps in the characterization and identification of bacteria [10].

The Gram stain separates bacteria into two groups: the Gram-positive bacteria that retain the color of the first stain used (crystal violet), and the Gram-negative bacteria that assume the color of a second stain (safranin). Bacteria stain differentially because of the differences in the structure and chemical composition of their cell wall. The Gram-positive bacteria have a thick cell wall that consists primarily of peptidoglycan (figure 6.13). Many Gram-positive bacteria, however, have in their cell wall a polymer called teichoic acid in their cell walls which may account or as much as 50% of the wall's weight. The peptidoglycan layer of the cell wall consists of polysaccharides, made up of alternating N-acetylglucosamine and N-acetylmuramic acid units, cross-linked by short peptides, while teichoic acid are long polymers of alternating phosphate and carbohydrates (e.g., ribitol or glycerol). The cell wall of Gram-positive bacteria is generally between 20 and 80 nm [10].

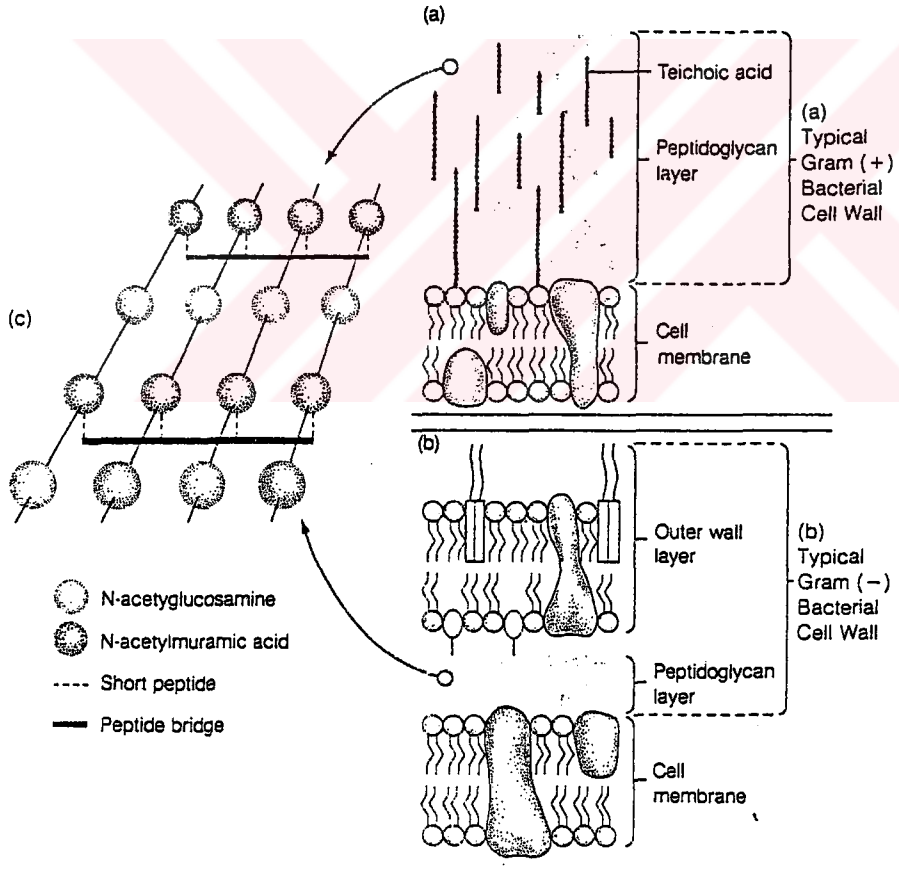


Figure 6.13 Cell wall of (a) Gram-positive and (b) Gram-negative bacteria. (c) Peptidoglycan structure [10].

The walls of Gram-negative have much less peptidoglycan than what those of Gram-positive bacteria have. The peptidoglycan layer, usually about 2 nm thick, is surrounded by a complex lipid bilayer called the outer membrane. Some microbiologists regard this outer membrane as part of cell wall while other scientists consider the outer membrane to be a separated and distinct envelope. No teichoic acids are associated with the cell wall of Gram-negative bacteria. The Gram stain procedure is illustrated in Figure 6.14 [10].

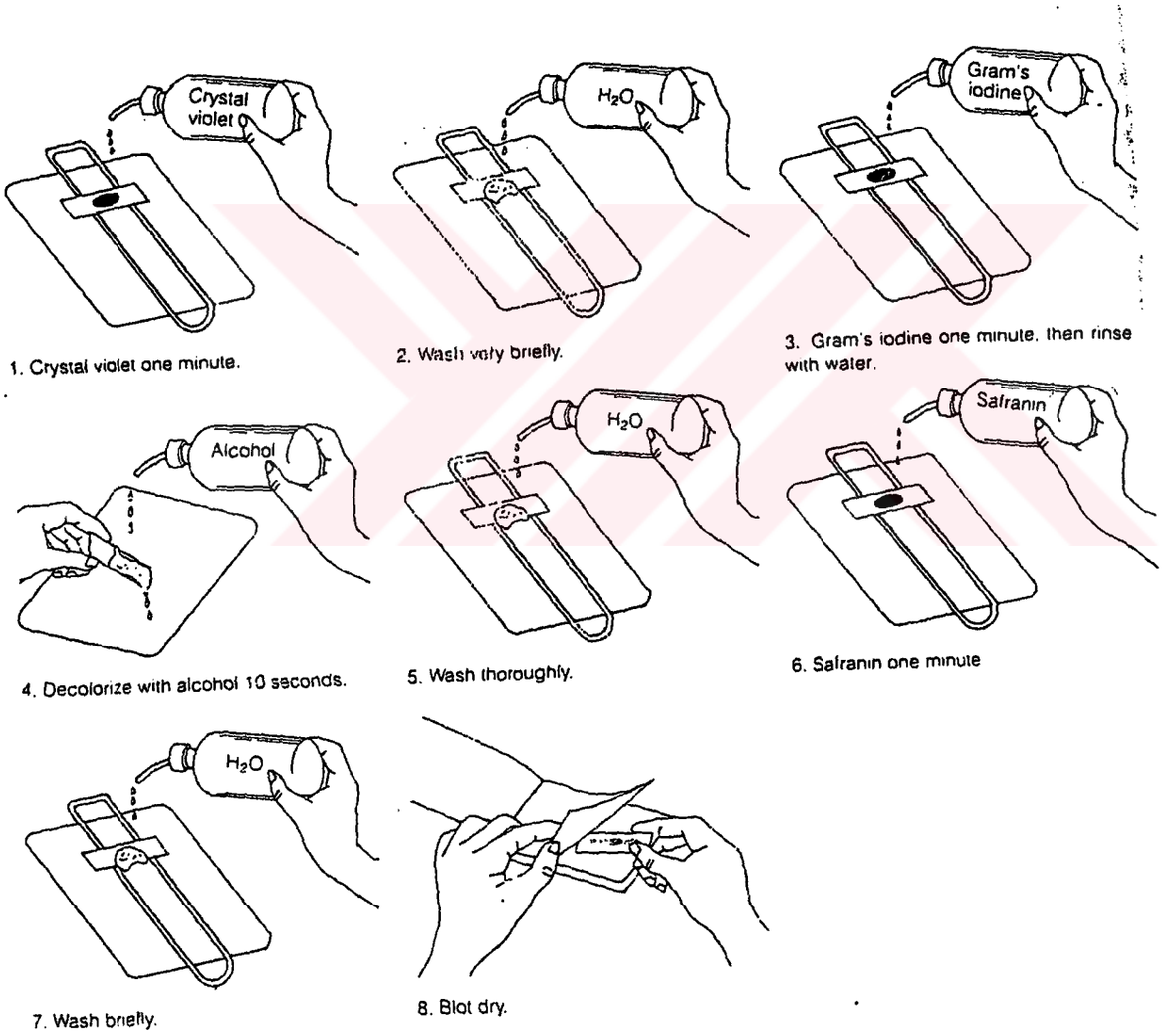


Figure 6.14 Gram Stain Procedure [10].

In order to obtain a reliable Gram stain, it necessary to use a young culture of organisms, no older than 24 to 48 hours. If older cultures are used, there is the possibility of ambiguous results. As culture age, progressively more cells sustain damage to their cell walls. This damage apparently allows dyes to be more easily leached out of the cell by decolorizing agents. Thus, gram-positive cells with damaged cell walls tend to lose their ability to retain crystal violet-Gram's iodine complex and consequently stain as if they were Gram-negative. Damaged cells in a pure culture of Gram-positive bacteria are sometimes the reason a mixture of blue-violet is seen in Gram stained smear of pure microorganisms [10].

6.4 Culturing Bacteria

6.4.1 Introduction

The cultivation of microorganisms in the laboratory requires that the needed nutrients and suitable environmental conditions be provided. In the laboratory, the nutrients are supplied to microorganisms in culture media. The culture medium may be in a liquid or in a solid gel form. Solid media are usually made by the addition of a polysaccharide called agar that is derived from some red algae [9, 10].

When the culture medium has been prepared, it is not ready for use, since microorganisms commonly associated with the nutrients, water, glassware, media maker, and air are likely to have contaminated it. It is necessary to sterilize the medium before it is used. Laboratory sterilization procedures generally are carried out using autoclaves (figure 6.13). Autoclaves sterilizes are carried out for 15 to 20° C at a pressure of 15 pounds per $inch^2$ above normal atmospheric pressure, which allows the steam temperature to reach 121° C. Heat-sensitive solutions are commonly sterilized using special filters that have pores with diameters of 0.45 micrometer. These filters prevent microorganisms from passing into the collecting chamber [10].

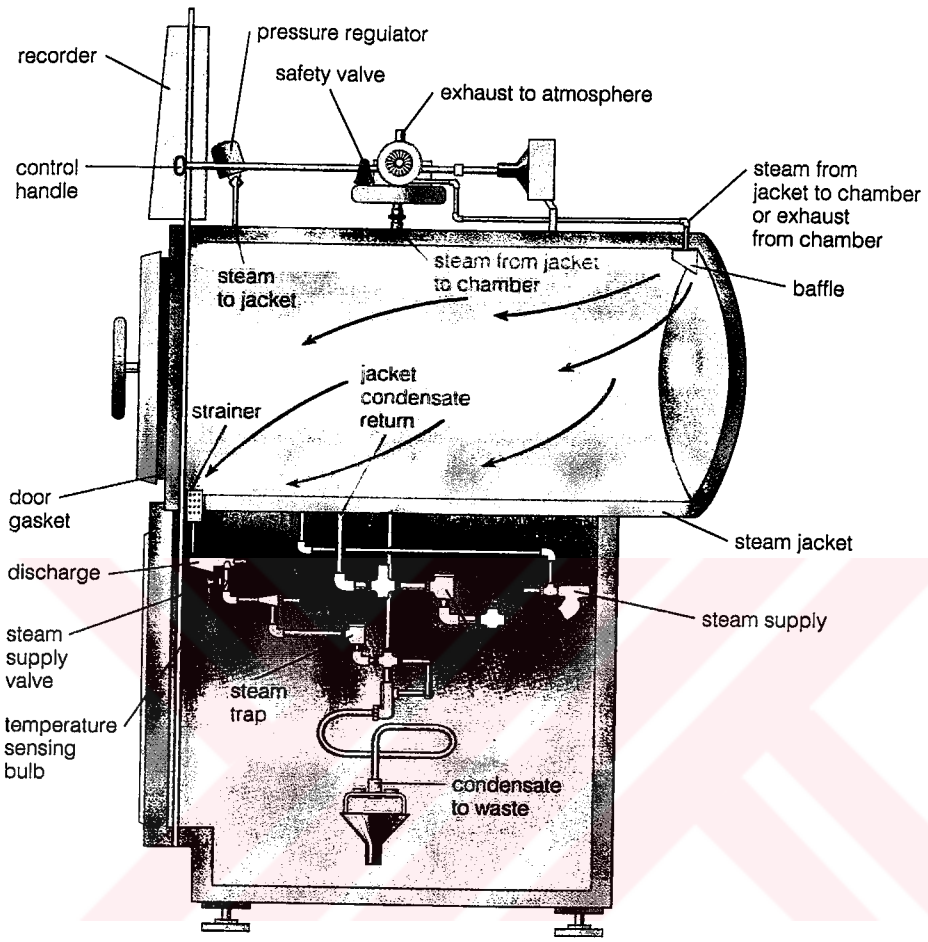


Figure 6.15 Autoclave [9].

Two categories of culture media based upon their formulation are routinely used in the laboratory. These categories are chemically defined and chemically complex (undefined) media. A chemically defined medium is formulated so that the concentration and chemical nature of each added compound is known. The ingredients used to prepare chemically defined media are highly purified (reagent grade) inorganic salts and simple organic compounds. Chemically defined media are often necessary in the study of the

physiology and genetics of microorganisms. However, if a defined medium calls for many ingredients, it can be tedious to prepare. Moreover, reagent grade chemicals are often expensive. Chemically complex culture media are prepared using natural products of unknown chemical composition. Common sources of complex nutrients are beef extracts, partially digested proteins called peptones, and yeast extracts and boiled vegetable and animal materials (infusions). Some culture media are further supplemented with blood, serum, vitamins, amino acids, and nucleosides in order to support the growth of special groups of microorganism [10].

Microorganism growing on agar media from masses of cells called colonies. Colony morphology may be used as an aid to the sole identifying criterion; it is used to recognize many common types of microorganism [10].

6.4.2 Obtaining Pure culture

6.4.2.1 The streak-plate method

Cultures consisting of a single species (or stain) are considered pure cultures. One technique for isolating pure cultures from mixtures involves the streak plate technique (fig. 6.16). The streaking technique is performed by spread small amounts of culture with an inoculating loop over an agar surface. As the microorganisms are spread over the surface, the concentration of microorganisms on the inoculating loop decreases. Eventually, a single microbial cell will be deposited on an area of the agar medium, multiply, and from a colony. This colony, derived from a single cell, can then be picked with a bacteriological loop (or a needle) and a subculture onto a fresh culture medium. In this way, a pure culture of the isolated colony can be obtained and maintained [9].

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Microorganism growing on agar media from masses of cells called colonies. Colony morphology may be used as an aid to the sole identifying criterion; it is used to recognize many common types of microorganism [10].

6.4.2 Obtaining Pure culture

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6.6 Laboratory Work Involving Culturing and Counting Bacteria

6.6.1 Materials and Methods

The bacterial species used in our experiments is *Staphylococcus epidermidis.* A bacterial culture of the species was provided by the Center for Culturing and Storing Microorganisms-Istanbul Faculty of Medicine, Istanbul University.

In all the steps of these experiments, the aseptic techniques like using disinfectants (70% ethyl alcohol) and detergents to clean the stuff (tubes, flasks, etc.) and the area of work. Also, UV light was turned on overnight to sterilize the experimental chamber. Further, a Bunsen flame was used near the equipment of bacterial manipulation and Petri plate preparation to minimize microbial contamination from the surrounding.

In order to obtain a pure bacterial culture, the streak plate method was applied. A loopful form the provided culture was inoculated into a sterile Trypticase Soy Broth (TSB) and incubated for 24 hours.

Then, a 0.1-ml aliquot from the suspension was pipetted on an agar plate (Petri dish) and spread, then incubated for 24 hours. From the surface of the plate, a colony (lenticular in form) was picked and again inoculated into a TSB tube and incubated for 24 hours. Afterwards the spread plate method was applied. The number of dilutions necessary to obtain a countable number of colonies on the Petri plate was estimated to be three hundred-fold dilutions, in each dilution a 0.1-ml of the initial suspension is diluted by TSB to obtain a 10-ml new suspension. Then, a 0.1-ml aliquot from the last suspension was pipetted onto a Petri dish and incubated for 24 hours. The above procedure was repeated three times.

6.6.2 Result

The average of the counted numbers of bacterial colonies in each plate was 155 colonies. The number of CFU/ml in the original suspension is $155\times100\times10^6 = 1.55\times10^{10}$ CFU/ml.

6.7 The Effects of Time and Concentration on the Antibacterial Efficacy of Colloidal Silver

6.7.1 Materials and Method

The bacterium used was *Staphylococcus epidermidis* ATCC 2311 in a 24-hour incubated suspension in Trypticase Soy Broth (TSB). Two solutions of colloidal silver (CS) of approximately 30 and 20 ppm CS (measured using a spectrophotometer of the TSE). Two 10-ml tubes the above solutions and seven tubes, each a range of dilutions were used to test for antimicrobial activity of CS. The diluted solutions were of 10, 7.5, 5, 2.5, 1, 0.5 and 0.1 ppm CS. The diluent was TSB. Each tube containing intact or diluted silver solution and additional control tube containing only TSB was inoculated by 0.1 ml form a two 100-fold diluted suspension of bacteria. An aliquot of 0.1 ml from each tube was pipetted onto a petri plate containing mannitol salt agar. This kind of agar does not allow the growth of any bacteria except Staphylococci, and hence contamination was reduced to the maximum. With the exception of the blank (control) tube, this procedure was repeated for each tube after 5, 10, 20, 40, 60, 80, and 120 minutes. The whole precedent procedure was repeated 10 times for statistical purposes.

6.7.2 Results

The results of the above experiment (the number of colonies in each tube) are presented in the following tables and graphs.

Table 6.2 Number of colonies resulting from Tube 1 (30 ppm).

Trials / Time (mn+)	1	2	3	4	5	6	7	8	9	10	Avg*	Std Dev**
0	111	106	98	104	102	103	100	108	113	94	104	5.8
5	54	42	47	44	49	38	42	45	41	51	45	4.9
10	7	4	11	8	7	10	6	90	5	8	8	2.2
20	0	0	1	0	0	5	3	0	0	0	1	1.8
40	0	0	2	0	4	0	0	1	0	0	1	1.3
60	0	0	1	1	0	0	3	0	1	0	0	1.0
80	0	0	0	0	1	0	0	0	2	0	0	0.7
120	0	0	2	0	0	0	0	0	0	2	0	0.8

*Avg= Average, **Std Dev= Standard Deviation, +mn= minute

The above table (of Tube 1) is represented graphically in Figure 6.22.

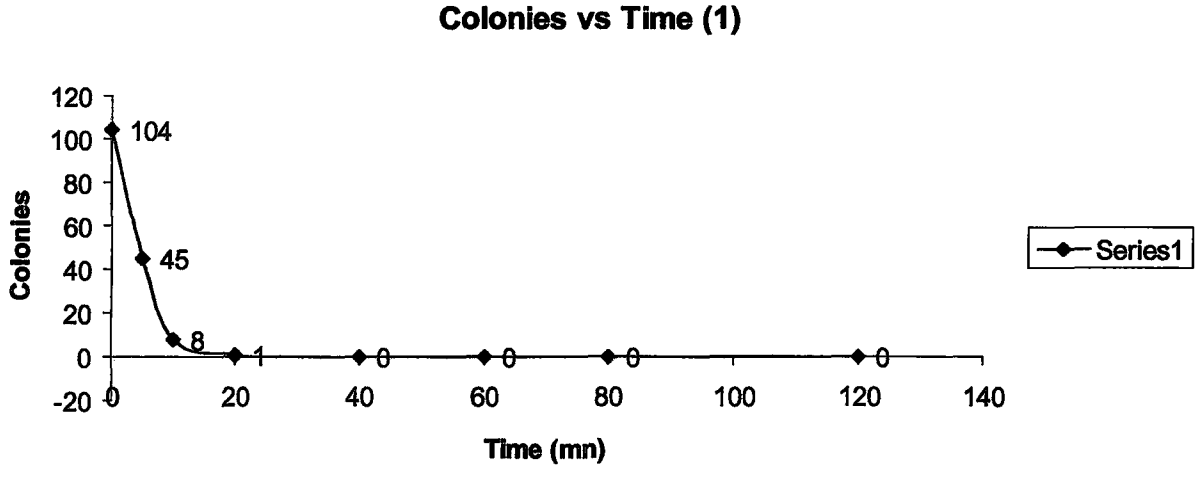

Figure 6.22 Average number of colonies versus time (Tube 1).

Table 6.3 Numbers of colonies resulting from Tube 2 (20 ppm).

Trials / Time (mn)	1	2	3	4	5	6	7	8	9	100	Avg	Std Dev
0	103	109	112	105	97	107	101	104	96	106	104	4.9
5	63	57	60	64	61	59	69	62	65	67	63	3.7
10	24	28	21	19	25	31	23	27	25	30	25	3.8
20	8	5	9	7	3	7	5	6	2	4	6	2.2
40	0	0	0	3	0	4	2	0	0	0	1	1.5
60	0	0	0	0	1	0	0	0	2	0	0	0.7
80	0	5	0	0	0	3	0	0	0	0	1	1.8
120	0	0	0	0	0.	0	3	0	0	0	0	1.0

The above table (Tube 2) is represented graphically in figure 6.23.

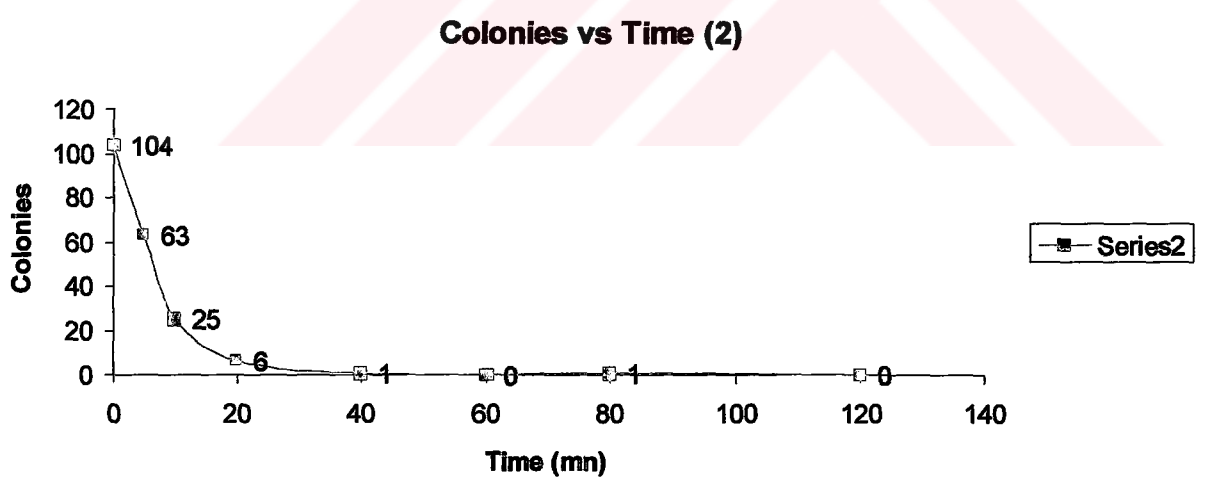

Figure 6.23 Average number of colonies versus time (Tube 2).

Table 6.4 Number of colonies resulting from Tube 3 (10 ppm).

Trials / Time (mn)	1	2	3	4	5	6	7	8	9	10	Avg	Std Dev
0	113	107	107	111	118	115	119	114	118	105	114	5.0
5	52	47	43	46	47	54	41	51	53	39	46	5.2
10	16	21	14	20	25	23	19	26	17	21	20	3.8
20	12	7	15	10	13	5	6	11	7	9	10	3.3
40	0	0	3	0	7	2	0	0	1	2	2	2.2
60	0	0	0	0	0	1	0	0	0	0	0	0.3
80	0	0	0	4	0	2	0	0	0	0	1	1.4
120	0	0	0	0	0	2	0	0	0	0	0	0.6

Table 6.4 is represented graphically in figure 6.24.

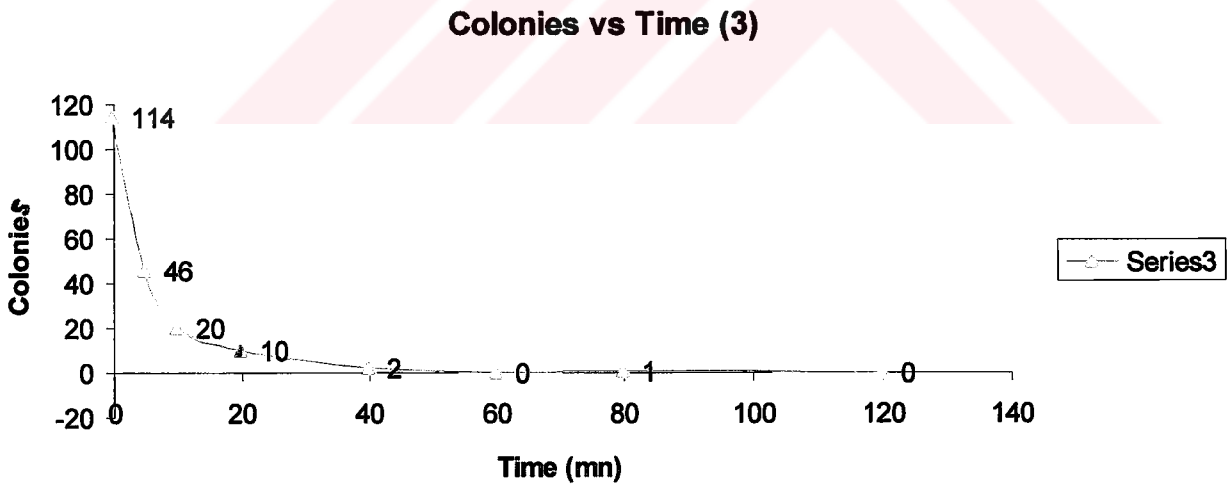

Figure 6.24 Average number of colonies versus time (Tube 3).

Table 6.5 Number of colonies resulting from Tube 4 (7.5 ppm).

Trials / Time (mn)	1	2	3	4	5	6	7	8	9	10	Avg	Std Dev
0	122	127	118	131	125	129	117	132	126	121	125	5.2
5	67	54	58	63	63	55	62	69	55	57	60	5.3
10	26	18	23	22	23	31	24	29	30	21	25	3.7
20	7	5	13	10	15	4	8	10	6	6	8	3.6
40	0	0	0	0	2	0	0	1	0	4	1	1.3
60	0	0	1	0	0	0	0	0	3	0	0	1.0
80	0	0	0	0	0	1	0	0	0	0	0	0.3
120	0	0	0	0	0	0	0	0	0	0	0	0.0

Table 6.5 is represented graphically in Figure 6.25.

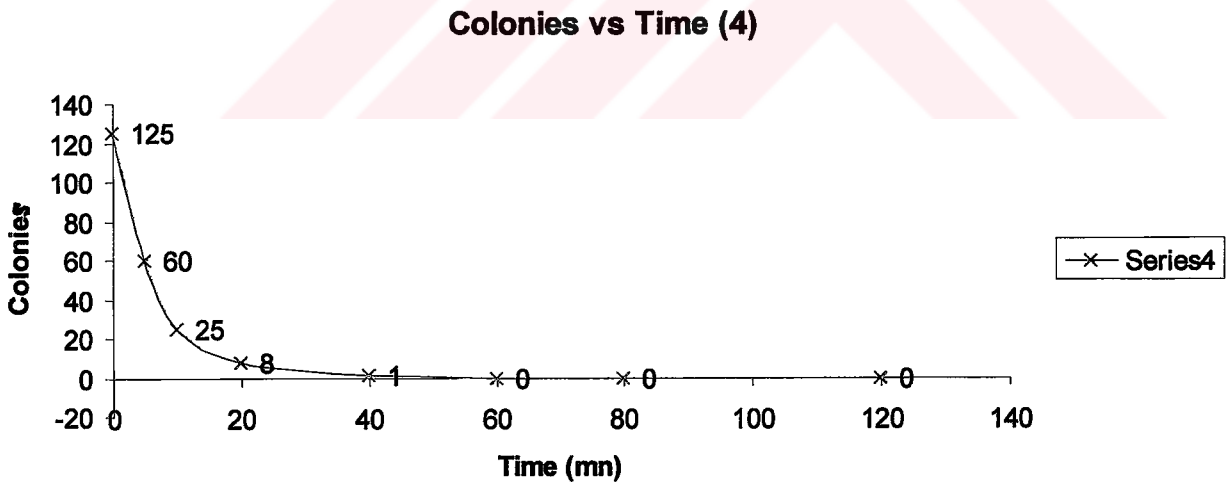

Figure 6.25 Average number of colonies versus time (Tube 4).

Table 6.6 Number of colonies from Tube 5 (5 ppm).

Trials / Time (mn)	1	2	3	4	5	6	7	8	9	10	Avg	Std Dev
0	134	129	127	123	130	126	131	135	124	121	128	4.6
5	72	73	65	77	63	64	69	76	61	66	69	5.6
10	33	34	26	29	38	37	32	25	36	35	32	4.5
20	18	12	16	16	19	23	20	14	17	21	18	3.3
40	7	13	6	10	4	12	11	9	7	5	8	3.1
60	3	3	0	5	2	6	4	0	0	2	2	1.8
80	0	0	0	4	0	0	2	0	0	0	1	1.4
120	0	0	0	0	0	0	0	0	0	0	0	0.0

Table 6.6 is represented graphically in Figure 6.26.

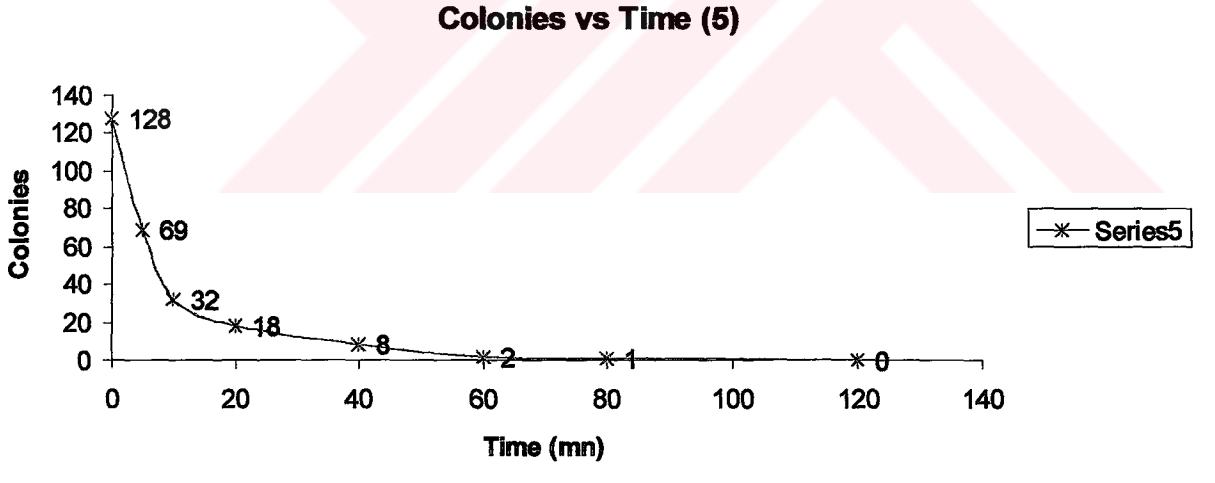

Figure 6.26 Average number of colonies versus time (Tube 5).

Table 6.7 Number of colonies resulting from Tube 6 (2.5 ppm).

Trials / Time (mn)	1	2	3	4	5	6	7	8	9	10	Avg	Std Dev
0	134	129	133	124	127	125	138	136	134	128	131	4.8
5	88	91	82	81	86	87	89	79	85	84	85	3.8
10	52	53	59	56	48	47	55	54	61	60	54	4.7
20	35	36	32	28	26	31	34	21	32	37	31	5.4
40	10	16	13	9	11	8	17	12	12	14	12	2.9
60	4	7	3	6	8	5	10	7	6	11	7	2.5
80	0	2	2	0	0	5	1	3	0	0	1	1.7
120	0	0	0	0	0	0	0	0	0	0	0	0.0

Table 6.7 is graphically represented in Figure 6.27.

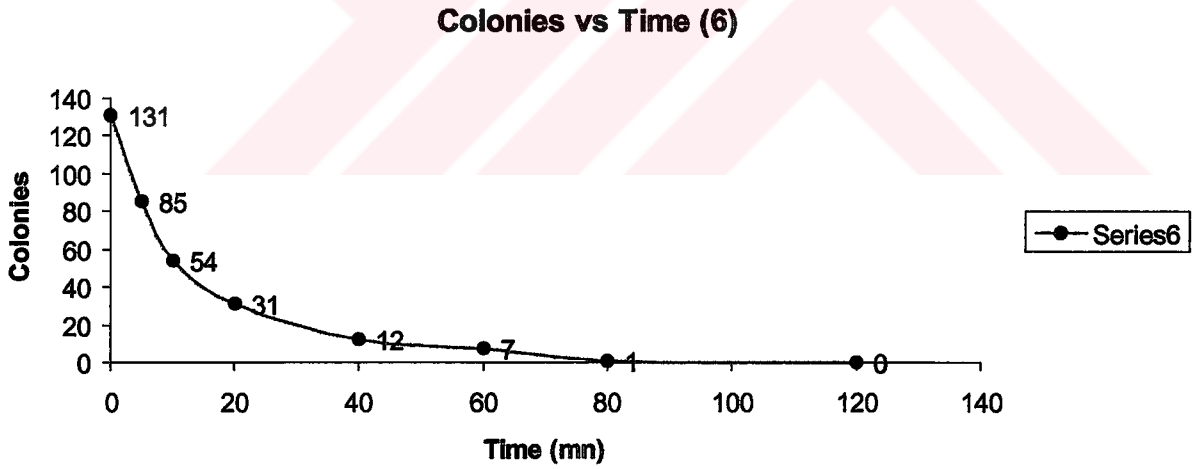

Figure 6.2 Average number of colonies versus time (Tube 6).

Table 6.8 Numbers of colonies resulting from Tube 7 (1 ppm).

Trials / Time (mn)	1	2	3	4	5	6	7	8	9	10	Avg	Std Dev
0	141	145	146	139	142	136	136	142	148	143	142	4.0
5	135	132	139	138	143	133	137	141	144	136	138	4.0
10	131	134	128	133	136	131	126	132	137	127	132	3.7
20	127	124	128	131	133	122	129	122	130	125	127	3.8
40	124	118	126	123	122	118	127	125	120	124	123	3.2
60	117	112	119	115	123	122	114	115	118	120	118	3.6
80	112	110	117	118	114	120	113	120	109	115	115	3.9
120	110	112	116	115	108	117	106	115	116	113	113	3.7

Table 6.8 is represented graphically in Figure 6.28.

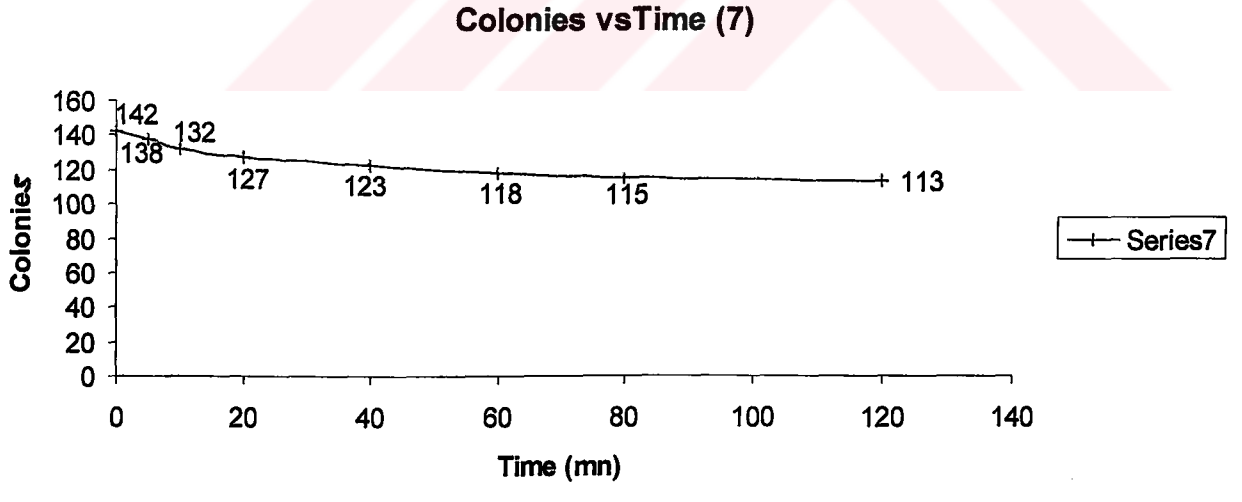

Figure 6.28 Average number of colonies versus time (Tube 7).

Tube 8 (0.5 ppm): The number of colonies is 120 and 140 and this result show little or no antimicrobial effect.

Table 6.9 Number of colonies resulting from Tube 9: blank.

Trials	1	2	3	4	5	6	7	8	9	10	Avg	Std Dev
Result	147	147	152	154	149	144	145	146	155	153	149.2	4.0

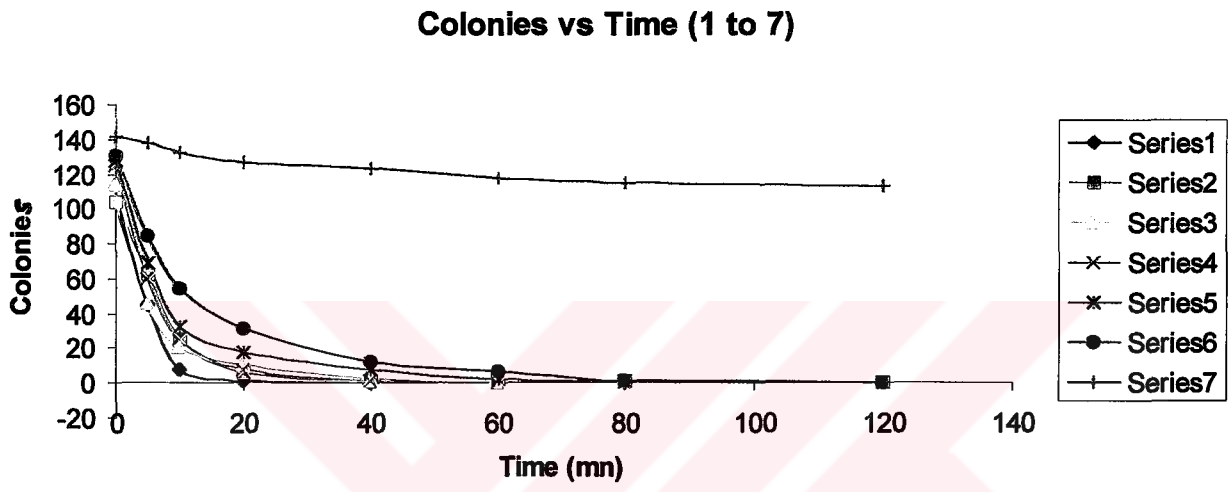

Figure 6.29 Average number of colonies versus time (Tube 1 to 7). In this graph, the curves corresponding to each table (drawn before separated) are placed together for comparison. The descending trends of curves are decreasing as the CS concentrations of their corresponding tubes are increasing.

6.7.3 Discussion

All tubes that contain CS of concentration more than 1 ppm show antimicrobial effects, as they caused noticeable decrease in the number of colonies. This antimicrobial effect reached the degree of total elimination after 120 minutes of exposition to CS as the number of bacterial colonies obtained was less than 10. Following the gradual decrease in the concentration of CS used in each tube, the decreasing trends of each of the corresponding graphs show differences, which proves the decrease of effect of CS as its concentration decrease. In addition, bacterial cells seemed to be affected by the metabolite released from dead cells in suspensions as time passed, which also account for the dramatically decreasing trends of the graphs. Also, the decrease in the number of

colonies on the table inoculated with suspension at the starting point (0 mn) as the concentration of CS increased showed that CS particles continued to slightly act after spreading on the plate surfaces (see Figure 6.29 and Tables 6.2 to 6.8). The above experiment indicated also the range of concentrations that should be used for the coming experiment aiming to determine the minimum inhibitory (bacteriostatic) and killing (bactericidal) concentration of colloidal silver. This range is between 5 and 2 ppm CS.

6.8 Minimum Bactericidal and Bacteriostatic Concentrations of Colloidal Silver

6.8.1 Materials and Method

In the first step, a range of dilutions was done using CS solution (10 ppm) and TSB as diluent. Seven tubes, each containing 10 ml of 5, 4.5, 4, 3.5, 3, 2.5, and 2 ppm CS. A suspension of the bacteria *S. epidermidis* was prepared as outlined before and incubated for 24 hours. Then from this suspension, two 100-fold dilutions was performed. Form the diluted suspension, an 0.1-ml aliquot was pipetted into each of the seven prepared tubes. Then all the tubes were incubated for 48 hours. In the second step, after incubation, A 0.1-ml aliquot from the tubes that shows no turbidity as a sign of bacterial growth was pipetted into a sterile 10-ml TSB and incubated for 48 hours. The above procedure was repeated ten times for accuracy.

6.8.2. Results

The concentrations that resulted in the inhibition of bacterial growth are in the interval between the minimum bactericidal (killing) concentration (MBC or MKC) and the minimum bacteriostatic (inhibitory) concentration (MIC). The lack of bacterial growth, as an indication of an effective concentration (between MIC and MKC) of the CS, is noted by obtaining a clear TSB solution. Whereas the bacterial growth is noted by the turbidity of the suspension, indicating an ineffective concentration, that is less than the MIC. These observations are tabulated in Table 6.10.

Table 6.10

Table 6.10 Bacterial growth in tubes containing 5 to 2.5 ppm CS, respectively (Step 1). In each column, the presence or absence of bacterial growth is indicated for each tube corresponding to each used concentration of CS (first step).

Trials / C/ppm	1	2	3	4	5	6	7	8	9	10
5	–	–	–	–	–	–	–	–	–	–
4.5	–	–	–	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–	–
3.5	–	–	–	–	–	–	–	–	–	–
3	–	–	–	–	–	–	–	–	–	–
2.5	–	–	–	–	–	–	–	–	–	–
2	+	+	+	+	+	+	+	+	+	+

– no growth, + growth

From Table 6.10, it is clear that a concentration less than 2.5 ppm CS has no antibacterial efficacy against *S. epidermidis*. Hence, the MIC is estimated to be approximately 2.5 ppm. On the contrary, all suspensions containing 2.5 ppm and more of CS shows no bacterial growth.

Table 6.11 Bacterial growth in the tubes of Step 2. In each column, the presence or absence of bacterial growth indicated for the tubes of sterile TSB that were inoculated from the previous tubes that showed no bacterial growth (second step). See Table 6.10.

Trials / C/ppm	1	2	3	4	5	6	7	8	9	10
5	–	–	–	–	–	–	–	–	–	–
4.5	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+
3.5	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+
2.5	+	+	+	+	+	+	+	+	+	+

From Table 6.11, CS concentrations that are below 5 ppm shows bacterial growth when the treated bacteria were inoculated in a TSB medium free of antimicrobials. Thus, these concentrations were effective only in inhibiting bacterial growth not for total elimination of bacteria.

From the above results, the MBC and MIC of CS estimated to be approximately 5 and 2.5 ppm, respectively.

6.8.3 Discussion

According to the literature, both the MIC and MBC of CS for *S. aureus* are found to be 5 ppm [49]. However, the time of incubation of the suspensions treated with CS was 24 hours, which seems not to be enough for showing the antibacterial effect of the CS of concentrations less than 5 ppm. As mentioned before (section 6.8.1), the incubation time of the first step of the experiment is 48 hours, as recommended in Ref. 10. This long period of incubation is necessary to show the inhibitory effect of CS on bacteria. On the contrary of the previous experiment that involved spreading bacteria onto plates, in which the time of exposure in suspensions did not exceed 120 mn, this experiment that showed the effect of antibacterial CS in suspension needed that long

period of incubation. The reason for this is that the presence of bacterial growth in suspensions, indicated by the presence of turbidity, cannot be seen after a short time of exposure. Whereas transferring bacteria on agar plates after these relatively short time of exposure is enough because the effect of the used antimicrobial is greatly reduced by spreading aliquots of bacteria over agar surface. This allowed the approximate determination of the time needed for the antimicrobial, depending on its concentration to exert its effect on bacteria.

6.9 Minimum Bastericidal and Bacteriostatic Concentration of Silver Nitrate

6.9.1 Materials and Methods

A 0.1 % w/w (i.e. 1000 ppm) solution of $AgNO_3$ was used as a stock solution, from which a range of dilutions was made for testing antimicrobial activity. Again, *S. epidermidis* was used as the testing organism. The concentrations of the diluted $AgNO_3$ (with TSB as diluent) are 500, 450, 400, 350, 300, 250, 200, 150, and 100, respectively. These solutions, together with the original stock solution were used to determine the MIC and MBC in the same way as outlined before for CS.

6.9.2. Results

The results of this experiment are tabulated as follows.

Table 6.12 Bacterial growth in the tubes of the first step. In each column, the presence or absence of bacterial growth for each tube corresponding to each concentration of $AgNO_3$ is indicated.

Trials / C/ppm	1	2	3	4	5	6	7	8	9	10
1000	–	–	–	–	–	–	–	–	–	–
500	–	–	–	–	–	–	–	–	–	–
450	–	–	–	–	–	–	–	–	–	–
400	–	–	–	–	–	–	–	–	–	–
350	–	–	–	–	–	–	–	–	–	–
300	–	–	–	–	–	–	–	–	–	–
250	–	–	–	–	–	–	–	–	–	–
200	–	–	–	–	–	–	–	–	–	–
150	–	–	–	–	–	–	–	–	–	–
100	+	+	+	+	+	+	+	+	+	+

From this table (6.12), the MIC of the $AgNO_3$ is estimated to be approximately 150 ppm because it is the lowest concentration that allowed bacterial growth.

Table 6.13 Bacterial growth in the tubes of the second step. The presence or absence of bacterial growth in the sterile TSB tubes inoculated with aliquots from the tubes with no bacterial growth from the first step is indicated.

Trials / C/ppm	1	2	3	4	5	6	7	8	9	10
1000	–	–	–	–	–	–	–	–	–	–
500	–	–	–	–	–	–	–	–	–	–
450	–	–	–	–	–	–	–	–	–	–
400	+	+	+	+	+	+	+	+	+	+
350	+	+	+	+	+	+	+	+	+	+
300	+	+	+	+	+	+	+	+	+	+
250	+	+	+	+	+	+	+	+	+	+
200	+	+	+	+	+	+	+	+	+	+
150	+	+	+	+	+	+	+	+	+	+

Table 6.13 shows that the concentrations of $AgNO_3$ below 450 ppm allowed bacterial growth when an aliquot of the suspensions were transferred to sterile TSB mediums without antimicrobials. Thus, from the above tables, the MBC and MIC of $AgNO_3$ are 450 and 150 ppm, respectively, which are much larger than those of CS.

6.10 The Efficacy of Antimicrobial Ceramics

6.10.1 Materials and Method

As explained before ceramics are rendered antimicrobial by treatment with silver ions. Ceramics of this sort (Vitra from Eczacibasi) was used in this experiment. Discs

of diameter of 3 cm were placed in Petri plates. A mannitol salt agar was poured into each of the plates in which the antimicrobial ceramic discs were placed. Then a three 100-fold diluted bacterial suspension (*S. epidemidis*) were used as the source of 0.1-ml aliquots that were spread over the plates, one of which was a control plate without antimicrobial ceramics. The plates were incubated for 48 hours. The whole procedure was repeated many times for accuracy.

6.10.2 Results

The results (number of colonies in each plate) of the precedent experiment are in the table below.

Table 6.14 The number of colonies resulting on the plates with the antibacterial ceramic disks, after 48-hour incubation.

Trials / Plates	1	2	3	4	5	6	7	8	9	10
1	4	0	0	2	0	0	1	2	0	7
2	0	0	0	0	5	0	0	0	0	3
3	1	0	0	0	1	0	4	0	0	0
4	0	2	5	6	0	0	6	0	4	0
5	0	7	5	0	3	0	0	0	0	2
6	0	0	0	4	0	0	0	3	5	0
7	3	2	3	4	0	2	0	0	3	0
8	5	0	1	0	0	0	0	0	0	2
Blank	147	142	151	150	144	148	140	145	148	152

6.10.3 Discussion

From the above table, it is concluded that the used antimicrobial ceramic is efficiently bactericidal, since the number of colonies on each of the plates that contains the ceramics did not exceed 10 colonies. Also, this antimicrobial activity is continuous

and durable. A continuous release of silver ions and their diffusion through agar during incubation caused the inhibition of bacterial growth and even the killing of a large portion of the bacterial population. In fact, this result conforms to the test done by TUBITAK that used microscopic direct bacterial count and showed no bacterial growth after incubation [50].

6.11 Antimicrobial Activity of Ceramics Covered with Colloidal Silver, Silver Nitrate, and Silver Sulfadiazine

6.11.1 Materials and Method

Normal ceramics under the form of discs 3-cm diameter where treated with a CS solution (30 ppm), silver nitrate (0.1%), and silver sulfadiazine in the form of a commercial cream used for treatment of burns (Silverdine). As for the precedent experiment with antimicrobial ceramics, the treated ceramic disks were placed in petri dishes and bacterial growth (*S. epidermidis*) at the upper surface of agar was tested. For each silver compound, seven plates were used, with an additional blank (control) plate. The whole procedure was repeated ten times for accuracy.

6.11.2 Results

The numbers of colonies obtained on the surface of the plates containing treated ceramics were not significantly different from the blank plate (in the range between 120 and 150 colony).

6.11.3 Discussion

The above result shows an obvious ineffectiveness of silver liquid agents and creams for continuous and durable antimicrobial treatment of solid surfaces if continuous reapplications or coating techniques, as discussed in previous sections, are not employed. Effective silver ions should have diffused or washed away during manipulation of the

treated ceramic plates, and hence no antimicrobial effect was manifested over the growing bacterial colonies on agar surfaces. In addition, a better method, especially for ceramics is the impregnation of silver ions during the manufacturing of antimicrobial ceramics.

7. CONCLUSION

The experimental work involving the use of silver against bacteria showed clearly the antibacterial efficacy of this element, in different forms and compounds, in disinfecting mediums and solid ceramic surfaces.

First, the application of colloidal silver to prevent bacterial growth and killing bacteria was tested by growing and incubating treated bacteria both in suspension and on agar plates. This antibacterial effect was dependent on concentration and the time of exposure, as was shown in the experiments that involved plating. The values of the MIC and MKC of CS were close to those found in the literature.

Second, from the estimation of the MIC and the MKC of $AgNO_3$, which were much higher than those of CS, the superiority of CS over silver salts as antimicrobial was proved, which conformed to the literature.

Third, the durable effect of antimicrobial ceramics were silver ions are impregnated within the ceramics during manufacturing was reproved experimental using viable plate counts. This durable antimicrobial effect is not shown for silver when used in liquid (solutions or colloidal suspensions) or pharmaceutical creams.

In these in vitro experimental works, bacteria were used in the planktonic form. As mentioned before bacteria in sessile form found in biofilms are more resistant to antibacterial treatment. Therefore it is recommended to experiment different forms of silver against biofilms grown on solid surfaces. In addition, it is recommended to experiment the antimicrobial silver treatment of biomaterials such as ceramics, metal, polymer, or composite form in vivo to prove its effectiveness in producing biocompatible antimicrobial implants

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