

**EFFECTS OF CHLORIDE ION AND
THE TYPES OF OXIDES
ON THE ANTIBACTERIAL ACTIVITIES OF
INORGANIC OXIDE SUPPORTED AG
MATERIALS**

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ABSTRACT

EFFECTS OF CHLORIDE ION AND THE TYPES OF OXIDES ON THE ANTIBACTERIAL ACTIVITIES OF INORGANIC OXIDE SUPPORTED SILVER MATERIALS

This study deals with the silver containing oxide supported materials and their antibacterial efficacies. Major goal of this study is to prepare silver chloride nano-crystals on oxide supports to provide a long—lasting protection due to the slow and controlled release of silver ions from the materials. Two oxide supports, titanium dioxide and zinc oxide – silica matrices were synthesized via a sol-gel route which allows one to easily tailor textural and chemical properties. Two approaches were followed to form AgCl containing materials; to synthesize AgCl containing materials in a single step sol – gel route and to form AgCl in the materials by HCl treatment of metallic Ag containing oxide materials.

The characterization of the samples were performed by using XRD, SEM, and BET techniques. Antibacterial efficacies of the powders were investigated by disk diffusion (zone inhibition) method against Gram negative *E. coli* bacteria. In the antibacterial activity tests, the powders containing metallic Ag and/or AgCl nano-crystals were compared with the repeated usage to study the feasibility of their long-term protection.

In this study, it was found that AgCl nano-crystals containing samples showed a larger zone of inhibition than Ag containing samples in the standard media whose ingredient's inhibitory effect on the antibacterial activity is the lowest. However, in the high broth containing media, metallic Ag nano-crystals containing samples showed higher zone of inhibition than AgCl containing samples which reveals the dependence of antibacterial activity on the type of media. In ZnO – SiO₂ samples, the highest antibacterial activity was found unexpectedly at the lowest ZnO concentration. Moreover, Ag containing ZnO – SiO₂ was found to be more active than Ag containing TiO₂ samples. As the antibacterial tested samples were investigated by XRD, it was seen that Ag crystals were formed into AgCl crystal because of the Cl⁻ ions present in the broth media. AgCl formation indicates that there is an interaction between the content of growth media and the antibacterial materials.

ÖZ

KLORÜR İYONUNUN VE OKSİT ÇEŞİTLERİNİN İNORGANİK OKSİT DESTEKLİ GÜMÜŞ MALZEMELERİNİN ANTİBAKTERİYEL AKTİVİTELERİ ÜZERİNDEKİ ETKİSİ

Bu çalışma gümüş içeren oksit destekli malzemeler ve antibakteriyel etkilerini içermektedir. Çalışmada, gümüş iyonlarının yavaş ve kontrollü salınımindan yararlanarak uzun süreli bir etki sağlamak amacıyla oksit desteklerin üzerinde gümüş klorür nano-kristalleri oluşturulması amaçlanmıştır.

İki çeşit oksit destek malzemesi -titanyum dioksit ve çinko oksit/silika- ve dar bir aralıkta parça boyut dağılımı (değişken yükleme oranları kullanılarak) elde etme olanağı veren sol-gel yöntemi ile hazırlanmıştır. Titanyum oksit matriksindeki gümüş klorür kristalleri tek basamaklı sol-gel yöntemi ile jelleşme sırasında ve/veya olmadan önce oluşmuştur. Gümüş klorür oluşturmak için kullanılan bir diğer yöntem ise Ag nano-metal kristalleri bulunan tozlara HCl işlemi uygulamaktır.

Hazırlanan örneklerin karakterize edilmesi XRD, SEM ve BET ile yapılmıştır. Örneklerin antibakteriyel etkileri disk yayılma yöntemi ile Gram negatif *E.coli* bakterisine karşı uygulanmıştır. Antibakteriyel aktivite testlerinde gümüş ve gümüş klorür içeren örnekler uzun süreli etkileri aynı malzemeyi birden fazla kullanarak karşılaştırılmıştır.

Bu çalışmada gümüş klorür içeren örnekler standart besiyer ortamında (diğer bir deyişle: engelleyici iyon etkisi en az olan) en yüksek antibakteriyel etki alanı göstermiştir. Fakat, yüksek konsantrasyona sahip besiyeri ortamında Ag nano-kristal parçacıkları içeren örnekler daha fazla aktivite göstermiştir. ZnO – SiO₂ örneklerinde ise en fazla antibakteriyel aktivite beklenenin tersine en düşük ZnO konsantrasyonunda görüldü. Bundan başka, destek malzemelerin antibakteriyel aktivitelerinin karşılaştırılmasında, gümüş içeren ZnO – SiO₂ destekli malzemelerin gümüş içeren TiO₂ e göre daha aktif olduğu bulundu. Antibakteriyel test yapılmış malzemeler XRD ile incelendiğinde, gümüş nano-kristallerinin besiyerinden gelen Cl⁻ iyonları nedeniyle AgCl kristallere dönüştüğü gözlenmiştir. AgCl oluşumu kullanılan besiyerin içeriğine bağlı olarak test edilen malzemeler arasında bir etkileşim olduğunu göstermektedir.

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CHAPTER 1

INTRODUCTION

Microorganisms are an intricate part of life on Earth. Some of the microorganisms, such as the nitrifying bacteria of the genus *Nitrosomonas*, help ecologic cycles by decomposing wastes or dead organisms into nitrogen, oxygen and carbon but some others are responsible for diseases among people, animals and plants. For example, cold, influenza, measles are some well-known viral infectious diseases affecting millions of people and similarly, pneumonia, ear infections, diarrhea, urinary tract infections and skin disorders are common bacterial infections. As well as microorganisms, viruses cause serious infections. However, viruses are significantly different than other living organisms in a way that they cannot sustain a life cycle without a host cell. They require a host cell as a medium to survive and reproduce. On the other hand, bacteria are simple organism with one cell. They can survive in any environment, such as plants, insects, animals, pets and even in the human digestive system and upper respiratory tract.

Bacteria are classified into two groups by using a specific staining procedure called as Gram – stain technique. According to Gram stain technique, gram – negative (G^-) bacteria which are initially blue - purple loses its color and become transparent after washed with ethyl alcohol while the color of gram positive (G^+) bacteria remain same. The classification of bacteria is important because of their characteristics like resistance against disinfectants and penicillin. (Alcamo 2004).

Growth and death of bacteria are influenced by environmental factors. Outside the range of a suitable environmental condition, bacteria could survive in a dormant state or lose the ability to reproduce, hence resulting in their death. Temperature, pH, oxygen level, salinity, pressure and radiation are the most important factors affecting the microbial growth. For example, some bacteria (aerobes) require oxygen for growth whereas others (anaerobes) grow in the absence of oxygen. Even some microorganisms, such as blue-green algae, require light for photosynthesis (Brooks et al. 1991). Not only the absolute amounts of these variables in the living environment of the bacteria are important but also their optimum values are crucial for the bacteria to survive and

multiply. For example, *E.Coli* bacteria require 10 – 45 °C temperature range to sustain the cellular activities.

Diseases causing microorganisms affect the human health through several contamination pathways; respiratory droplets, dust, contact with contaminated objects, animals, contaminated water and food etc. (Alcamo 2004). In a microbial disease development cycle, the first step is the requirement of a suitable reservoir of infection in which microbes survive and possibly multiply. In the second step, microbes are transmitted to a host from the infection reservoir. This follows by accessing into the body and then the microbial disease cycle is completed by the portal of exit step in which pathogen leaves the body to spread the disease (Alcamo 2004).

The transmission is an important process in which infectious agent is spread among people or animals or plants through environmental media. The transmission pathways describe how people get the diseases. There are two types of transmission pathways; direct and indirect transmission. In the direct transmission, while an infectious agent is directly transferred from a portal of exit to a portal of entry. On the other hand, in the indirect transmission, the transfer of the infectious agent occurs between a reservoir to an intermediate agent and then to a host. The intermediate agent can be living or nonliving media in the indirect transmission (Alcamo 2004, Krasner 2002). In Table 1.1, the examples of direct and indirect transmissions are given:

Table 1.1. Several direct and indirect transmission modes (Krasner 2002)

Direct Transmission modes	Indirect Transmission modes
<ul style="list-style-type: none"> • Contact (by kissing, touching, sneezing, coughing, sexual contact) • Animal Bites 	<ul style="list-style-type: none"> • Vehicles (fomites, door knobs, eating utensils, toys) • Airborne (by aerosols) • Vectors (mosquitos, ticks, flies)

In the prevention of contamination or the sterilization of contaminated materials, antimicrobial agents are used. The antimicrobial agents could be physical or chemical. Heat and radiation treatments are physical ways of decontamination. For instance, a temperature of 120 °C can kill both bacteria and spores whereas ultraviolet light or ionized radiations could be used in the sterilization. Commonly used chemical antimicrobial agents are alcohols, phenols, heavy metal ions, oxidizing agents,

alkylating agents and detergents. Among these, heavy metal ions, such as copper, iron and silver are well known for their high antibacterial activities (Brooks et al. 1991). Antimicrobial agents can be effective on bacteria through two ways: bacteriostatic and bactericidal actions. Bacteriostatic action is the inhibition of the bacterial multiplication but it could be resumed by the removal of the bacteriostatic agent. However, bactericidal action which is an irreversible action kills the bacteria and destroys the microorganism in such a way that the reproduction is no longer possible. In fact, these bactericidal agents could function through five possible mechanisms; the damaging of DNA, the denaturation of proteins, the disruption of cell wall or membrane, the removal of free sulfhydryl groups, and the chemical antagonism defined as inactivation of the enzymes.

Antibacterial materials could be used in food packaging or handling, medical tools, hospitals where people are more vulnerable to infections, possible areas like bathrooms where personal hygiene is important, air and water filtration etc. A pure antibacterial material, such as a silver or copper plate or silver nitrate solution, could be directly used but it is not preferred because of the factors like high cost, discoloring or losing antibacterial activity caused by environmental effects, such as the formation of an inactive atop layer (Tanimato et al. 1996). Therefore, the dispersion of an antibacterial agent in a carrier support material, such as polymer or silica, is the most common and economical way.

In developing an antibacterial material with a support carrier, the following several criteria must be considered. First, the support material used to disperse antibacterial agents must be chemically durable and non-toxic to human beings. Second, the supported antibacterial materials are supposed to be easily handled and environmentally sound materials. Third, the antibacterial material should possess a good effectiveness (defined as 30% decrease of a microbial concentration over a time provided by sufficient amount of agent) against a variety of bacteria involved in the specific application. Finally, a long term antibacterial activity is desired to achieve long lasting protection against bacteria (Kawashita et al. 2003).

Among many materials known to be effective against a variety of bacteria, silver is a unique material because it is non-toxic for human beings. Historically, silver has been used to prevent open wounds from infections. Besides, silver solutions have been reported to improve the healing of indolent wounds and to repair damaged tissues (WEB_2). In fact, silver ions in silver solutions were found to be selectively toxic for

prokaryotic microorganisms but less effective on eukaryotic cells (Yoshida et al. 1999). In addition, slow release rate of ionic silver was shown to provide a long term antibacterial effect (Jeon et al. 2003). It is clear that silver-doped inorganic materials seem to be promising candidates because of their user friendly handling, non-toxicity and the adjustable control of long-term release rate.

In the production of silver doped inorganic materials, there are several methods; high-temperature-glass fusion, implantation, ion exchange, sputtering, sol-gel etc. Among these preparation methods, the sol-gel route is the most suitable and preferable method to prepare support materials and/or one pot synthesis of the supported antibacterial agents because it is usually performed at low temperatures, e.g. less than 100 °C, and also possible to tailor the textural and chemical properties of the material. Besides, in preparing metal doped materials, the sol-gel approach allows narrow crystallite size distributions even at high metal loadings; hence resulting in high surface area of the active antimicrobial agent (Mitrikas et al. 2001). For a supported antibacterial agent, the choice of the support material is generally dependent on the application area. It must not be toxic or be able to make complex formation with the antibacterial agent in order to keep its bactericidal activity. Similarly, the bactericide itself must be non-toxic but effective for either specific bacterium or a variety of bacteria.

In this study, silica and titania were chosen as the support materials because they are used in ceramic and paint industries. For bactericides, silver and/or zinc oxide were used due to their non-toxicity and relatively high antibacterial activity. Apart from having high bactericidal activity, the slow release rate of the antibacterial agent is necessary to ensure a long lasting protection against bacteria. To achieve the controlled release rate, silver was compounded with chloride ions during the preparation step. It is known that the equilibrium dissolution rate of silver chloride (AgCl) is very low but this low concentration of silver (in terms of ions) is enough to destroy bacteria. As a result, it is expected to achieve a long term bactericidal activity. Two preparation approaches were used to prepare supported AgCl materials. In one of the approaches, the support material was synthesized using sol-gel method and then it was impregnated with a silver precursor to obtain the supported Ag material. This material was later impregnated with a dilute HCl acid to convert Ag crystallites to AgCl on the support material. In the second approach, the supported AgCl materials were prepared in one pot using a single step sol-gel procedure. Also, the effect of zinc oxide on the antibacterial activity and the

dispersion of AgCl crystallites of silica supported AgCl materials prepared using the single step sol-gel method was explored. In addition, zinc oxide was used as the additives for the silica supported AgCl materials since it is known that zinc oxide is antibacterial and also textural promoter (effective on crystallite size and oxide stability). This additive was introduced in the single step sol-gel method using titanium butoxide and zinc nitrate precursor.

Antibacterial activities of silver doped oxide materials were explored using the disk diffusion method. In this evaluation approach, the antibacterial tests are performed against *G⁻ Escherichia coli (E.coli)* bacterium which is known to be responsible from most of the infections in our daily lives. The short term (one day long) and the long term (repetitive usage of the same material) activities are investigated. In fact, the control of slow release of silver ion using the preparation methods mentioned above is indirectly determined. In other words, it is aimed at studying the size effect of AgCl crystallites and also the additives on the silver release rate for the supported AgCl materials are studied using the two preparation methods.

This thesis contains five chapters. In chapter one, a general introduction for bacteria, their significance in our daily lives, the prevention methods and also the aim of this thesis are introduced. In chapter two, a literature survey on the types of antibacterial materials and their antibacterial mechanisms, and also the material preparation methods are presented. In chapter three, the specifications and pretreatment procedures (if any) of the chemicals used in this study and also the material preparation methods, such as the single step sol-gel and the impregnation methods, is explained in details. Also in this chapter, the material characterization methods, such as X-Ray diffraction and the surface area measurement using N₂ adsorption, the chemical analysis of solution and/or materials and the disk diffusion method to determine the antibacterial activities of the materials are given. In chapter four, the antibacterial activity, and the material properties, such as silver and silver chloride crystallite sizes, are presented and it is discussed if there is a relationship between the antibacterial activity and the material properties of the oxide supported AgCl materials studied in this thesis. Finally, the conclusions and some recommendations are listed in chapter five.

CHAPTER 2

LITERATURE SURVEY

2.1. Antibacterial Materials

The prevention of bacterial contamination in our daily activities is an interesting challenge for scientists and engineers. The research and development studies have resulted in better understanding on the contamination pathways and also the development of novel or new antimicrobial materials. Although there are many well known antibacterial materials used against a spectrum of bacteria nowadays, research studies on new or novel materials with better textural and biocidal properties have been increasing each year.

Wilks et al. investigated infection risk of *E. coli O157:H7* which is a serious of pathogen causing haemorrhagic colitis from contaminated metal surfaces, including stainless steel because it is one of the most common construction materials used in food processing and handling industries, abattoirs, hospital environments, public transit systems, drinking water systems and in domestic premises, where bacterial contamination could pose an important health risk (Wilks et al. 2005). They reported that in a desiccated state, *E. coli O157* was found to be able to survive on stainless steel surface at refrigeration and room temperatures for more than 28 days. Although some reduction in bacteria concentration occurred during 28 days, the remaining population density stayed constant at 10^4 CFU which shows a potentially serious health risk (Wilks et al. 2005).

Physical methods, such as heat and radiation could be used to achieve sterilization through destroying microbes but they are not as adequate as the prevention of contamination. In fact, the effectiveness of the physical methods would not last for a long time. Thus, the usage of antibacterial materials seems to be better solution against bacterial contamination. Antibacterial materials could be classified into two groups; organic and inorganic materials. Organics are phenols, halogenated compounds, quaternary ammonium salts and in recent years, studies about antibacterial materials have been focused on natural materials, such as chitosan and chitin. For inorganic

agents, metals, metal oxides and metal phosphates are known common antibacterial materials.

Among inorganic materials, nano size metals and metal oxide were found to show high antibacterial efficacy against bacteria (Stoimenov et al. 2002, Yamamoto et al. 2004, Sondi et al. 2004) and also the oxide materials can substitute the conventional organic antibacterial agents (Yamamoto et al. 2004). Yamamoto et al. studied the effect of lattice constant of zinc oxide on antibacterial characteristics and found that zinc oxide as an antibacterial material had several advantages; zinc is an essential element to the humans and shows antibacterial activity in pH = 7 (in neutral region) without light. Besides, Atmaca et al. reported that zinc was required for the growth of the bacteria at low concentrations; however, at higher concentrations zinc oxide damaged bacteria (Atmaca et al. 1998). The antibacterial effect of zinc oxide was explained by the generation of hydrogen peroxide (H₂O₂) which was very effective at inhibition of bacterial growth (Sawai et al. 1998). In fact, hydrogen peroxide was found to damage the cellular proteins in bacteria (Trapalis et al. 2003a, Trapalis et al. 2003b). In the study of Yamamoto et al. heat was applied to ZnO powders at different temperatures. (Yamamoto et al. 2004). Antibacterial activities of the powders were tested against *E.coli* as a function of lattice constant of hexagonal structure and its effectiveness on the production of H₂O₂. It was found that H₂O₂ generation occurred in all samples. However, samples heated at 800 °C possessed a higher antibacterial activity than those heated at 1400 °C (Yamamoto et al. 2004).

Stoimenov et al. studied the antibacterial and sporicidal activities of MgO nanoparticles and its halogenated derivatives synthesized by an aerogel procedure (Stoimenov et al. 2002). They reported that the materials had an excellent activity against *E. coli* and *B. megaterium*, and fairly good activity against spores of *B. subtilis*. These materials possessed the high surface area, hence bringing about the enhanced surface reactivity that increased the adsorption capacity for active halogens. Due to the small particle size, the total contact surface area increases, thus resulting in a high antibacterial activity. This study showed that metal nanoparticles exhibited the excellent bacterial activity against G⁺ and G⁻ bacteria (Stoimenov et al. 2002). Similarly, Sondi et al. examined the antibacterial efficacy of silver nanocrystallites on *E.coli* by developing a simple preparation method to synthesize low-cost and non-toxic materials, such as stable silver hydrosols with high concentration (Sondi et al. 2004). Antibacterial activity tests were performed by determining optical density of *E.coli* bacterial cultures in liquid

medium and also as a second method agar plates were used. In the liquid medium, silver nanoparticles delayed the bacteria growth whereas they completely inhibited the growth on agar plates, thus proving that silver nanoparticles could be used as antibacterial materials.

Studies on natural antibacterial materials, such as chitin, chitosan and their derivatives, have recently increased. Chitosan is a natural nontoxic biopolymer derived by deacetylation of chitin and it has been approved as a food additive in Korea and Japan since 1995 and 1983. No et al. examined the antibacterial activities of chitin and chitosan with the varying molecular weights against four G^- (*E.coli*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, and *Vibrio parahaemolyticus*) and seven G^+ bacteria (*Listeria monocytogenes*, *Bacillus megaterium*, *B. cereus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *L. brevis*, and *L. bulgaricus*). They found out that chitosans possessed higher antibacterial activity than chitosan oligomers (No et al. 2002). Liu et al. also studied antibacterial activity chitosans by using a range of initial concentrations and molecular weights (Liu et al. 2006). In fact, the molecular weight of chitosan were changed using the method of acetic acid hydrolysis similar to the one used by Chen et al. According to the results obtained by Liu et al, it seems that chitosan has a good antibacterial property at high concentrations and the antibacterial activity of chitosan occurs by flocculating and killing bacteria.

Antibacterial activity of a material could be provided by using antibacterial agent using following several preparation methods:

- Adding the agent directly into the material.
- Coating the material with an antibacterial material.
- Blending the material with a support containing antibacterial agent.

Kumar et al. prepared a polyamide composite material containing silver. In their study, silver was added to the melted polyamide directly (Kumar et al. 2004). They reported that the silver doped polymers showed antibacterial activity by the release of silver ions. Antibacterial activity tests of the polyamide/silver composites were applied against *E.coli* and *S. aureus* bacteria. These tests showed that the composites were effective against pathogens (Kumar et al. 2004). Similarly, Jayakumar et al. studied metal containing polymers. Metal ions used as antibacterial agent were Ca^{2+} , Cd^{2+} and Pd^{2+} . Resultant product was metal containing polyurethanes (PU) which were synthesized by the reaction of hexamethylene diisocyanate or tolylene 2, 4-diisocyanate with Ca^{2+} , Cd^{2+} and Pd^{2+} salts of mono (hydroxyethoxyethyl) phthalate in

the presence of a catalyst. Their antibacterial activities were tested against *E.coli*, *Pseudomonas fluorescence*, *Streptococcus sp.*, and *Salmonella sp.* by using zone inhibition method. Results showed that PU containing Cd^{2+} and Pd^{2+} had better activity than that containing Ca^{2+} which showed mild activities. They concluded that metal ion containing PUs possessed fairly good antibacterial activities (Jayakumar et al. 2004). Jiang et al. also studied antibacterial polymers containing quaternary ammonium as an antibacterial agent instead of metals. The polymer was insoluble crosslinked polystyrene (Ps) and it was loaded with antibacterial agent using a grafting technique. Their antibacterial activities were evaluated against *Staphylococcus aureus* by a colony count method. It was determined that the resultant polymers had bacteriostatic effect on bacteria which meant the agent inactivates the bacteria without killing them (Jiang et al. 2006). In addition, Bucheaska developed a polyamide fiber possessing antibacterial properties. Polyamide fibers were first impregnated with a benzene solution and then grafted with acrylic acid. The resultant polymers were modified by penicillin (Pe), neomycin (Ne) and gentamycin (Ge) used as the antibacterial agent additives. Their antibacterial activities were investigated against *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. According to the results, Ne and Ge added fibers showed antibacterial efficacy against *E. coli*, *P. aeruginosa* and *S. aureus*. However, fibers with Pe showed antibacterial effect only against *E.coli* and *S. aureus*.

Dowling et al. studied antibacterial coatings on polymeric substances (Dowling et al. 2001). Silver were deposited on the sheets of polyurethane and silicone in addition to the tubes of polyvinyl chloride (PVC), polyurethane pellethane and cycle-aliphatic polyurethane. These polymers were coated by a combination of magnetron sputtering and a neutral atom beam source. Antibacterial activities of the silver coated final products were investigated against *Staphylococcus epidermidis*. They reported that the performance and the success of the antibacterial coatings were dependent on the silver content. However, at very high silver loadings, it was found to show some toxic effects on certain human cells. Thus, cytotoxicity tests were performed and reported that silver coated thermally sensitive polymers possessed antibacterial activity and these polymers were non-toxic to human cells (Dowling et al., 2001).

Another method to prepare an antibacterial material is to blend a polymer with a supported antibacterial agent. Hagiwara developed an antibacterial polymer composite comprising a polymer and an antibacterial mixture (Hagiwara 1999). The antibacterial mixture was consisted of a support material, such as silica gel; a coating of an

aluminosilicate on the surface of silica gels; and antibacterial metal ions, such as silver, copper and zinc. They investigated several polymers, such as low density polyethylene (LPDE), polypropylene (PP), polycarbonate (PC) and acrylonitrile butadiene styrene (ABS). They found out that LPDE containing antibacterial mixture showed a good antibacterial effect against *S. aureus* and *E.coli*. In contrast, PP antibacterial composite were very effective against *E. coli*. Antibacterial activity tests of PC composites were performed against *S. aureus* and good results were obtained. Also, they reported that ABS composites were found to have a good antibacterial activity against *A.niger* (Hagiwara 1998).

It is generally preferable to prepare antibacterial materials in a supported antibacterial agent form. There are several reasons to use a support material. A support material enables to prepare materials in such a way that it makes them more convenient for handling than unsupported antibacterial materials. For instance, a solid oxide supported metal ions, such as silver on silica, are more convenient for handling and applying than unsupported antibacterial solution, such as silver nitrate solution (Tanimato et al. 2000). Another advantage of using a support is that the effectiveness of the antibacterial material is increased because the support material increase the active surface area of the antibacterial material and consequently, the contact area between the antibacterial agent and the medium will increase. However, the support material could also control the release rate of the agent to the medium which provide a long-term antibacterial effect. In the cases where no support is used, the agglomeration of the antibacterial agent could occur; hence resulting in loss of surface area, i.e. contact area, (an undesired condition for the performance of the antibacterial material). In addition, a more uniform and narrow dispersion of the antibacterial agents could be obtained using an appropriate support material. For instance, a metal ion doped solid supported polymers are more effective than unsupported metal colloids because of the uniform dispersion which could be controlled by adjusting the particle size of the support (Hagiwara 1998).

For supported antibacterial materials, zeolites have been intensively studied as a support material because they possess active sites where cationic exchange could occur. Zeolites could be treated by the solutions containing antibacterial agents such as metal ions and the exchange of metal ions in the zeolite structure can be achieved to prepare zeolite supported metal materials (Jacobson et al. 1997). Tanimoto et al. developed silver containing antibacterial zeolites. They claimed that zeolite caused a little

discoloration when blending with an organic polymer under the application of heat or ultraviolet light. Silver containing zeolite was prepared by an ion – exchange method. Antibacterial properties of the zeolites were evaluated against *E.coli*, *A. niger* and *P.aeruginosa*. It was concluded that the prepared materials had a good antibacterial activity. (Tanimoto et al. 2000). In another study, Garza et al. investigated the antimicrobial effect of the Mexican zeolitic mineral exchanged with silver ions. The zeolites were prepared by Na⁺ / Ag⁺ ion exchange process and antibacterial activities of the zeolites were evaluated against *E.coli* and *Streptococcus faecalis*. They found that Ag- zeolites had good antibacterial properties (Garza et al. 2000). Kawaraha et al. developed an antibacterial zeolite suitable for the dental applications. Antibacterial activity tests were applied against *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* which were known as major periodontopathogens. In fact, *Streptococcus mutans*, *Streptococcus sanguis* and *Actinomyces viscosus* are pathogens causing dental caries, and *Staphylococcus aureus* causes respiratory infections. This study showed that silver – zeolites could be successfully used as antibacterial materials in aerobic and anaerobic conditions (Kawahara et al. 2000). Also, Top et al. studied the antibacterial effects of zeolites prepared by an ion – exchange process focusing on zeolites doped with Zn and Cu and silver (Top et al. 2004). In the study of Top et al, Na form of clinoptilolite was used and selectivities of the ions were determined as Ag⁺ > Na⁺ > Zn²⁺ > Cu²⁺. Antibacterial activity tests showed that Ag – clinoptilolite had antibacterial activity at the used exchange levels. However, Zn and Cu – clinoptilolites showed antibacterial activity as exchange level was increased. Furthermore, it was claimed that there should be a limit for the exchange level, since it could result in a decrease in porosity, hence decreasing antibacterial activity. It was concluded that Ag – clinoptilolite was a suitable material as an antibacterial material with a low cost (Top et al. 2004).

In addition to zeolites, other oxide materials, such as silica and titania, are also used as support materials. TiO₂ is also known to be photocatalyst which is used in degradation of environmental contaminants. For example, under UV light, TiO₂ decomposes many organic compounds, such as isopropyl alcohol. The photocatalytic activity of TiO₂ depends on several factors such as the crystal structure, surface area, size distribution porosity (Trapalis et al. 2003b). Although SiO₂ does not have catalytic or antibacterial activity, it is preferred materials because it is chemically durable (Kawashita et al. 2003), and could be added to the mediums, such as plastic molded

articles, or into a coating on a material without leading to any deterioration or decomposition (Ito et al. 2004). Moreover, SiO₂ is used in preparation of bioactive glass in combination with CaO, P₂O₅ and Na₂O due to its biocompatibility.

Yoshida et al. focused on antibacterial dental resin composites incorporated with supported silver materials (Yoshida et al. 1999). Dental resin composites containing silver on support materials were prepared and evaluated against *S. mutans* which is the major oral pathogen. In the study, silver supported antibacterial materials Novaron and Amenitop were used in combination with resin composites which were used for preventing secondary caries in dentistry. No silver release or insignificant release of silver from the material was determined after 1 day and 6 months in water. The non-releasing supported silver material containing dental resins showed long lasting antibacterial activity. Moreover, materials containing Novaron showed good mechanical properties after being immersed in water for 6 months. Antibacterial activity and good mechanical properties of dental resin composites containing silver on support materials is claimed to be promising antibacterial materials in dentistry (Yoshida et al. 1999).

Keleher et al. investigated antibacterial properties of silver coated TiO₂ particles against *E. coli* and *S. aureus* (Keleher et al. 2002). The immobilization of silver to the support material provides a long – term antibacterial activity to the antibacterial material. They prepared through the photo - reduction of Ag⁺ and MIC and disk diffusion tests were used to evaluate the antibacterial performance of the materials. According to the MIC analysis, it was said that Ag/TiO₂ particles were more efficient than Ag metals and comparable to AgNO₃. Disk diffusion tests showed that Ag/TiO₂ particles possessed comparable antibacterial effect as compared with other antibacterial materials, such as tetracycline, chloramphenicol, erythromycin and erythromycin.

2.2. Role of Silver in Antibacterial Activity

Antibacterial activity of the silver compounds has been well-known and already been used in medicine for a long time. In silver based antibacterial systems, ionization of elementary silver is required for antibacterial efficacy. There are several silver delivery systems exist that deliver silver from ionic compounds, such as silver calcium phosphate and silver chloride, and those that deliver silver from metallic compounds, such as nanocrystalline silver. Silver in the bulk form has no activity in biological

means. Silver that has antibacterial capability must be in ionic form of Ag^+ or Ag^0 clusters. Ag^0 clusters is the uncharged silver that is found in nanocrystalline silver structures. (Atiyeh et al. 2006)

It is known that nanoparticles possess unique properties different from their bulk materials. Nanocrystalline silver differs in both physical and chemical properties from micro and macrocrystalline silver and from silver salts. Nanocrystalline silver products provide Ag^0 form of silver which is less deactivated by the ions and organic materials than ionic silver (Atiyeh et al. 2006). One unique property of nanocrystalline silver is the release of Ag^0 clusters and Ag^+ ions. The release of Ag^0 clusters provides an enhanced antibacterial capability. Nevertheless, nanocrystalline silver was found to be thermally unstable (Taylor et al. 2005). Moreover, Sondi et al. found that at even high concentrations silver nanoparticles in liquid media only delay the growth of the bacteria (Sondi et al. 2004).

Silver nitrate is one of the silver compounds showing antibacterial activity. Aqueous solutions containing silver nitrate shows antiseptic effect. However, such solutions are unstable, and causes color changes on the applied surface which makes is unpractical (Atiyeh et al. 2006). Another silver compound showing bactericidal action is silver sulfadiazine which possesses the activity due to sulfadiazine ion and silver ion. Unlike silver nitrate, silver sulfadiazine has low solubility that provides a long-term activity (Capelli et al. 2005).

Silver in ionic form could be found as the clusters of silver ions. An ion or atom of silver has a diameter of ~ 0.25 nm whereas metallic silver (i.e. colloidal silver particles) composes of many atoms; thus having a bigger particle size (WEB_1). In fact, it is better to use ionic silver because colloidal silver agglomerates in aqueous media and also in the presence of bacteria, hence losing the total antibacterial activity and colloidal stability.

There are several studies on silver which are focused on the inhibitory effect of silver on bacteria and its bactericidal mechanism. Several mechanisms have been proposed in order to explain how Ag^+ ions are effective on bacteria (Feng et al. 2000) but still the mechanism is partially known (Sondi et al. 2004). Feng et al. investigated antibacterial effects of silver ions on *E.coli* and *S.aureus*. They found two important experimental observations;

- Silver reacted with proteins by combining $-\text{SH}$ groups causing inactivation of the proteins (Feng et al. 2000)

- Ag^+ ions affected the DNA molecule and DNA lost its replication abilities (Feng et al. 2000).

DNA is the most important component in the cell since it stores the genetic data. Mutation or death of an organism could be caused by a possible damage and/or alteration to DNA. It is known that some stimulated proteins, which are produced by cells in the presence of silver ions, surrounds the nuclear region in order to protect DNA; hence resulting in condensation of DNA molecule in the center of the cell. The condensed DNA loses its replication abilities. This ultimately leads to death of the cells, such as bacterial cells. It is known that the replication occurs when the DNA is in relaxing state. Feng et al. claimed that bactericidal mechanism explains the effect of silver ion on bacteria and the general defense of the cell (Feng et al. 2000). Besides, silver ion is a heavy metal that could cause the deposition of proteins in the cells. Thus, the penetration of silver ion into the cell causes denaturated proteins.

Silver cation (Ag^+) binds to electron donor groups containing sulfur, oxygen, and nitrogen due to its reactive electronic structure. These 3 components that silver ion binds are found in the biological molecules in the form of thio, amino, imidazole, carboxylate and phosphate groups (Schierholz et al. 1999). In this way, silver ion reacts with thiol groups, which leads to the inactivation of the bacteria (Jeon et al. 2003, Keleher et al. 2002, Feng et al. 2000). Silver ion also causes the formation of hydrogen peroxide that catalyzes the destructive oxidation of microorganism (Kokkoris et al. 2002, Feng et al. 2000). Silver ion and hydrogen peroxide both damages the cellular proteins (Feng et al. 2000). Since the antibacterial effect of silver ion is proportional to Ag^+ concentration, it is possible to have the multiple attacks of silver ions on the multiple targets, such as DNA or cellular proteins. Therefore, the antibacterial efficiency of the material seems to increase as the silver ion content is raised (Schierholz et al. 1999).

There are many studies focused on antibacterial mechanism of silver ion. However, the inhibitory mechanism of silver nanoparticles is not fully understood. In the study of Sondi et al., the inhibitory mechanism of the silver nanoparticles is explained by the interaction between the particles and cell membrane causing structural changes and degradation leading to death of the cell. In the TEM analysis of Sondi et al., elementary silver was detected in the cell membrane by using EDAX. (Sondi et al. 2004)

2.3. Antibacterial Materials Prepared by Sol-gel Method

In the literature, antibacterial materials are generally in the forms of antibacterial agent doped support systems. In preparing agent loaded oxide materials, sol – gel method is the most proper method because of the following several reasons. First, sol-gel method could allow producing highly homogenous (in molecular level) materials. Second, it is possible to produce high-purity products. Sol-gel also enables one to prepare oxide support matrixes doped with metal particles which could have narrow particle size distributions (Mitrikas et al. 2001). Moreover, the modification of the products could be easily performed by altering the solution chemistry using temperature or pH or the precursors. The product properties like porosity, particle size, and pore size could be tailored for the specific applications using the preparation parameters.

Silver containing silica glass materials were prepared by Kawashita et al. (Kawashita et al. 2000). They prepared the colorless and chemically durable silver – doped silica glasses which were desirable in dental applications. They showed that using Al in the composition allowed preparing colorless materials and also, Al content in the compound affected the release rate of silver ion into the medium. They also reported that when there was no Al, Ag^+ ions rapidly released into the water. However, at the ratios of $\text{Al} / \text{Ag} \geq 1$, the release rate of silver was lower which enabled a long-term antibacterial activity. Moreover, they claimed that the release rate of Ag^+ ion was controlled by the inter-diffusion of the Ag^+ and H_3O^+ ions within the solid compound. In order to investigate the antibacterial activity of these materials, they were applied tests against *Streptococcus Mutans* which are known to be responsible for the dental caries. They concluded that the silica based materials were useful as antibacterial materials for the medical applications (Kawashita et al. 2000). Later, Kawashita et al. reported that they prepared silver-containing silica glass microspheres with a diameter of $<1 \mu\text{m}$. It was thought that as the diameter of microspheres decreased, their surface area for the mass transfer of the ions increased; consequently, antibacterial activity increased. The advantage of preparing silica glass in the form of microspheres $< 1 \mu\text{m}$ is that they can be mixed with organic polymers which can be formed into a thin film or fine fibers. In fact, the antibacterial tests of these silver doped silica glass microspheres showed that they possessed excellent antibacterial activity against *E.coli* (Kawashita et al. 2003).

In the study of Jeon et al., silver – doped silica thin films made by sol-gel method was tested if they had antibacterial activity (Jeon et al. 2003). In the sol-gel method, the sols were first prepared and then the films were produced by the dip coating process. In preparing the sols, nitric acid was used as the hydrolysis catalyst and silver nitrate was used as the silver source since nitrates easily decomposes during heating step of the preparation. In their study, the effects of thermal treatment were also examined. After the dip coating process, the thermal treatment was applied in the range from 200 to 600 °C. In order to determine the antibacterial activity of the films, the film attachment method was used. The bacteria used in their study were *E.coli* (*E. coli*) which is G⁻ and *Staphylococcus aureus* (*S. aureus*) which is G⁺. The thermal treatment tests showed that at lower temperatures, Ag⁺ ions were not trapped in the silica matrix and the color of the films was dark. They claimed that untrapped silver ions could diffuse to the surface and form silver metal there. Therefore, they proposed that the silver ions had to be completely trapped in the silica matrix and this was obtained by a thermal treatment at 600 °C. The antibacterial activity tests applied to the Ag-doped SiO₂ films showed that they were successful against both *E. coli* and *S. aureus* by the 100% reduction of bacteria (Jeon et al. 2003).

Kokkoris et al. also studied the AgSiO₂ thin coatings prepared by sol-gel method (Kokkoris et al. 2002). The films were prepared with a similar method that Jeon et al. used. In the study of Kokkoris et al., the antibacterial activities of the films were examined against *E.coli* with the antibacterial drop test. It was reported that the drop test was an appropriate method in order to determine the inhibitory capability of antibacterial materials with the corrosion resistance and the low diffusion rate of the corrosion species. Moreover, the atomic force microscopy (AFM), Rutherford backscattering spectroscopy (RBS) and the heavy ion RBS (HIRBS) techniques were used to examine the structure of the films to determine the relation between the layer inter-diffusion after the thermal treatment and antibacterial activity. According to the antibacterial activity test results, they concluded that AgSiO₂ films prepared by sol-gel method possessed the higher antibacterial activity than that treated thermally and treated in reductive atmosphere.

Instead of silver, Trapalis et al. used copper as antibacterial agent in the silicate thin film coatings (Trapalis et al. 2003b). Copper containing silicate coatings were prepared with a similar method that Kokkoris et al. used. Cu (acac)₂ was used as copper source instead of cupric nitrate. Cu (acac)₂ was preferred in order to control the reaction

rate during the synthesis of the coatings. They also used RBS and HIRBS techniques to study the structures of the coatings. The antibacterial activities of the coatings against *E. coli* were investigated using antibacterial drop method. They found out that copper had as high antibacterial activity as silver in silicate coatings.

Trapalis et al. also investigated Fe³⁺ doped titania thin films prepared with a sol-gel method and examined the antibacterial activity of the films (Trapalis et al. 2003a). The effects of the concentration of Fe³⁺, the number of coating cycles and the calcination temperature on the film structure were also investigated. Antibacterial tests were applied to the *E. coli* bacteria with the antibacterial drop method. Once titania sols were prepared with a sol-gel method, the soda-lime glass was coated with the sol. According to the results, the coatings showed good antibacterial properties. Moreover, it was seen that the thickness of the films increased with the content of the Fe³⁺. Also, as the number of coating cycle was increased, the antibacterial activity of the film increased. The heat treatment at 500°C provided the formation of anatase crystalline structure which in turn increased the antibacterial activity of the titania in anatase phase. They claimed that the photocatalytic activity of titania was better in anatase phase than that in rutile phase (Trapalis et al. 2003a).

Silver oxide (Ag₂O) - doped bioactive glass and their antibacterial activities were studied by Bellantone et al. (Bellantone et al. 2002). In their study, Ag containing bioactive glasses (AgBG) composed of SiO₂, CaO, P₂O₅ and NaO₂ were synthesized using a sol – gel method. The purpose of the study was to develop a material possessing bioactivity and bactericide. MIC antibacterial test method was used in the evaluation of antibacterial activity of materials against *E.coli*, *P. aeruginosa* and *S. aureus*. They found out that AgBG materials inhibited bacterial growth whereas BG materials without silver had no effect on bacterial growth.

Catauro et al. studied the antibacterial activity and bioactivity of the sol-gel made silver containing Na₂O-CaO-2SiO₂ glasses (Catauro et al. 2004). The support material was claimed to be modified with calcium to achieve good biocompatibility and bioactivity in vitro. Antibacterial activity and bioactivity tests were applied to the glass powders using *E.coli* and *Streptococcus mutans*. Antibacterial activity test results showed that silver containing silica glass possessed high antibacterial activity. For the bioactivity tests, silver containing Na₂O-CaO-2SiO₂ glasses placed in the stimulated body fluid (SBF) and their FTIR spectra seemed to confirm the formation of

hydroxyapatite (HA) and also EDS results indicated that the layer formed was composed of calcium and phosphorous.

Amezaga-Madrid et al. investigated the photocatalytic activity of TiO₂ films under the UV-irradiation (Amezaga-Madrid et al. 2003). It is known that titania is an excellent photocatalytic material (He et al. 2002) and also it possesses good antibacterial activity to kill the bacteria to distances of 50 µm (Fujishima et al. 2000). Amézaga-Madrid et al. reported that titania films were obtained by a sol-gel method and a glass slide was coated by the spin-coating. The antibacterial activity tests applied to the wild bacteria *Ps. aeruginosa* showed that the activity of the irradiated titania were not successful because of the high concentration of bacteria. In fact, they found out that the bacteria cells were not affected (Amézaga-Madrid et al. 2003). Similarly, Daoud et al. studied titania coatings prepared by a sol-gel method and investigated their antibacterial activity (Daoud et al. 2004). In order to determine photocatalytic activity, they placed the film coated cotton substrate and uncoated one on the same plate where *Klebsilla pneumoniae* G⁻ bacteria were seeded. As the samples were incubated under fluorescent light with UV intensity of 4.74 µW/cm², they determined that under the film coated cotton substrate, no bacteria were seen whereas a continuous growth of the bacteria was observed under the uncoated substrate (Daoud et al. 2004). This observation was also in agreement with the fact that the titania possessed the antibacterial activity and photocatalytic effect (He et al. 2002).

Nablo et al. studied the nitric oxide releasing sol-gel materials and investigated their antibacterial properties for orthopedic implants (Nablo et al. 2005). The significance of this study is that the antibacterial agent is in gaseous form. In the study, stainless steel slides were coated with the sols composed of butyltrimethoxysilane (BTMOS) and N-(6-aminohexyl) aminopropyltrimethoxy -silane (AHAP3). The films were modified by converting the diamine groups to the diazeniumdiolate NO donors by placing the films into the NO reactor. Then, the antibacterial tests were applied to the uncoated steel, the sol-gel coated and the modified sol-gel coated stainless steel samples. Antibacterial activity tests were performed against *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. They found that the modified the sol-gel coating decreased the bacterial adhesion as compared to the sol-gel coating and the bare stainless steel. However, after the release of NO from the sol-gel coating, the antibacterial activity of the coating was similar to the unmodified sol-gel coating.

Among the studies reported in the literature up until now, silver is the most common antibacterial agent successfully used in the supported antibacterial materials. In addition to silver, Fe and Cu were found to be effective as the bactericidal agents. Silica and titania are the common choice of the support material. Although titania could be used alone as photocatalytic material, the combination with an bactericidal agent improves the antibacterial property of titania. It is also seen that the materials could be modified with other compounds, such as the addition of calcium into silica, to improve the material property, such as biocompatibility, as reported by Catauro et al. (Catauro et al. 2004).

CHAPTER 3

MATERIALS and METHODS

3.1. Materials

In this study, two oxide supports were prepared; titania and silica oxide. In the synthesis of silver doped titania powders, tetrabutyl ortotitanate (TBOT) from Fluka was used as titania source. Ethanol (EtOH) as a solvent, and hydrochloric acid as a peptizer (HCl) were used. Silver nitrate (AgNO_3) in powder form from Fluka was used as a silver source.

Table 3.1. Properties of materials used in synthesis of titania powders

	TBOT	EtOH	Hydrochloric acid
Chemical Formula	$\text{C}_{16}\text{H}_{36}\text{O}_4\text{Ti}$	$\text{CH}_3\text{CH}_2\text{OH}$	HCl
Molecular Weight (g/mol)	340.36	46.07	36.46
Density (g/cm^3)	0.780-0.790	0.789	1.2
Purity	97%	99.50%	37%

In the preparation of silica powders, tetraethylortosilicate (TEOS) from Alfa Aesar was used as silica source. Ethanol is again used as the solvent. 1M of hydrochloric acid solution and 0.05 M of ammonium hydroxide (NH_4OH) solution were used as a catalyst. For zinc source, zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) and for silver source silver nitrate (AgNO_3) in powder form from Fluka Co. were used.

Table 3.2. Properties of materials used in synthesis of silica powders

	TEOS	Ammonium hydroxide	Zinc nitrate hexahydrate
Chemical Formula	(C ₂ H ₃ O) ₄ Si	NH ₄ OH	Zn(NO ₃) ₂ .6H ₂ O
Molecular Weight (g/mol)	208.33	35.05	297.48
Density (g/cm ³)	0.94	0.9	
Purity	99%	29.30%	98%

In the antibacterial activity tests, disk diffusion (zone inhibition) method was used. In the tests, *E.coli* bacteria were used. The turbidity of the bacteria colony was adjusted according to McFarland no 0.5 (10⁸ CFU/ml). For agar – broth media; Mueller – Hinton broth, nutrient agar and agar – agar were obtained from Mercks Co.

3.2. Methods

In this study, the experiments could be categorized into 3 groups as seen in Figure 3.1;

- Preparation of the antibacterial materials
- Characterization of the materials
- Antibacterial activity tests

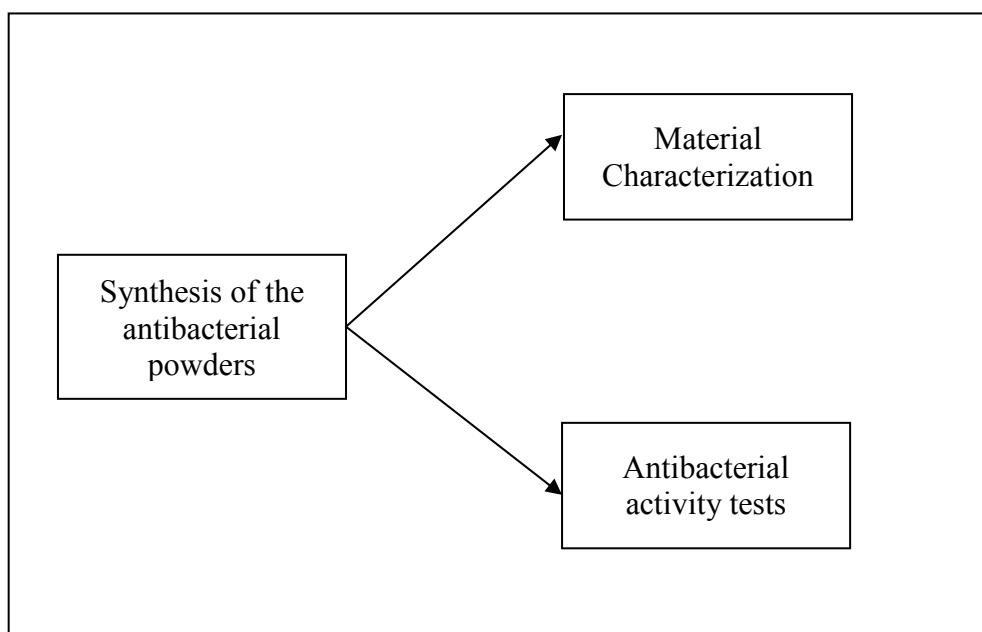


Figure 3.1. Experimental procedure

3.2.1. Preparation of the Antibacterial Materials

Titania supported and zinc oxide – silica supported Ag powders were synthesized via a sol-gel route. In Figure 3.2, experimental procedure for the synthesis of titania powders is shown. TiO₂ powders were synthesized by using two different acids; using HCl and HNO₃. HCl was used to prepare TiO₂ supported AgCl containing powders. Two types of gels can be prepared by the sol-gel method; colloidal and polymeric. If HCl is directly added before water gel becomes colloidal, and if HCl is added in an HCl – H₂O mixture, gel becomes polymeric.

The ratio of the reagents used in the experiment is given in Table 3.3. In the synthesis, TBOT and EtOH were first mixed at room temperature. For polymeric samples, HCl solution was added after 10 minutes. HCl solution was prepared in order to add HCl and water together in one step. This solution consisted of half of water and all of HCl that were required in the procedure. For colloidal samples, by using 10 minutes of intervals, half of total HCl, half of total water, half of total HCl were added respectively. After 10 minutes of adding this solution, silver was added. Silver nitrate dissolved in half of the water used in the experiment. Mixing was continued at room temperature until gelation. After that, calcination was applied to the gels. The gels were calcined at 400 °C with a heating rate of 10 °C/min for 3 h. The powders obtained were ground and were sieved with a stainless steel screen with 75 µm size. The same procedure was used to synthesize the TiO₂ powders by using nitric acid instead of HCl. Samples were prepared by using nitric acid to compare the powders consisting AgCl and metallic Ag.

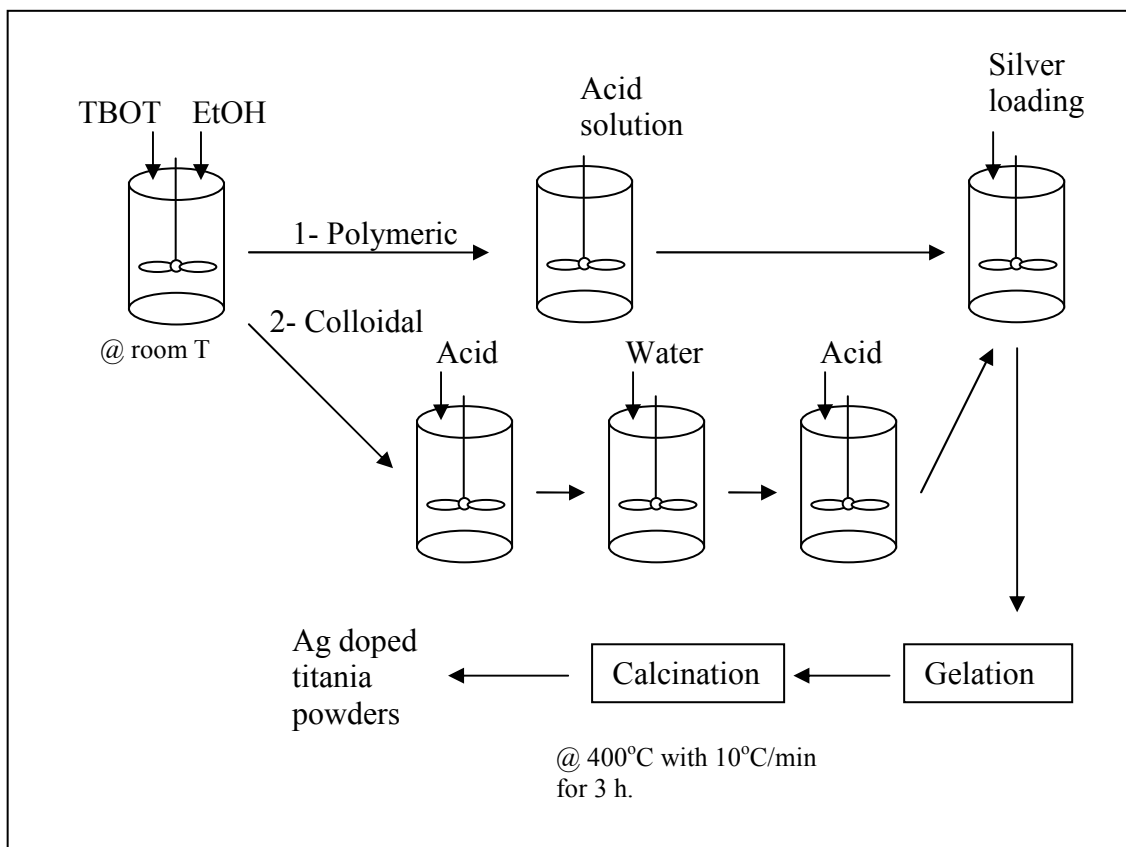


Figure 3.2. Experimental procedure for the preparation of titania supported samples

Table 3.3. Molar ratios of the reagents used in the synthesis of TiO₂ supported powders

(HCl) / (Ti)	0.25
(H ₂ O) / (Ti)	5
(EtOH) / (Ti)	27.3

In Figure 3.3, the experimental procedure for the synthesis of silica powders is seen. The ratios of the reagents used in making silica supported Ag sample are given in Table 3.4. First, TEOS, EtOH, H₂O and 1 M HCl solution were mixed. After for 10 minutes, the mixture was heated to 85°C. The temperature was kept constant at 85°C during for the rest of the sol-gel process. The solution was stirred for 2 hours, and then zinc nitrate precursor was added. This was followed by the addition of 0.05 M NH₄OH. Gelation occurred within 4 hours. Gels were left for overnight for ageing. Then, these gels were dried for 24 hours at 120°C and calcined at 500°C with a heating rate of 8°C/min for 12 h. Finally, samples were ground and sieved to 75 μm mesh size. The amount of water required for impregnation is found by wetting the sample until the sample is saturated (i.e. no water absorbed further). After drying the powders, a

specified amount of AgNO₃ were dissolved in an amount of water found earlier. Finally, this Ag containing solution was impregnated to the powders and they were calcined at 200 °C for 2 hours. For the SiO₂ samples, four ZnO loadings were used; 0%, 30%, 50%, and 70%.

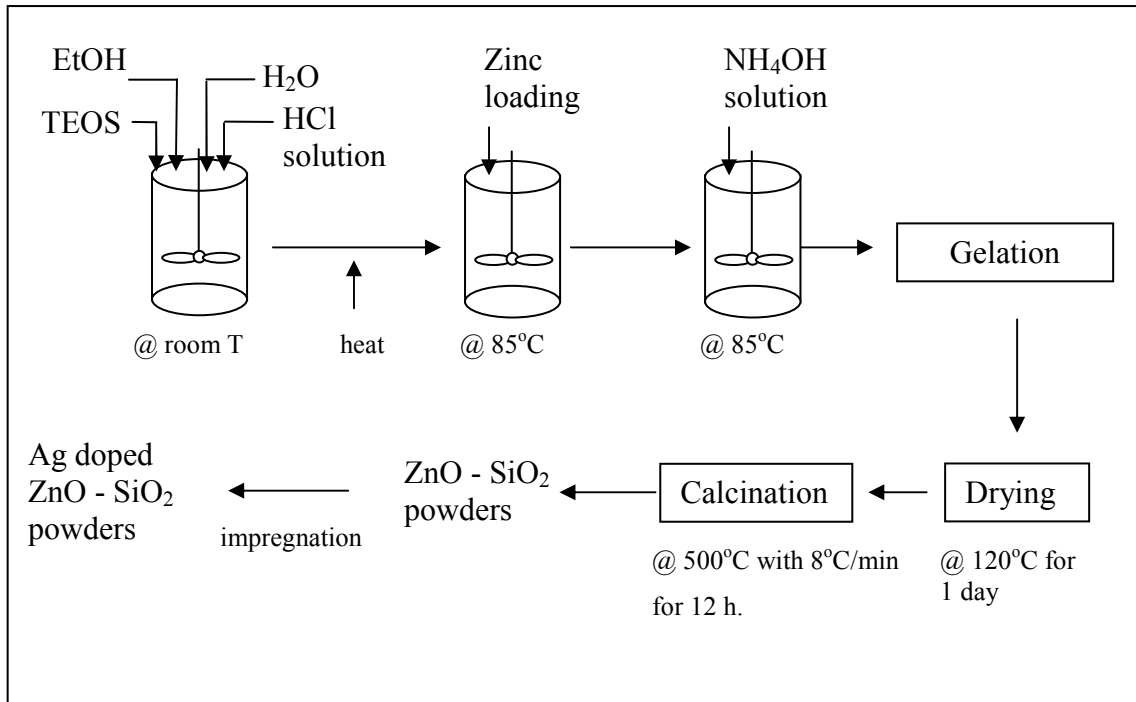


Figure 3.3. Experimental procedure for the preparation of silica supported samples

Table 3.4. Molar ratios of the reagents used in the synthesis of ZnO - SiO₂ supported powders

(HCl) (1 M) / (Si)	$7.9 * 10^{-4}$
(H ₂ O) / (Si)	15.6
(NH ₄ OH) (0.05 M) / (Si)	$2.5 * 10^{-3}$
(EtOH) / (Si)	22.00

HCl treatment was applied to some samples in order to form AgCl from Ag particles in the powders. Acid treatment was applied to TiO₂ and ZnO-SiO₂ powders using the same procedure used in Ag impregnation. However, the concentration of the acid used for TiO₂ and ZnO - SiO₂ powders were different. 37% HCl was used for TiO₂, whereas 1% HCl was used for ZnO – SiO₂ powders due to the solubility of ZnO in

acids. When 1% HCl was used, HCl impregnation was repeated for three times. Then, HCl treated samples were calcined at 200°C for 2 hours.

Ag/TiO₂ (molar) ratios in those experiments were 0.01, 0.1 and 0.3. Their weight percentages were equal to 1.2%, 12% and 29% (Ag/ (Ag + support)) respectively. Silver loading for SiO₂ samples were adjusted according to the TiO₂ loadings. Two silver loadings, 1.2% and 12% were used.

Antibacterial activities of the powders prepared were compared with a natural zeolite. The zeolite was prepared by ion-exchange with the help of Miss Yelda Akdeniz. Briefly, the following procedure was used. First, 10 days of treatment with 1 N NaCl was applied (NaCl was replaced with fresh one every 3 days). After that, zeolites were placed into 0.01 M AgNO₃ solution for 3 days at 60°C and then, dried at 65°C for 24 h. Its final Ag loading was determined as 3.178% (w/w).

3.2.2. Characterization of the Materials

In the characterization of the samples, X-ray diffraction (XRD) and scanning electron microscopy (SEM) were used. XRD pattern of the samples were determined using a Philips Xpert XRA- 480 Model X-ray diffractometer. The surface morphology was examined by using a Philips XL30S model scanning electron microscope. Pore volume and pore diameter of the oxide powders were determined by ASAP2010 in the presence of N₂ at 77.34 K.

3.2.3. Antibacterial Activity Tests

In the evaluation of antibacterial activity of the samples, disk diffusion (zone inhibition) method was used. For the application of this method media and other necessary steps were given in the following parts. *E.coli* bacteria were obtained from specialist Mert Sudagidan in Biotechnology Department in İzmir Institute of Technology.

3.2.3.1. Broth - Agar Media

In this method, first agar – broth for the growth media was prepared. Two types of media were used. The media were composed of Mueller – Hinton broth and agar agar, and Mueller – Hinton and nutrient agar, respectively. In Mueller – Hinton broth / agar agar preparation, 1 L of deionized water were used with 13 g of agar agar and 21 g of broth. For Mueller – Hinton broth / nutrient agar, 1 liters of deionized water, 20 g of nutrient agar and 21 g of broth were added. Then, the solution was left in the autoclave for sterilization at 121°C for 15 minutes. After that, the broth agar (cooled to about 40°C) was poured to the petri dishes at equal amounts (approx. 20 ml.). These petri dishes were left for drying and solidifying at room temperature.

In addition to Mueller – Hinton media, Luria – Bertani media was also used. 5 g yeast extract and 10 g of tryptone were added to 900 mL of deionized water. After that, 7 N NaOH solution was used to adjust pH to 7. After that, final volume was completed to 1 L.

3.2.3.2. Turbidity Standard for Inoculum Preparation

It is desired to standardize the inoculum density in the antibacterial activity tests, 0.5 McFarland standards was used. McFarland standard was prepared by mixing 0.5 ml aliquot of 0.048 mol/L BaCl₂ with 99.5 ml of 0.18 mol/L H₂SO₄ (1 % v/v).

Table 3.5. Ingredients of 0.5 McFarland (10⁸ CFU/ml)

0.5 Mc Farland Standard	Equivalent to 10 ⁸ CFU/ml
Barium Chloride, 0.048 M solution	0.5 ml
Sulfuric Acid, 0.18 M solution	99.5 ml

3.2.3.3. Inoculum Preparation and Inoculating Agar Plates

E.coli bacteria taken from the stock was applied on the petri dishes by using a needle holder. The petri containing *E.coli* was incubated for one day at 37°C to obtain single colonies. 37 °C is the optimum growth temperature of *E.coli*. Single colonies were taken from the grown bacteria colonies, and diluted in water to obtain a bacterial

suspension having turbidity equal to that of McFarland no: 0.5. Bacterial suspension was streaked on the surface of agar-broth in two directions by using sterile cotton swabs to obtain uniform growth. Then, samples pressed as a pellet (0.8 cm in diameter) were placed on the petri dishes. Finally, after one day incubation at 37°C, the width of inhibition zone of each sample in the plates was measured (NCCLS, 2000).

CHAPTER 4

RESULTS and DISCUSSION

AgCl nano-crystals in the oxide support materials, used as a stabilizing medium were prepared to study their antibacterial properties against *E.Coli* bacteria. Two kinds of oxide supports, titanium oxide and zinc oxide-silica mixed oxide powders were synthesized via a sol-gel route.

In the synthesis of TiO₂ powders, several different approaches were used to form AgCl nano-crystals. Single step sol-gel routes for making polymeric and colloidal gels which differed in the addition of HCl and H₂O were followed. Moreover, HCl treatment was applied to the samples which contain metallic silver and/or AgCl nano-crystals. The samples prepared by using HCl in synthesizing the powders contained not only AgCl nano-crystals, but also metallic silver was formed during the calcination of the powders because of the availability of the reducing agent, such as alcohol, and heat. With HCl treatment, AgCl crystals were formed due to the presence of chloride ions. The sintering effect of Cl⁻ ions during calcination steps leads to the formation of large AgCl crystals. The samples which were prepared by using HNO₃ contained only metallic silver.

In the synthesis of ZnO-SiO₂, the procedure followed in synthesis of TiO₂ powders was different in such a way that silver loading to the powders were applied by impregnation after obtaining ZnO-SiO₂ powders, because ZnO-SiO₂ were synthesized at 85 °C which is known to cause the reduction of silver. In addition, the amount of HCl used for silica gels was too low to form AgCl crystals in the matrix. Thus, by considering these points, Ag addition was done by the impregnation method. Zinc oxide has a high solubility in acids; therefore, dilute HCl solution (1 %) was used to form AgCl nano-crystals in ZnO-SiO₂ matrix. Acid treatment using with dilute HCl solution (1 %) was repeated 3 times to ensure the formation of AgCl from Ag crystals. In order to examine the effect of HCl on the crystal structure of the materials, XRD analyses were performed.

The color of TiO₂ powders without silver was white – yellow. The samples containing a low amount of silver were grey, and at the highest silver content, they were black. After HCl treatment and calcination, their color became lighter than their initial color. The dark color of the samples was caused by the reduction of weakly attached silver ions on the support to metallic silver because of calcination at high temperature.

Although ZnO – SiO₂ powders were white, even the powders containing a low silver amount were light grey. However, at high Ag loading in the powders, the color of the samples turned darker. Similar to TiO₂ samples, HCl treatment resulted in lighter color in the samples due to the formation of AgCl nano-crystals from metallic silver crystals as confirmed by XRD measurements. In Figure 4.1, XRD pattern single step synthesized polymeric samples with silver loading of 29% are shown.

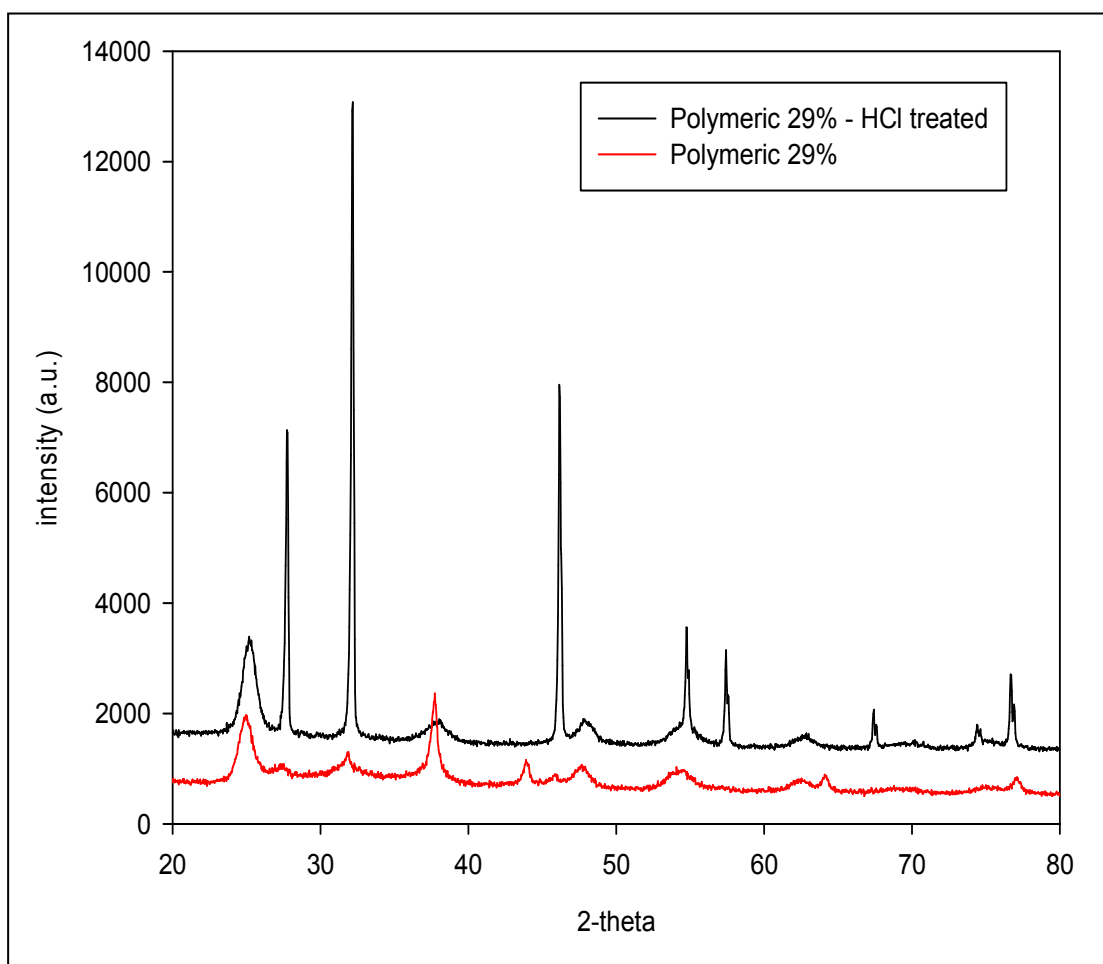


Figure 4.1. XRD pattern of untreated and HCl treated Ag 29% - TiO₂ samples

4.1. Antibacterial Activity Tests Using Disk Diffusion Method

The results of the disk diffusion tests are presented in two sub-sections according to the types of oxide supports; titania and zinc oxide/silica. Disk diffusion method was performed according to the NCLLS standards (NCLLS, 2000). In the antibacterial activity tests, the zone diameters of the samples were measured with an electronic caliper. Zones were measured from the four sides of the zones in order to

minimize reading errors. Using the four measurements, 95% confidence interval of the measurements were calculated. In order to apply disk diffusion method, samples should be in a disk shape having a constant diameter and weight. Thus, the powder samples were first formed into pellet in a hydraulic press using a dye with 0.8 cm in diameter. In disk diffusion method, there are several parameters that can affect the results of the tests significantly. For instance, the depth of the agar-broth in a petri dish affects the results. Moreover, the concentration of bacteria on agar-broth can differ from one petri to another. Therefore, each petri was separately compared and examined to obtain more accurate results. For instance, the effect of silver concentration on antibacterial activity was investigated in one petri, whereas the effect of the support type was evaluated using fresh samples in another petri dish.

4.1.1. Antibacterial Activity Tests of Titania Supported Ag Samples

In TiO₂ supported Ag samples, the effect of preparation method was first investigated. At a constant silver loading, the single step polymeric gels made using HCl as a hydrolysis catalyst, the single step polymeric and colloidal gels made using HNO₃ as hydrolysis catalyst were compared. In addition, SGP gel powder synthesized using HCl was further treated with HCl to examine the effect of the treatment. The results of tests are shown in Table 4.1 below.

Table 4.1. Antibacterial test results of TiO₂ samples prepared via different methods

Samples	Zone dia. (mm)
Colloidal with HNO ₃ – Ag 29%	12.62 ± 0.06
Polymeric with HNO ₃ – Ag 29%	12.86 ± 0.00
Polymeric with HCl – Ag 29%	12.54 ± 0.13
HCl treated Polymeric – Ag 29%	11.41 ± 0.00

In Figure 4.2, antibacterial activities of polymeric and colloidal samples synthesized using HNO₃ as the hydrolysis catalyst are compared. It is seen that polymeric samples showed higher activity than the colloidal ones. The difference in activity between these two samples could be explained by different crystal thicknesses of Ag nano-crystal in the samples.

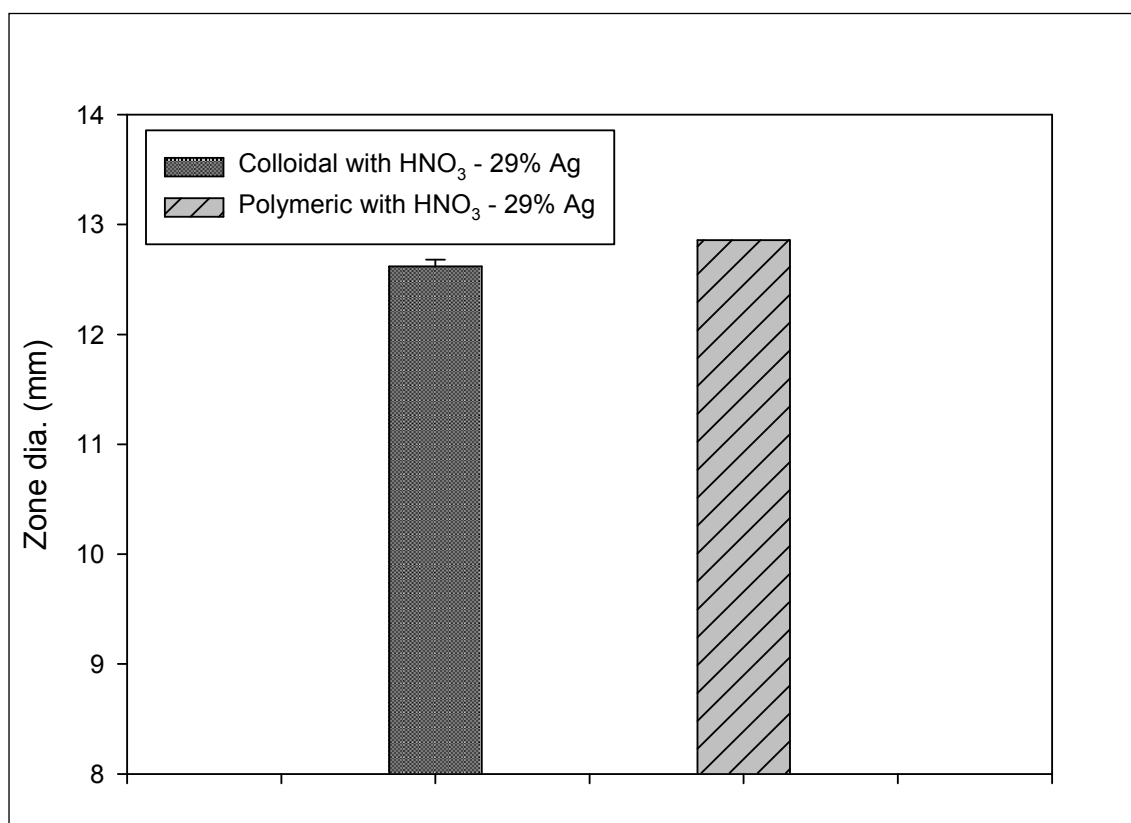


Figure 4.2. Metallic silver containing colloidal and polymeric TiO₂ with HNO₃ samples at silver loading of 29%

In Figure 4.3, XRD patterns of these samples are given. Using HNO₃ as hydrolysis catalyst during the synthesis of these powders favored the formation of only metallic silver nano-crystals in the samples. Thus, no AgCl nano-crystals were present. At 38.20 and 44.40 of 2 θ angles, metallic silver peaks (with additional peaks for the other crystal planes of silver at 64.60 and 77.6) are seen. Also, the other peaks of silver as listed in Appendix A are clearly seen in the diffraction pattern.

At 25.3 2 θ angle, the strongest peak of anatase phase of TiO₂ is seen clearly. Also, at 48.07, another peak of anatase phase with a high intensity is seen. If the crystalline phase in TiO₂ were rutile, the identifying peak for rutile phase would be located at about 27 of 2 θ angle. In the study of Chao et al., anatase phase formation was showed to begin at a calcination temperature of 400 °C for undoped TiO₂ (Chao et al. 2003). It was found that rutile phase did not appear until the calcination temperature of 750 °C whereas Ag doping lowered the formation temperature of rutile phase; for example at even 10% mol Ag doped samples, rutile formation was seen at 700 °C. The findings of this thesis are in parallel with that of Chao et al., that only anatase phase in

all the samples synthesized in this study was observed due to the low calcination temperature. It is desired to synthesize the TiO_2 in anatase phase since it has higher surface area than rutile phase. Synthesizing a support material having low surface area is not a desired condition because decreasing the surface area will decrease the mass transfer area which will affect the silver ion release.

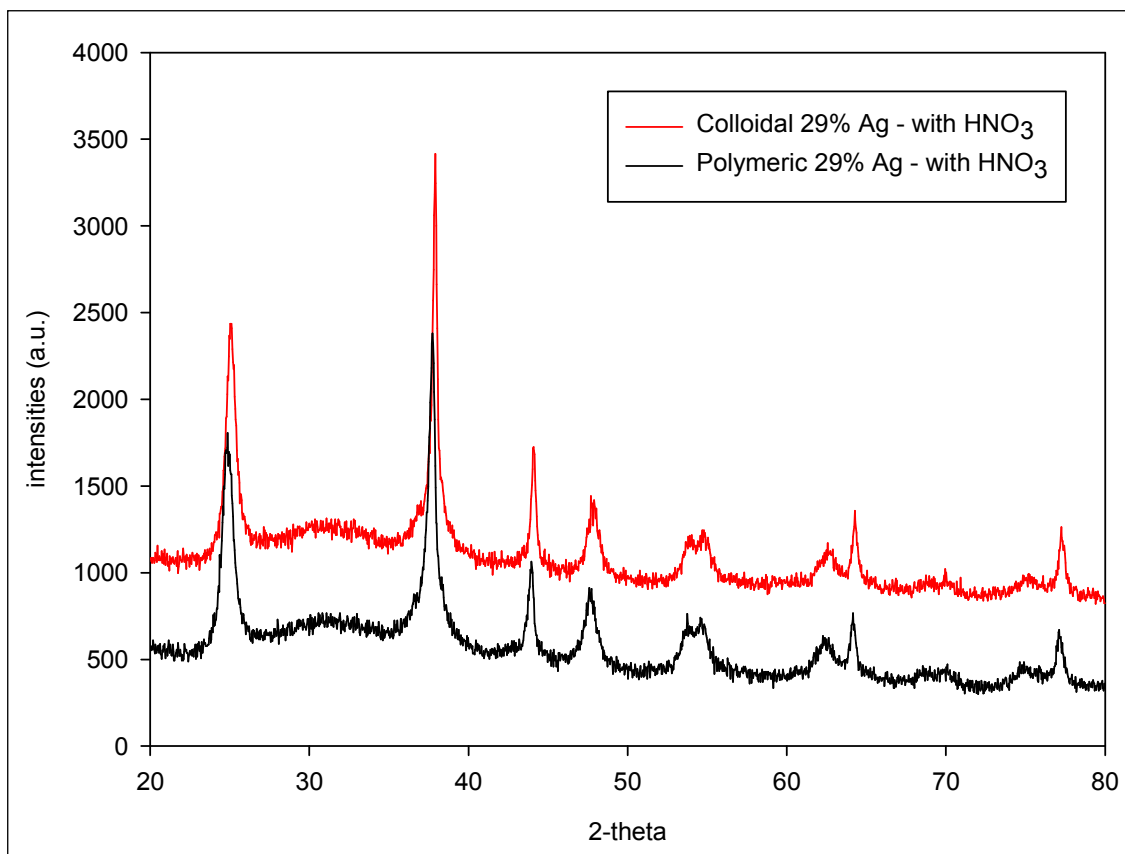


Figure 4.3. XRD pattern of colloidal and polymeric TiO_2 with HNO_3 at silver loading of 29%

In order to examine the effect of the form of silver in the samples, three polymeric gel powders were compared; a sample synthesized with using HNO_3 as hydrolysis catalyst, a sample synthesized with using HCl as hydrolysis catalyst and a sample impregnated with HCl acid. In Figure 4.4, XRD patterns of the three polymeric TiO_2 samples are seen. While the sample prepared with HNO_3 contained only metallic nano-crystals, the sample prepared with HCl contained both Ag and AgCl nano-crystals.

Crystalline thicknesses were calculated using Scherrer equation seen below:

$$\bar{d} = \frac{0.9\lambda}{\beta \cos \theta} \quad (4.1)$$

where

λ is the wavelength of X-ray (0.154 nm)

β is the peak width of half height (in radian)

θ is the diffraction angle

Calculated crystal thicknesses of different samples were given in Appendix A in Table A1. According to this equation, crystal thickness of Ag on polymeric TiO₂ synthesized with HCl was found to be 11.72 nm and AgCl crystal thickness to be 15.63 nm. In colloidal samples, AgCl and Ag crystal thicknesses were calculated to be 24.21 nm and 21.53 nm, respectively (XRD pattern of polymeric and colloidal sample is shown in Figure A6 in Appendix A). As polymeric and colloidal samples are compared, it is seen that crystal thickness of polymeric samples is small. It is caused by difference in the procedure applied in the synthesis of the powders. In polymeric samples, the pore sizes and consequently, particles sizes were smaller. As the polymeric gel network was formed, linear branches were formed between small oxide particles. In contrast, the branches were formed between relatively big oxide particles in colloidal gels.

In HCl used samples, some part of the AgCl crystals decomposed into Ag silver due to high calcination temperatures. In the XRD pattern of this sample, AgCl peaks as well as metallic silver peaks are clearly seen. Silver chloride peaks were the strongest at 2-theta of 27.83, 32.24 and 46.24. In order to form only AgCl nano-crystals, HCl treatment was applied to these samples. In the study of Li et al., the high reactivity of Ag nanoparticles was observed in the reaction with hydrochloric acid (Li et al. 2006). Li et al. studied the reaction of HCl with Ag and the reaction product was found to be AgCl. Figure 4.4 shows the XRD pattern of HCl treated polymeric TiO₂ – Ag 29% powder. As seen from Figure 4.4, the strong AgCl peaks were located at 27.83, 32.24 and 46.24 which confirmed the results of the study of Li et al. These peaks have larger crystalline thickness than that of untreated polymeric samples. In HCl treated polymeric samples, AgCl crystalline thickness was found to be 29.31, while it was 11.62 nm in polymeric sample with HCl.

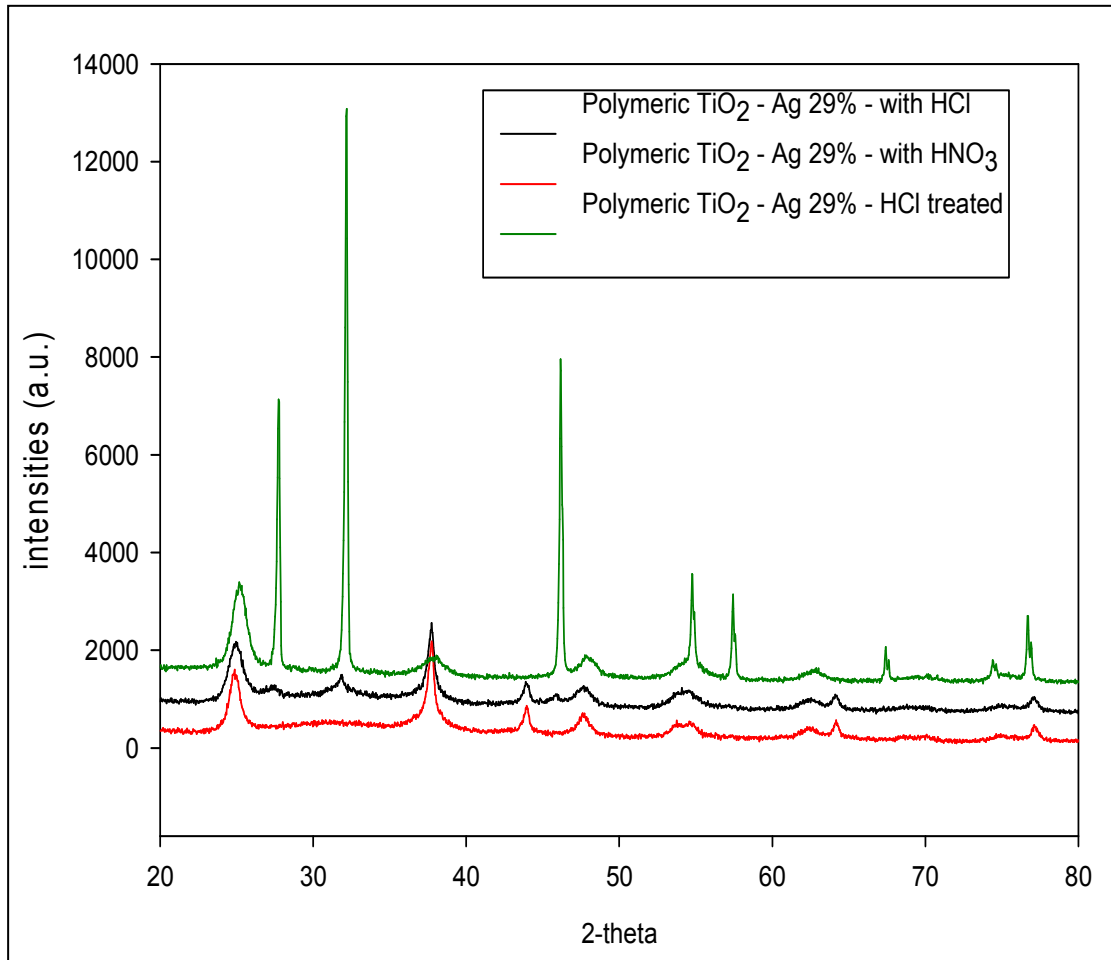


Figure 4.4. XRD patterns of polymeric samples containing Ag and/or AgCl nano-crystals.

In Figure 4.5, the antibacterial activity test results of the three polymeric TiO₂ – Ag 29% powders are seen. According to the results, polymeric TiO₂ – Ag 29% with HNO₃ that contain only metallic silver showed the higher antibacterial activity. HCl treated polymeric sample containing AgCl nano-crystals showed the lowest antibacterial activity. The lowest antibacterial activity of AgCl containing samples can be explained by the counter – ion effect introduced by the test medium for bacteria growth. In other words, antibacterial test results could be affected easily by the ions present in the agar-broth media where antibacterial tests were performed. If ions with complex compound formation ability are present in the media, it could inhibit diffusion of silver ions through the media from the powders.

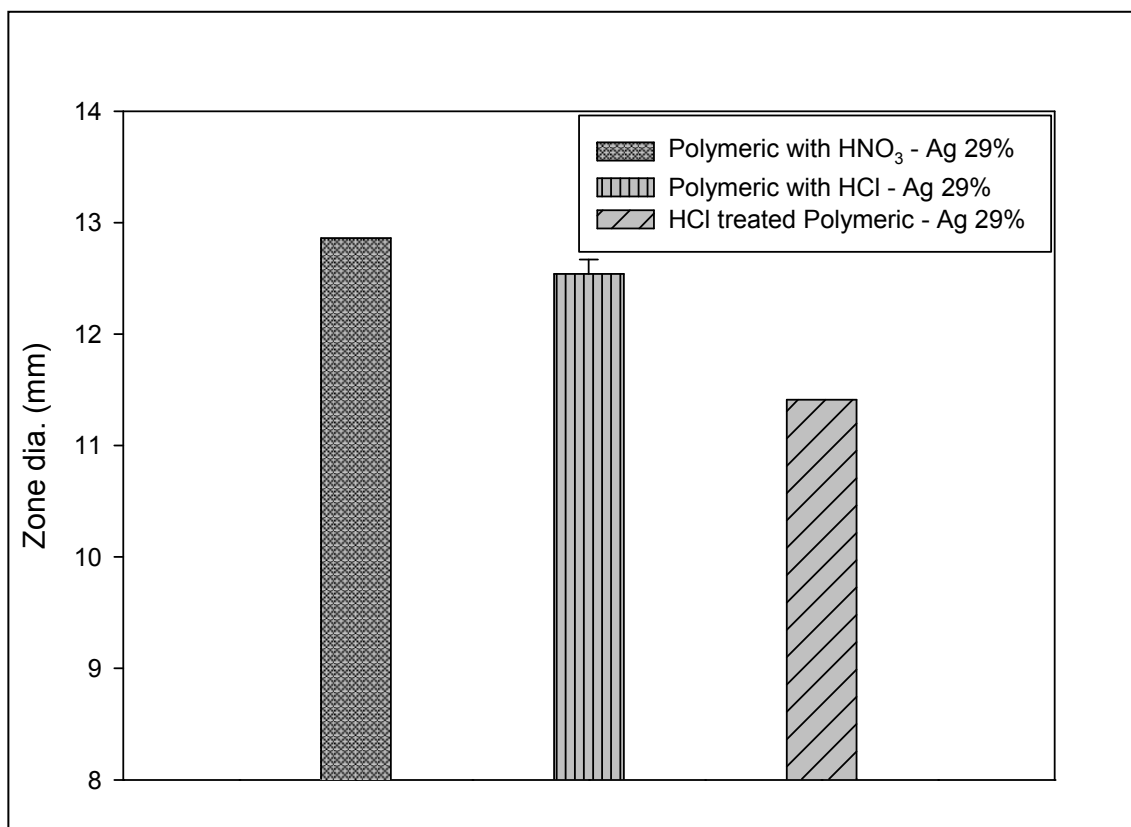


Figure 4.5. Effect of preparation method on antibacterial activities of polymeric TiO₂ – Ag 29% powders

The effect of silver loading on the antibacterial activity was also investigated. Antibacterial activity of polymeric TiO₂ powders were compared at three silver loadings; 1.2%, 12% and 29% (by weight). The results are given in Table A2 in the Appendix A. Figure 4.6 illustrates silver loading effect on the antibacterial performance of the polymeric TiO₂ powders. Pure TiO₂ powder without silver did not possess any antibacterial activity. In addition, HCl treated pure TiO₂ powder without silver did not show any activity. It was observed that as the silver concentration increased, the antibacterial activities of samples increased almost linearly. Keleher et al. studied silver - coated TiO₂ particles for antibacterial applications and found that Ag-TiO₂ particles possessed antibacterial activity but not as much as the common antibacterial agents (Keleher et al. 2002). They also determined that as the silver concentration increased antibacterial zone diameter increased; hence they claimed that the Ag⁺ induced antibacterial activity rate was directly proportional to Ag⁺ concentration, typically acting on multiple targets. Similarly, Schierholz et al. reported that as the silver ion concentration increased, antimicrobial efficacy increased (Schierholz, 1999). In fact, the results obtained in this thesis confirmed the findings of Keleher et al. and Schierholz et al.

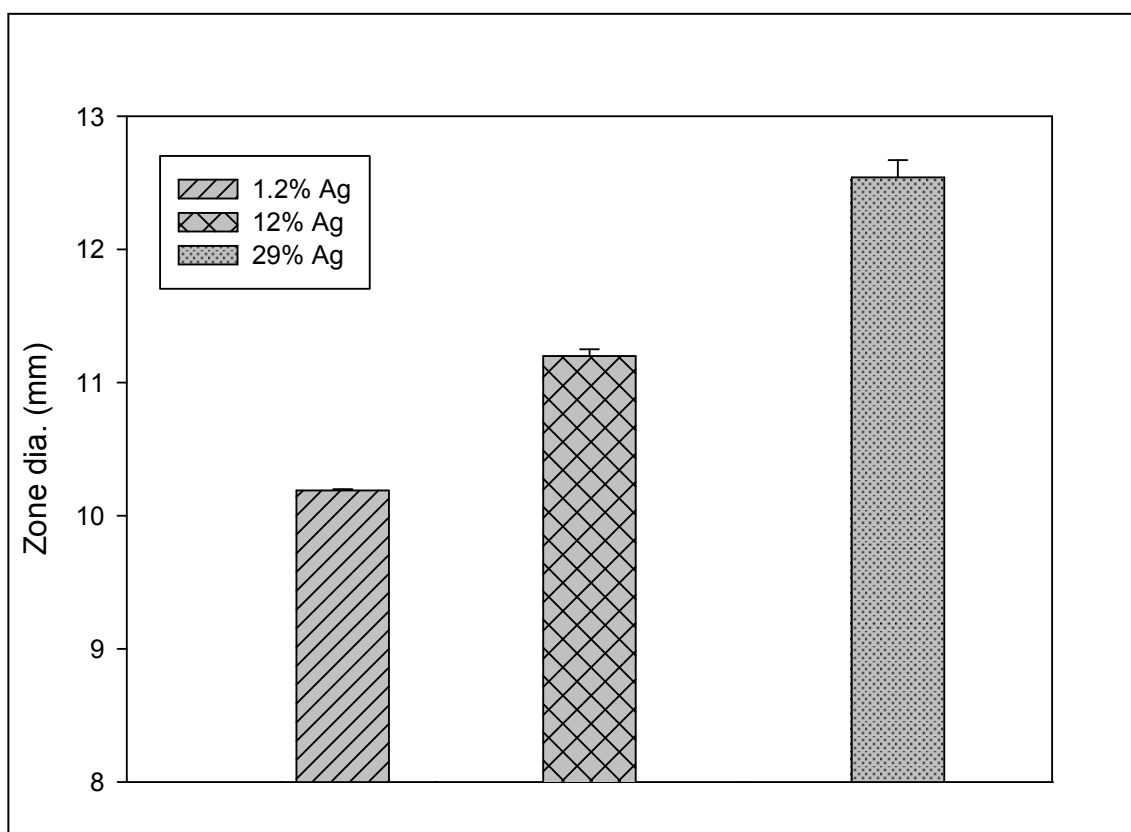


Figure 4.6. Effect of silver loading on the antibacterial performance of the polymeric TiO_2 powders

In order to examine the effect of the counter - ion concentration in the medium on the antibacterial activities of the oxide supported silver containing materials, a different medium was used. Mueller – Hinton broth was mixed with nutrient agar as described in Chapter III. These sets of experiments were not following a standard disk diffusion procedure, since agar – broth media contained more nutrients than that of the broth media used in the standard method. Nevertheless, the aim was to test the effect of the counter ions present in the media so that this nutrient rich medium where counter ion concentration was high was used. The results of the experiments showed the effect of content of the medium. In fact, the inhibitory effect of counter ion concentration (i.e. concentration of ions with the ability of complex formation with silver) on the antibacterial capability of the samples was observed. First, TiO_2 supported samples were tested on Mueller Hinton – nutrient agar media. The pellets were about 0.15 g in weight and 0.8 cm in diameter. In Table 4.2, the results are presented. In these tests, the same samples were tested 9 times; i.e. the same samples were used with the fresh medium in each test for a period of 9 days.

Table 4.2. Antibacterial tests results of the 9 tests applied to the TiO₂ samples using Mueller-Hinton / nutrient agar media

TiO ₂ Samples 29% Ag	Zone dia. (mm)			
	day 1	day 2	day 3	day 4
Colloidal with HCl	12.26± 0.10	10.77± 0.12	10.47± 0.03	10.04± 0.045
Polymeric with HCl	12.24± 0.16	10.48± 0.08	10.33± 0.09	10.22± 0.12
Polymeric with HNO ₃	11.85± 0.21	10.75± 0.18	10.56± 0.13	10.09± 0.07
Colloidal with HNO ₃	11.32± 0.10	10.74± 0.11	10.61± 0.11	9.86± 0.18
HCl treated Poly.	9.66± 0.11	9.54± 0.05	9.43± 0.12	9.66± 0.06

Table 4.2. (cont.)

	Zone dia. (mm)			
	day 6	day 7	day 8	day 9
Colloidal with HCl	10.03 ± 0.04	9.39 ± 0.04	9.34 ± 0.09	9.25 ± 0.02
Polymeric with HCl	10.08 ± 0.08	9.61 ± 0.22	9.56 ± 0.10	9.49 ± 0.01
Polymeric with HNO ₃	9.91 ± 0.04	9.76 ± 0.10	9.72 ± 0.02	9.75 ± 0.03
Colloidal with HNO ₃	9.76 ± 0.05	9.60 ± 0.05	9.82 ± 0.02	9.71 ± 0.03
HCl treated Poly.	9.58 ± 0.02	9.46 ± 0.06	9.40 ± 0.07	9.50 ± 0.11

In Figure 4.7, antibacterial activity test results for polymeric TiO₂ were plotted. In the first day tests, it was found that HCl treated samples showed the lowest antibacterial activity, while polymeric samples that contain metallic silver showed the highest activities. It means that HCl treated samples had the least antibacterial capability in Mueller Hinton – nutrient agar media. Large zone diameters were obtained with metallic silver containing samples in the first days of the 9 day tests. However, day-after-day these zone diameters decreased until they were close to the zone diameters of HCl treated samples (AgCl containing samples). AgCl containing samples possess less antibacterial activity but at a constant level for the 9 tests (i.e. 9 days). After the 6th test, activities of the Ag containing samples became constant and close to that of HCl treated ones. The low activity of the AgCl containing samples could be explained by the counter ion effect resulted in by the nutrient rich broth media, but the fast decrease of the antibacterial activity of the samples containing metallic silver could be explained by the formation AgCl from Ag metals due to counter ion present in the nutrient rich medium, as shown in the reaction below:



XRD analyses of the samples used in the experiments performed with Mueller Hinton – nutrient agar media showed that metallic silver crystals transformed to AgCl crystals during the antibacterial tests which meant there was a counter ion transfer between the samples and the medium. The formation of AgCl on Ag containing samples explains the decrease of their activity and ultimately, being almost equal to the activity of the samples prepared with HCl. These results indicate that the medium of the antibacterial test is essentially important for the accuracy of the experiments and the evaluation of the samples. The media must not contain compounds that have inhibitory effect on the performance of the samples.

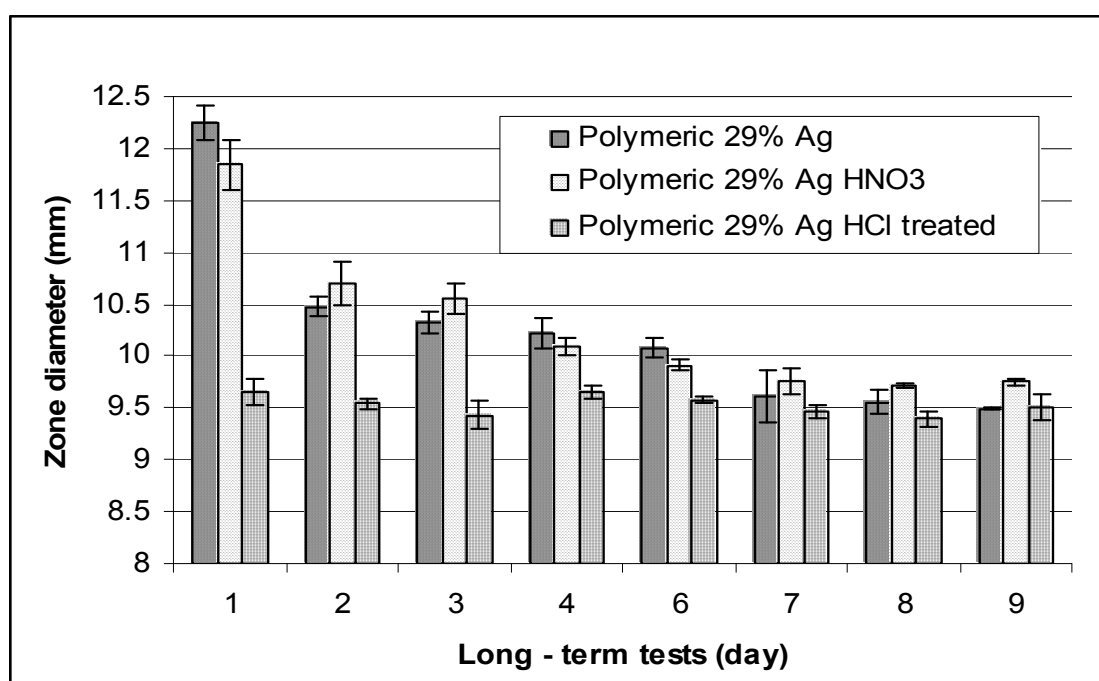


Figure 4.7. Antibacterial activities of polymeric gel powders on nutrient rich media

In Figure 4.8, XRD patterns of polymeric TiO₂ samples (29% Ag) before and after antibacterial tests on Mueller – Hinton / nutrient agar (nutrient rich media) are shown. The difference in the crystal structure of the same sample before and after being used in disk diffusion antibacterial tests was seen clearly. The formation of AgCl nano-crystals and the decrease of the intensity of silver peaks were observed. This pattern indicates the effect of the broth – agar media on the crystal structure of the samples. Fresh samples initially had no AgCl nano-crystals. However, after the antibacterial tests, it was determined that AgCl thickness of samples used in the highly nutrient rich

broth became 24.54 nm. According to this, it is seen that there seems to be ion transfer between the media and the sample. Kawahara et al. reported that there was no detectable Ag^+ release from silver – zeolites in water although Ag^+ release was determined when broth was used. It was explained that Ag^+ was released from silver containing zeolites by ion exchange with other cations and the amount of Ag^+ was dependent on the concentration of cations in the solution (Kawahara et al., 2000). Thus, it could be concluded that materials which release silver ions show long-term antibacterial protection in aqueous media with very low ion concentration.

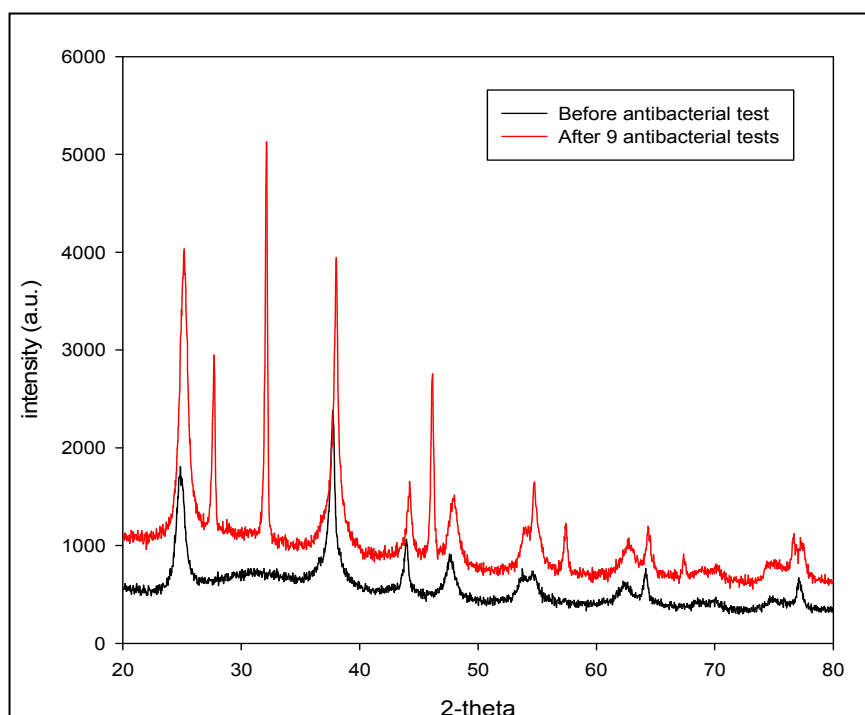


Figure 4.8. Comparison of polymeric TiO_2 samples (29% Ag) with HNO_3 before and after antibacterial tests on Mueller – Hinton / nutrient agar

In Figure 4.9, it is seen that the tests performed by using agar – agar instead of nutrient agar also affected the crystal structure of the Ag crystals in the sample. Again the formation of AgCl nano-crystals can be seen clearly; however, the crystal size of AgCl were determined to be 14.75 nm which is less than that found in the samples tested with Mueller – Hinton broth / nutrient agar. Thus, it could be concluded that, the ionic concentration of the antibacterial test media affects the antibacterial performance of the samples used in the disk diffusion antibacterial test procedure.

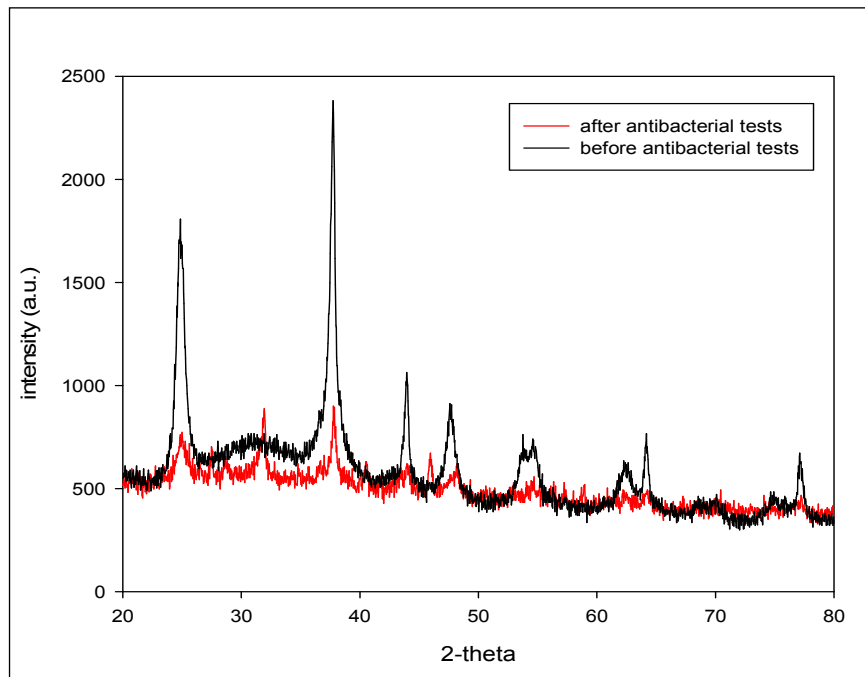


Figure 4.9. Comparison of polymeric TiO₂ samples (29% Ag) with HNO₃ before and after antibacterial tests on Mueller – Hinton / agar agar

In Figure 4.10, the same observations are found for polymeric TiO₂ Ag 29% powders. In this sample, AgCl crystals were formed after antibacterial tests. The crystal size of AgCl increased from 11.62 nm to 25.81 nm after being used in antibacterial test.

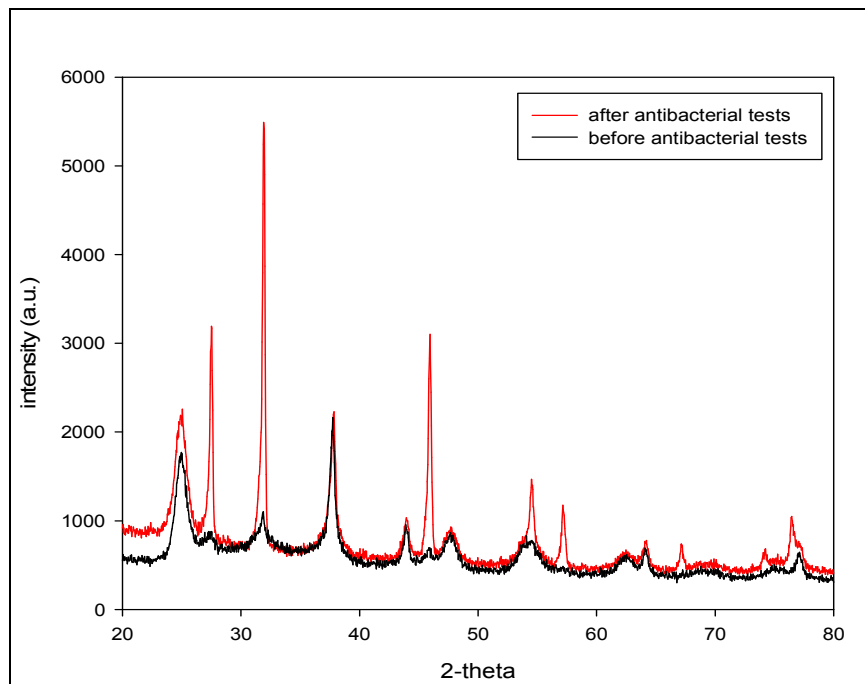


Figure 4.10. Comparison of polymeric Ag 29% with HCl samples before and after antibacterial tests on Mueller – Hinton broth / nutrient agar

Figure 4.11 shows antibacterial test results of polymeric and colloidal samples synthesized by using HCl or HNO₃ as hydrolysis catalyst is seen. In the first tests, samples made with HCl showed higher antibacterial activity than that prepared with HNO₃. However, after 7th test, it is seen that samples synthesized with HNO₃ became more active.

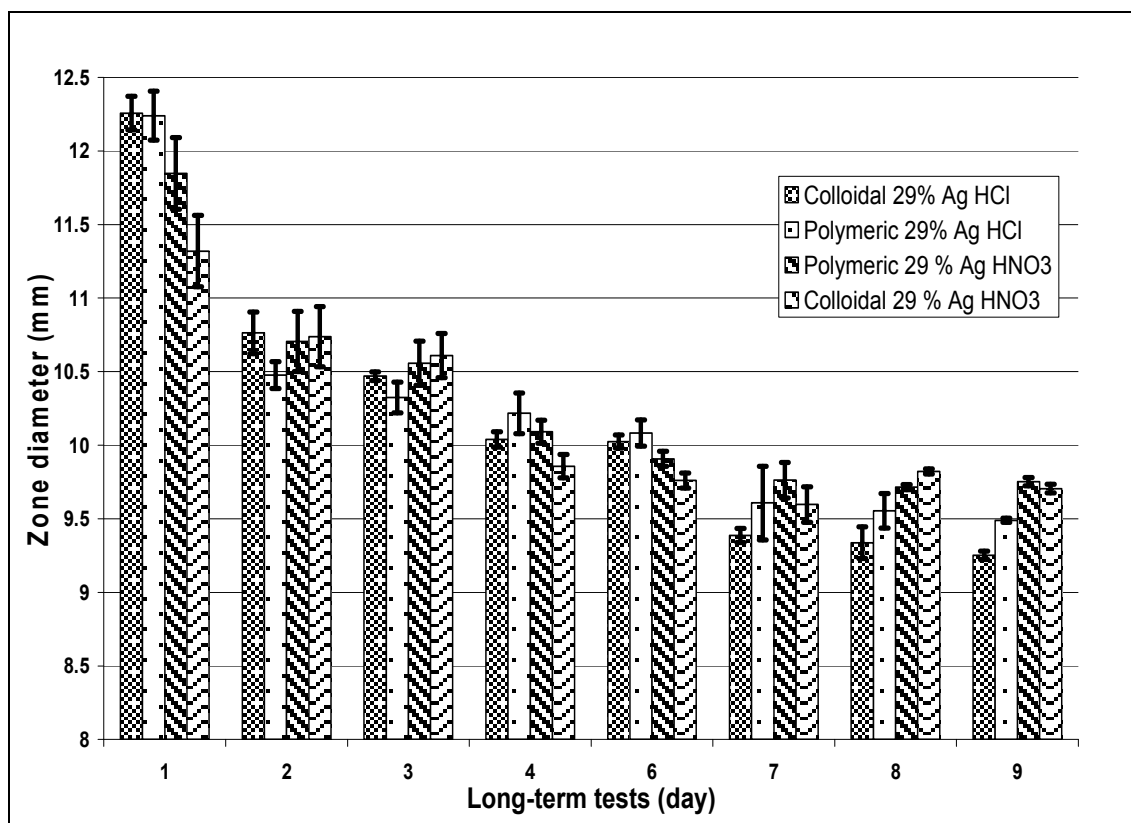


Figure 4.11. Antibacterial activities of polymeric and colloidal samples on nutrient rich media

In order to determine the repeatability of the antibacterial tests using Mueller-Hinton/nutrient agar media, 3 of the samples were examined for 9 days. Polymeric samples synthesized with HCl or HNO₃ and HCl treated samples were compared. As the results given in Table 4.2 and Table 4.3 are compared, it is seen that the samples show the same trend in antibacterial activity. HCl treated samples showed constant but the lowest antibacterial activity. Metallic silver containing samples were more active in the first day test, but their activities decreased during the 9 tests. In fact, on 9th day test, their activities were close to that of the HCl treated ones.

Table 4.3. Antibacterial tests results of the 9 tests applied to the TiO₂ samples using Mueller-Hinton / nutrient agar media for the repeatability.

Average Zone Diameters (mm)									
Samples – 29% Ag	test 1	test 2	test 3	test 4	test 5	test 6	test 7	test 8	test 9
HCl treated Polymeric	9.71 ± 0.06	9.55 ± 0.06	9.52 ± 0.04	9.43± 0.02	9.45± 0.02	9.34 ± 0.02	9.35 ± 0.03	9.38 ± 0.01	9.34 ± 0.01
Polymeric with HNO ₃	12.14± 0.07	10.84± 0.10		10.36± 0.02	10.22± 0.02	9.90± 0.02	9.90± 0.05	10.03± 0.06	9.85± 0.04
Polymeric with HCl	11.63± 0.14	10.55± 0.06		10.01± 0.02	9.77± 0.03	9.63± 0.03	9.46± 0.04	9.41± 0.01	9.36± 0.02

The results obtained in the antibacterial tests performed using two different media showed that AgCl containing materials gave large zones when standard Mueller Hinton broth/agar media was used. However, the results obtained with a mixture of Mueller Hinton broth/nutrient agar media (containing the high concentration of nutrient) are different than that obtained with the standard broth. It is seen that the ion concentration in the media directly affected the structure of the antibacterial material, hence affecting the silver ion release rate. These results mean that the antibacterial activity of a material is dependent on the type of media if there is a direct interaction between the media and the test material. If silver containing antibacterial material is used in an environment in which Cl⁻ ions are present, the antibacterial performance of the material is affected. The inhibition zone around the material could be low but this decrease means that the material releases silver ions slowly and in a more controlled way. The slow release of silver ions from AgCl containing antibacterial materials in Cl⁻ environment could be used to provide a long – lasting antibacterial protection. In fact, Cl⁻ ions are present in many places where antibacterial materials could easily find use. For example, the surfaces in contact with human skin might contain Cl⁻ ions that come from human sweat. Even human blood and body contains some amount of Cl⁻ ions.

Luria – Bertani (LB) media was also used to show the effect of media on the antibacterial activity of materials. For this, TiO₂ samples were tested simultaneously on Mueller Hinton / nutrient agar and Luria – Bertani media. The results are presented in Table 4.4.

Table 4.4. Zone diameters of TiO₂ samples on different media

	MH / Nutrient agar	LB media
Samples - 29% Ag	test 1	test 1
Polymeric with HCl	10.56 ± 0.02	9.25 ± 0.07
Collodial with HCl	10.66 ± 0.05	9.89 ± 0.04
Polymeric with HNO ₃	12.35 ± 0.05	12.51 ± 0.10
Collodial with HNO ₃	11.99 ± 0.14	12.00 ± 0.01

It is seen that LB media has also adverse effect on AgCl containing samples like tests done on the broth containing high nutrient concentration. The reason for this inhibition is due to high Cl⁻ ion concentration in LB media. In other words, the reverse reaction given in equation 4.2 is favored due to high Cl⁻ ion concentration (Le Chatelier's principle).

4.1.2. Antibacterial Activity Tests of Zinc Oxide - Silica Supported Ag Samples

The antibacterial activities of ZnO – SiO₂ powders are shown in Figure 4.13 (data is given in Tables A3 and A4 in Appendix A). In the tests, it is seen that pure ZnO is biologically active material, while pure SiO₂ is not. HCl treated SiO₂ also did not show any antibacterial property. 30 % ZnO containing SiO₂ powders possess higher antibacterial capability than 50% and 70% ZnO containing powders. In the study of Yamamoto et al., it was found that as ZnO concentration increased, antibacterial activity of the material increased (Yamamoto et al. 2004). As ZnO is found to be antibacterial, it was thought that increasing ZnO concentration would increase the antibacterial activity. However, an opposite result was observed in this study that 30 % ZnO-SiO₂ was the most active sample. This unexpected result might be caused by occlusion of some ZnO in the materials; hence resulting in inaccessible ZnO crystals. During the synthesis of the materials at high zinc oxide concentrations, zinc oxide particles may be trapped in the silica matrices. In the XRD pattern of zinc oxide – silica samples, at 70% ZnO – SiO₂, ZnO nano-crystals can be seen clearly. However, zinc oxide crystals could not be seen at 30% ZnO, since it is known that XRD technique is sensitive to crystalline size larger than 5 nm. In addition, no Ag peak was detected at silver loaded samples in XRD patterns (given in Figure A7 and Figure A8 in Appendix A) due to the same reason.

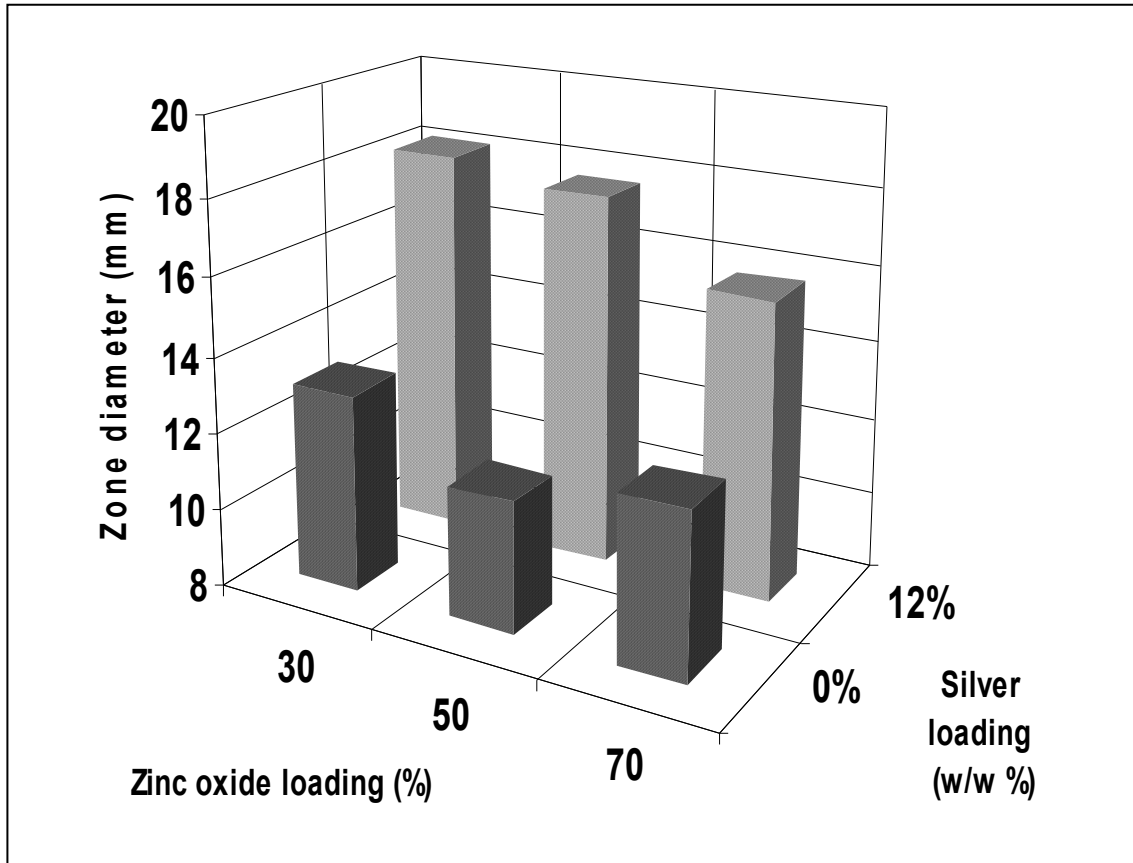


Figure 4.12. Antibacterial performance of Zn – SiO₂ oxide powders on MH / agar agar

At silver loading 12%, 30% ZnO containing samples still possessed higher antibacterial activity than the other ZnO loadings. However, 50% ZnO-SiO₂ possessed higher activity than 70% ZnO-SiO₂. It was observed that as ZnO content was increased, the activity decreased at silver loading 12%. Catauro et al. prepared silver free and Ag nano-particles containing Na₂O.CaO.2SiO₂ glasses by using a sol-gel route (Catauro et al. 2004). It was reported that the addition of silver oxide provided high antibacterial activity to the glass materials. Similarly, the antibacterial activity of silver added antibacterial ZnO-SiO₂ powders was found to increase considerably in this study. When the activities of samples are compared at the same ZnO concentration, it is seen that Ag addition increased the activity.

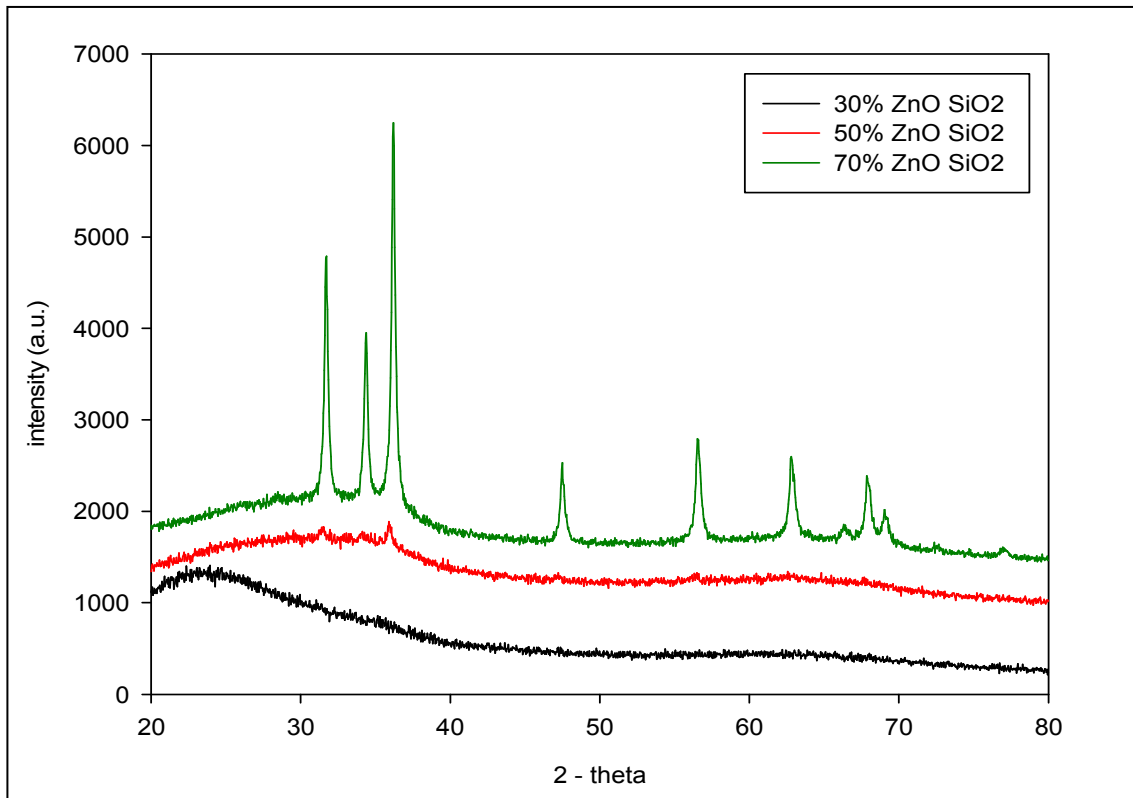


Figure 4.13. XRD pattern of ZnO – SiO₂ powders at different ZnO concentrations

Antibacterial activity tests on ZnO- SiO₂ powders were also performed using Mueller – Hinton / nutrient agar media. Results are given in Figure 4.14. The weights of ZnO-SiO₂ pellets were 0.15 g and 0.8 cm in diameter. Antibacterial activity results of ZnO – SiO₂ powders without silver are showed in Table 4.5.

Table 4.5. Antibacterial activities of ZnO – SiO₂ powders

Samples	Zone dia. (mm)
30% ZnO-SiO ₂	11.39 ± 0.01
50% ZnO-SiO ₂	10.83 ± 0.01
70% ZnO-SiO ₂	10.52 ± 0.01

It is seen that as the zinc oxide amount increased, antibacterial activities of the powders decreased on nutrient rich media. The results obtained with ZnO – SiO₂ powders containing a silver concentration are summarized in Table 4.6.

Silver loading 1.2% was chosen because at the high silver loadings (12% and 29%), no clear zone diameters could be measured because non-uniform zone formed around the pellets of ZnO – SiO₂ at silver loadings of 12% and 29% and also the pellets were disintegrated. This might seem to be due to the fast release of silver from the

samples or due to the excessive absorption of water and/or broth after the 1st test; but real cause needs to be investigated and at this time, it is not known.

Table 4.6. Antibacterial activities of ZnO – SiO₂ – Ag 1.2% powders on MH / nutrient agar

Samples	Zone dia. (mm)
30% ZnO-SiO ₂ – Ag 1.2%	13.08 ± 0.04
50% ZnO-SiO ₂ – Ag 1.2%	12.93 ± 0.03
70% ZnO-SiO ₂ – Ag 1.2%	12.63 ± 0.07

In addition, the sequential tests on the samples with high Ag loadings could not be performed since the pellets could not be reused because the pellets were cracked and disintegrated.

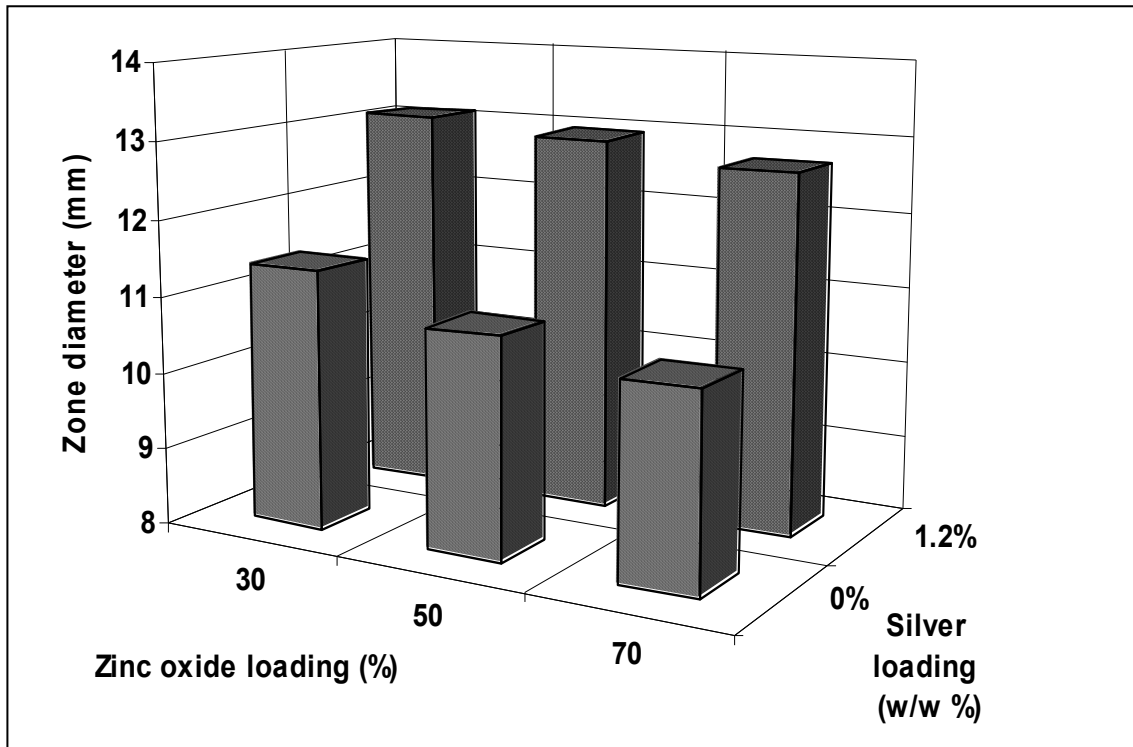


Figure 4.14 . Antibacterial performance of Zn – SiO₂ oxide powders on MH / nutrient agar

4.1.3. Comparison of Antibacterial Activity of Zinc Oxide-Silica Supported Samples and Titania Samples

In order to compare the effect of the kind of the oxide support, the most active two samples, 30% ZnO – SiO₂ and SGP TiO₂, were chosen for the antibacterial tests at constant silver loadings. In Table 4.7, antibacterial capabilities of ZnO-SiO₂ and TiO₂ powders at silver loading of 1.2% on standard media are given. Three duplicate tests were performed at the same day. As comparing the results obtained from 3 tests, it is seen that several data are different. However, the trend between the samples was same. The results showed that 30% ZnO – SiO₂ powders are more active than titania samples. Furthermore, it is observed that HCl treatment increased the activity of both titania and 30% ZnO – SiO₂ powders.

Table 4.7. Antibacterial capabilities of ZnO-SiO₂ and TiO₂ powders at silver loading of 1.2% (duplicate experiments) on MH / agar agar

Samples	Zone dia. (mm)		
	Test 1	Test 2	Test 3
Polymeric TiO ₂ - 1.2% Ag with HCl	9.43 ± 0.11	10.20 ± 0.08	10.23 ± 0.05
HCl treated polymeric TiO ₂ - 1.2% Ag	11.46 ± 0.05	11.50 ± 0.07	11.47 ± 0.11
30% ZnO – SiO ₂ -1.2% Ag	12.43 ± 0.01	13.09 ± 0.07	12.82 ± 0.07
HCl treated 30% ZnO – SiO ₂ -1.2% Ag	20.7 ± 0.09	20.74 ± 0.13	20.43 ± 0.03

In order to compare the effect of the kind of the oxide support, the most active two samples, 30% ZnO – SiO₂ and SGP TiO₂, were chosen for the antibacterial tests at a constant silver loading. In Table 4.7, the antibacterial capabilities of ZnO-SiO₂ and TiO₂ powders at silver loading of 1.2% in standard media are given. Three duplicate tests were performed on the same day. The results obtained from 3 tests show that the magnitude of the antibacterial activity (i.e. zone diameter) fluctuate among the tests for each sample but the activity trend among the samples for each test is the same. The results showed that 30% ZnO – SiO₂ powders are more active than titania samples. Furthermore, it is observed that HCl treatment increased the activity of both titania and 30% ZnO – SiO₂ powders.

Table 4.7. Antibacterial capabilities of ZnO-SiO₂ and TiO₂ powders at silver loading of 1.2%(duplicate experiments) on MH / agar agar

Samples	Zone dia. (mm)		
	Test 1	Test 2	Test 3
Polymeric TiO ₂ - 1.2% Ag with HCl	9.43 ± 0.11	10.20 ± 0.08	10.23 ± 0.05
HCl treated polymeric TiO ₂ - 1.2% Ag	11.46 ± 0.05	11.50 ± 0.07	11.47 ± 0.11
30% ZnO– SiO ₂ -1.2% Ag	12.43 ± 0.01	13.09 ± 0.07	12.82 ± 0.07
HCl treated 30% ZnO – SiO ₂ -1.2% Ag	20.7 ± 0.09	20.74 ± 0.13	20.43 ± 0.03

In Table 4.8, antibacterial capabilities of ZnO-SiO₂ and TiO₂ powders at silver loading of 12% on MH / agar-agar are shown. The same samples were tested 3 times on fresh media each time to examine their activities for the repeated usage. The same trend was again determined as seen in Figure 4.15 that 30% ZnO – SiO₂ powders are more active than titania samples. However, antibacterial activity of 30% ZnO – SiO₂ powders decreased sharply in the 2nd and 3rd tests. Decrease in the activities of titania samples was relatively small. At silver loading of 12%, the same results were also observed for the HCl treatment of the samples. It could be explained by the high release rate of Ag ions and/or Ag complexes from AgCl nano-crystals.

Table 4.8. Antibacterial capabilities of ZnO-SiO₂ and TiO₂ powders at silver loading of 12% on MH / agar agar

Samples	Zone dia. (mm)		
	Day 1	Day 2	Day 3
Polymeric TiO ₂ - 12% Ag with HCl	11.47 ± 0.15	10.70 ± 0.13	9.99 ± 0.11
HCl treated polymeric TiO ₂ - 12% Ag	12.02 ± 0.05	10.94 ± 0.15	10.22 ± 0.04
30% ZnO– SiO ₂ -12% Ag	14.34 ± 0.12	11.66 ± 0.04	11.03 ± 0.07
HCl treated 30% ZnO – SiO ₂ -12% Ag	16.38 ± 0.10	12.20 ± 0.07	10.93 ± 0.01

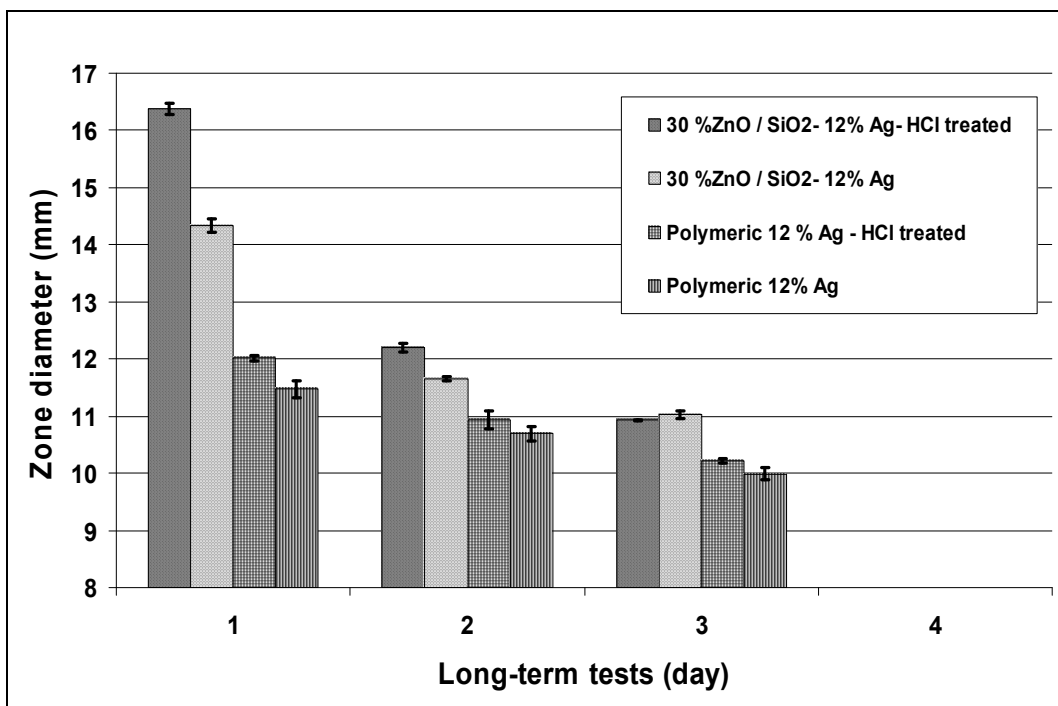


Figure 4.15. Antibacterial activity test results of a 3 days period for ZnO-SiO₂ and TiO₂ powders at silver loading of 12%.

XRD pattern of HCl treated 30% ZnO– SiO₂ with 12% Ag is showed in Figure 4.16. As seen in the figure, only AgCl peaks are present. Before the HCl treatment, there was no detectable Ag peaks, yet after HCl treatment AgCl peaks were detected.

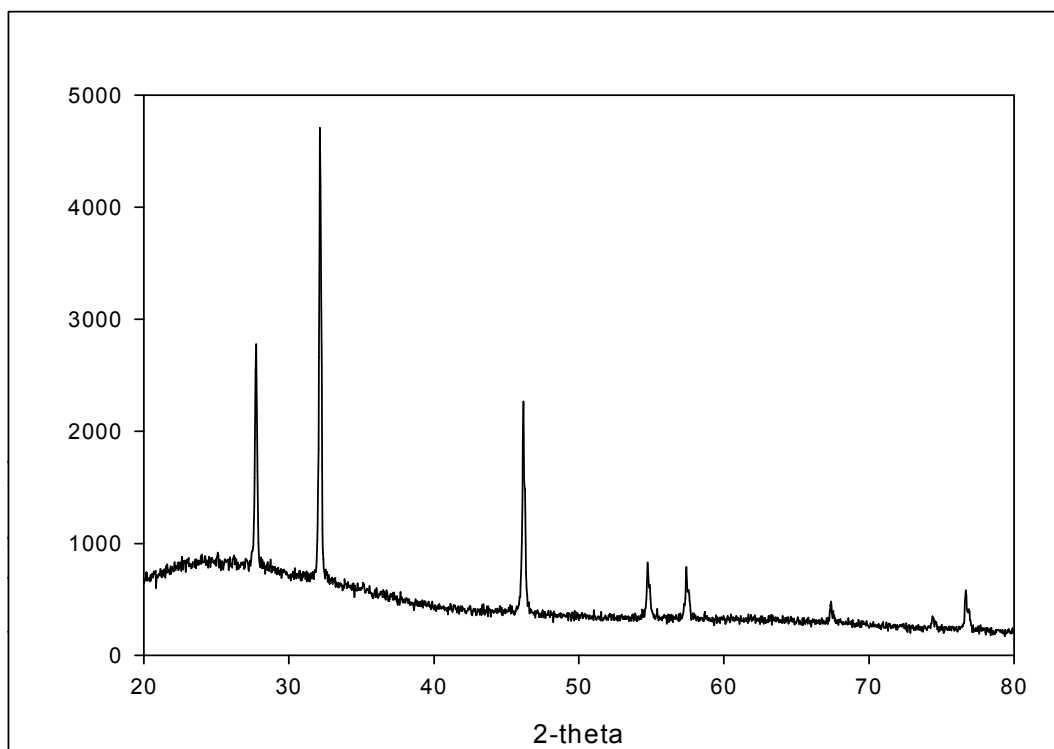


Figure 4.16. XRD pattern of HCl treated 30% ZnO– SiO₂ -12% Ag

If the results shown in Table 4.7 and 4.8 are compared, it is apparent that there is a decrease in antibacterial zone diameters although silver content increases. As stated before, the samples in each petri dish should be compared and examined to make a sound comparison.

Since zeolite based materials have been used as a support material in the literature, antibacterial activity of a zeolite sample was also examined here. Zeolites were formed into pellets in a press using a dye with 0.8 cm diameter and 0.15 g. The results of the antibacterial activity tests performed on the nutrient rich broth are seen in Table 4.9.

Table 4.9. Antibacterial activity of silver containing zeolites

Zeolites	1st day
1	9.75 + 0.06
2	9.76 + 0.04
3	9.72 + 0.02
4	9.75 + 0.03

One should know that the Ag loadings in the zeolites and in the oxide samples were different. In fact, loadings in oxide samples were considerably higher than that in zeolites. However, as seen in Table 4.9 and 4.6, ZnO – SiO₂ are more active than zeolites even at a low Ag loading. The difference between the antibacterial activities of these materials can be explained by the difference on the preparation of the materials. Ag containing zeolites were prepared by ion-exchange and Ag was loaded to ZnO-SiO₂ by the impregnation method. Thus, the strength of Ag attachment to the surface of the supports is different; hence affecting the release of the silver.

4.2. Textural Property Analyses

BET analysis was used to calculate the porosity and the specific surface area of the oxide samples. The measurements were performed at 77.35 K in the presence of N₂ for 4 samples; 30% ZnO-SiO₂, 50% ZnO-SiO₂, 70% ZnO-SiO₂, and TiO₂ which did not contain silver. Pore volumes and surface areas of these 4 samples are summarized in Table 4.10.

Table 4.10. Pore volume and surface areas of oxide materials.

	BET Surface Area (m ² /g)	Average pore diameter (Å)	Pore volume (cm ³ /g)
Polymeric TiO ₂	97.4	52.7	0.1285
30% ZnO-SiO ₂	362.9	32.0	0.291
50% ZnO-SiO ₂	141.5	49.5	0.1753
70% ZnO-SiO ₂	82.6	88.7	0.1833

It is seen that SiO₂ powders having 30% zinc oxide possess the highest surface area. In addition, as ZnO content increased, the surface area decreased. If we compare the surface areas of the oxide samples, the surface area of TiO₂ powders are found to be higher than that of 70% ZnO containing SiO₂ samples, but lower than that of 50% ZnO containing SiO₂ samples. Surface areas of the samples play an important role in antibacterial activity since increasing surface area enables the accessibility of inner parts of the sample; hence increasing release rate of silver from the samples. In fact, comparing SiO₂ supported samples studied in this thesis, it was found that 30% ZnO – SiO₂ showed better activity.

4.3. SEM Results

SEM-EDX analyses of the several samples reveal information on the spatial distribution of the elements, such as Ag, Ti, Zn, Si and O, in TiO₂ and ZnO – SiO₂ powders.

In Figure 4.17 and 4.18, SEM image of colloidal TiO₂ – 12% Ag powder is seen. There are two points marked as 1 and 2. Surface atomic concentrations at these two points were determined with SEM-EDX and presented in Table 4.11. It is seen that the particle at point 1 is a silver complex containing Ti, O and Cl atoms. However, silver concentration is higher at point 1 than at point 2. During the preparation, the surface silver enrichment seems to have occurred at point 1. On the other hand, point 2 is mostly TiO₂ rich.

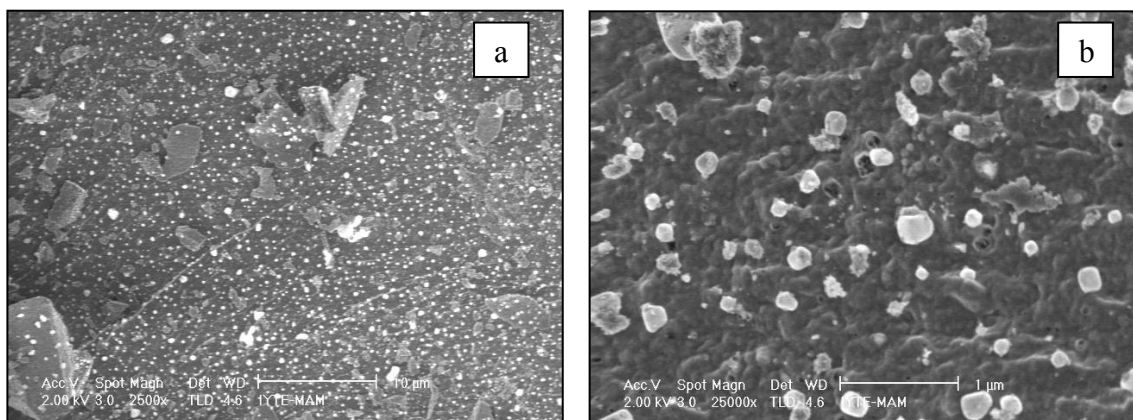


Figure 4.17. SEM images of colloidal TiO_2 – 12% Ag powders (a) At magnitude of 2500 (b) At magnitude of 25000.

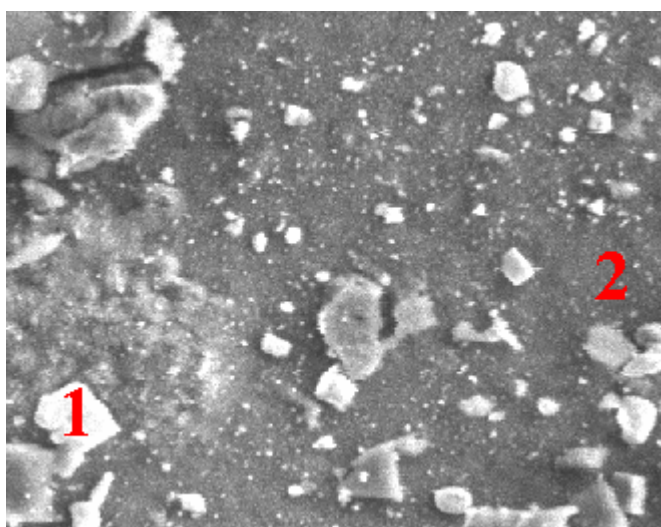


Figure 4.18. SEM-EDX image of colloidal TiO_2 – 12% Ag.

Table 4.11. Surface atomic concentration of colloidal TiO_2 – 12% Ag. (@ 2 points and on overall)

Element	Surface atomic concentration (mol %)		
	Point 1	Point 2	Overall
Ti	10.87	17.94	7.40
Ag	52.55	3.42	0.78
O	32.52	75.03	89.74
Cl	4.05	3.61	2.07

The effects of the preparation method for silver doped colloidal and polymeric samples on surface morphology were investigated by comparing SEM images of colloidal and polymeric TiO_2 powders. It is seen that the surface structure of colloidal samples was rough compared to the polymeric samples. In Figure 4.19, it is seen that polymeric sample had smooth surface structure.

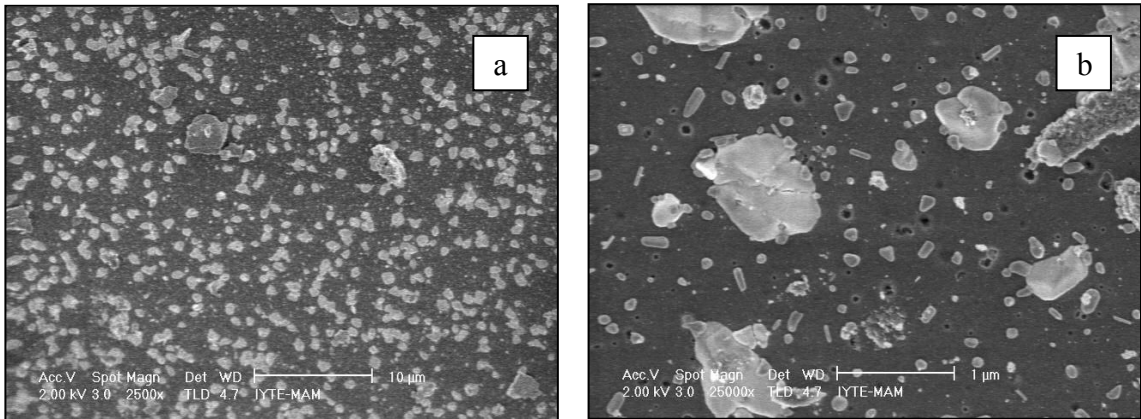


Figure 4.19. SEM images of polymeric $\text{TiO}_2 - 12\% \text{Ag}$ powders (a) At magnitude of 2500 (b) At magnitude of 25000.

In Figure 4.20, SEM image of polymeric $\text{TiO}_2 - 12\% \text{Ag}$ is seen and EDX results of surface atomic concentration at point 1 and 2 are given in Table 4.12. It is seen that both points had similar compositions, yet at point 2, silver concentration was higher. The overall surface concentrations of polymeric and colloidal 12% Ag samples were exactly the same. It is also seen that Cl^- atoms could not completely be removed during the calcination step.

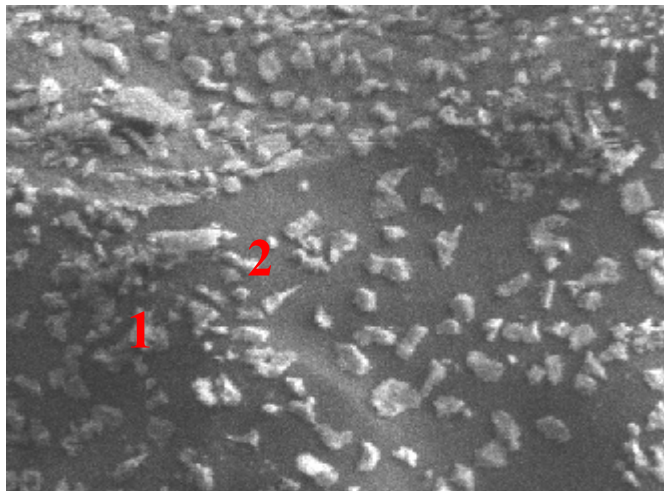


Figure 4.20. SEM-EDX image of polymeric $\text{TiO}_2 - 12\% \text{Ag}$

Table 4.12. Surface atomic concentration of polymeric TiO₂ – 12% Ag (@ 2 points and on overall)

Element	Surface atomic concentration (mol %)		
	Point 1	Point 2	Overall
Ti	8.75	8.37	8.77
Ag	0.88	2.66	1.08
O	89.30	87.94	87.70
Cl	1.07	1.03	2.46

In Figure 4.21, SEM image of 30%ZnO – SiO₂ – 1.2% Ag sample is seen. In the figure, surface concentration was determined at point 1 by EDX. Surface atomic concentration at point 1 and also overall surface concentration are given in Table 4.13. It is seen that the particle at point 1 is a zinc rich particle

Table 4.13. Surface atomic concentration of 30% ZnO – SiO₂ – 1.2% Ag

Element	Surface atomic concentration (mol %)	
	Point 1	Overall
Si	30.15	36.15
Ag	1.83	1.22
Zn	21.44	9.54
O	46.03	52.82
Cl	0.55	0.27

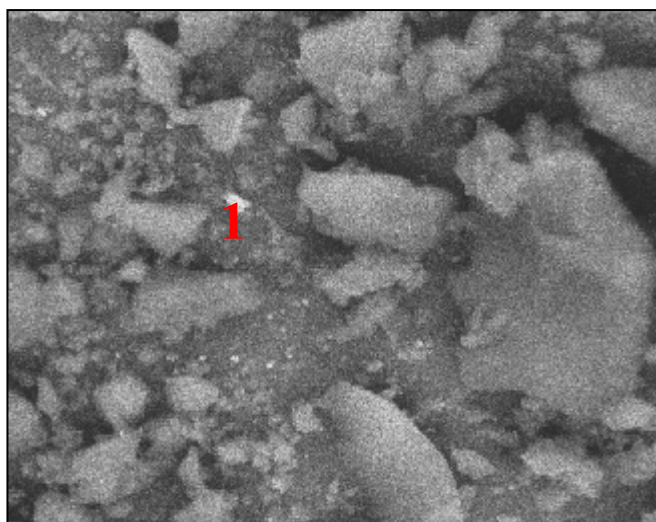


Figure 4.21. SEM images of 30%ZnO – SiO₂ – 1.2%Ag

In Figure 4.22, SEM image of HCl treated 30%ZnO – SiO₂ – 12% sample is given. It is seen that the sample does not have a uniform particle size distribution. It may be a result of impregnation. In Table 4.14, the surface atomic concentration of HCl

treated 30%ZnO – SiO₂ – 12% Ag is given. It is seen that Ag loading is higher than the bulk loading; hence indicating that during Ag impregnation, Ag particles were agglomerated at the surface.

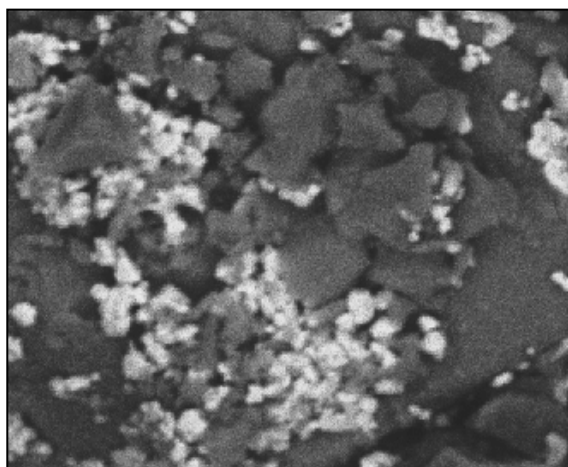


Figure 4.22. SEM image at magnitude of 2500 of HCl treated 30%ZnO – SiO₂ 12% Ag

Table 4.14. Surface atomic concentration of HCl treated 30% ZnO – SiO₂ –12% Ag (on overall)

	Surface atomic concentration (mol %)
Element	Overall
Si	22.87
Ag	6.29
Zn	9.64
O	47.79
Cl	13.41

After the antibacterial activity, SEM – EDX analysis was performed to the antibacterial tested samples. In Table 4.15, the surface concentration of 12% Ag-30%ZnO – SiO₂ is given.

Table 4.15. Surface atomic concentration of 30% ZnO – SiO₂ –12% Ag (after antibacterial tests)

	surface concentration (mol %)
O	57.44
Si	25.55
Cl	1.37
Ag	1.46
Zn	14.18

There are Cl⁻ ions present on the surface of 30% ZnO – SiO₂ – 12% Ag powders. As the amount of Ag and Cl⁻ are compared, they are closed to each other. It means Ag can be in AgCl form on the surface which confirms the results of XRD.

In Table 4.16 and 4.17, the surface atomic concentration of 12% Ag - polymeric TiO₂ and HCl treated 12% Ag - polymeric TiO₂ powders are given. It is seen that the amount of Cl is close to amount of Ag for 12% Ag - polymeric TiO₂. As it was seen in the XRD patterns, Cl⁻ ions present in media causes to form AgCl nano-crystals. In addition, oxygen enrichment was observed on the surface of both polymeric TiO₂ powders. One may expect to see that Ti : O molar ratio should be 1:2 but this ratio is about 1:2.5 for both polymeric TiO₂ powders which indicates the surface concentration of O atom is higher than the stoichiometric amount. It seems that this could be the result of chemisorption of atmospheric O₂ at the surface of titania.

Table 4.16. Surface atomic concentration of 12% Ag - polymeric TiO₂ (after antibacterial tests)

	surface concentration (mol %)
O	66.94
Cl	3.82
Ag	4.29
Ti	24.96

Table 4.17. Surface atomic concentration of HCl treated 12% Ag - polymeric TiO₂ (after antibacterial tests)

	surface concentration (mol %)
O	68.67
Cl	3.03
Ag	3.06
Ti	25.25

CHAPTER 5

CONCLUSION

In this study, the preparation and antibacterial activities of silver chloride and / or silver nano-crystals containing titanium oxide and silica - zinc oxide materials were investigated.

It was found that silver concentration directly affected the bacteria inhibition zone. As silver concentration increased, the antibacterial activity increased. In addition, AgCl containing materials showed slightly larger zone of inhibition than Ag containing materials in the tests performed on the standard broth media. However, the tests performed on the nutrient rich broth – media showed that Ag containing materials had a larger zone of inhibition. The difference indicates the dependence of antibacterial activity of materials on the media. Ionic compounds present in the broth affected the antibacterial activity of the materials directly. XRD patterns of used samples in the antibacterial tests confirmed that there was a counter-ion effect on the bacterial activity of the samples. Indeed, XRD results showed that AgCl nano-crystals were formed during the antibacterial tests because of the presence of Cl⁻ ions in the medium.

In the sequential tests of the materials with a high nutrient concentration containing broth media, the activities of Ag containing materials decreased and their activities were close to that of AgCl containing materials after several repeated tests (using the same samples with the fresh broth each time for several days). In addition, two oxide matrixes were compared at a single silver concentration. In this case, it was seen that Ag and/or AgCl containing zinc oxide/silica oxide materials showed larger zone of inhibition than that of titanium dioxide materials due to the combined effect of ZnO and Ag.

The repeated antibacterial test performed in this thesis shows that slow and controlled release of Ag ions and/or AgCl complexes, such as AgCl₂⁻, from AgCl nano-crystals in the oxide materials ensure a long lasting protection against *E.coli* bacteria. As a result, AgCl containing oxide materials synthesized in this study are promising antibacterial materials for long term usages in many applications, such as public phones.

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APPENDIX A

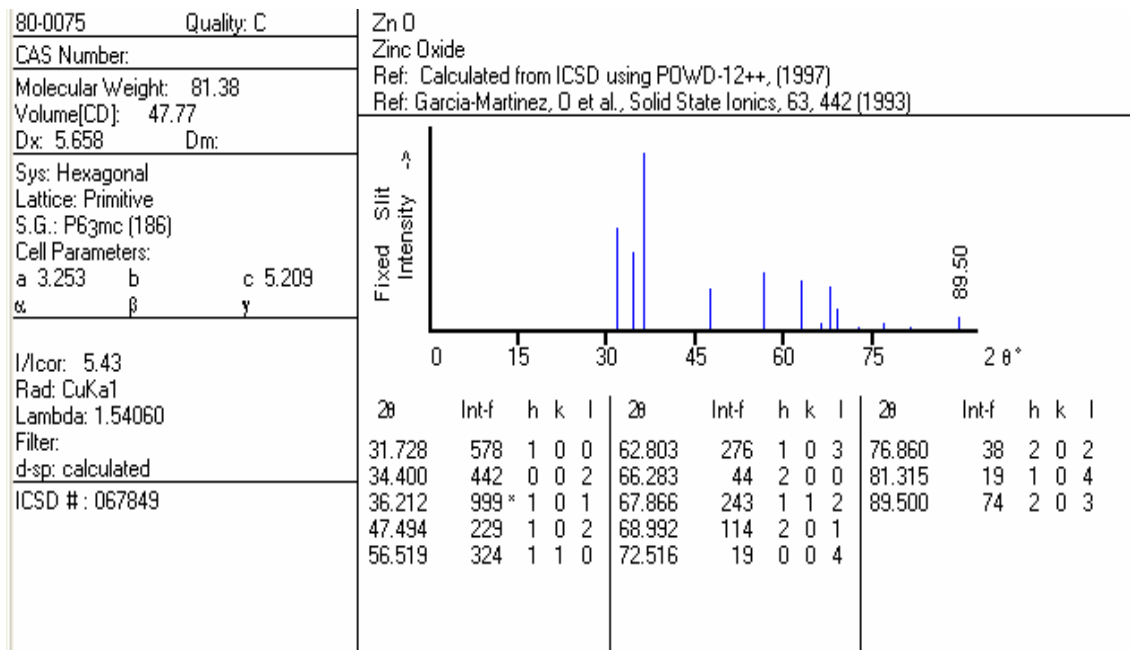


Figure A1. Powder diffraction data of ZnO

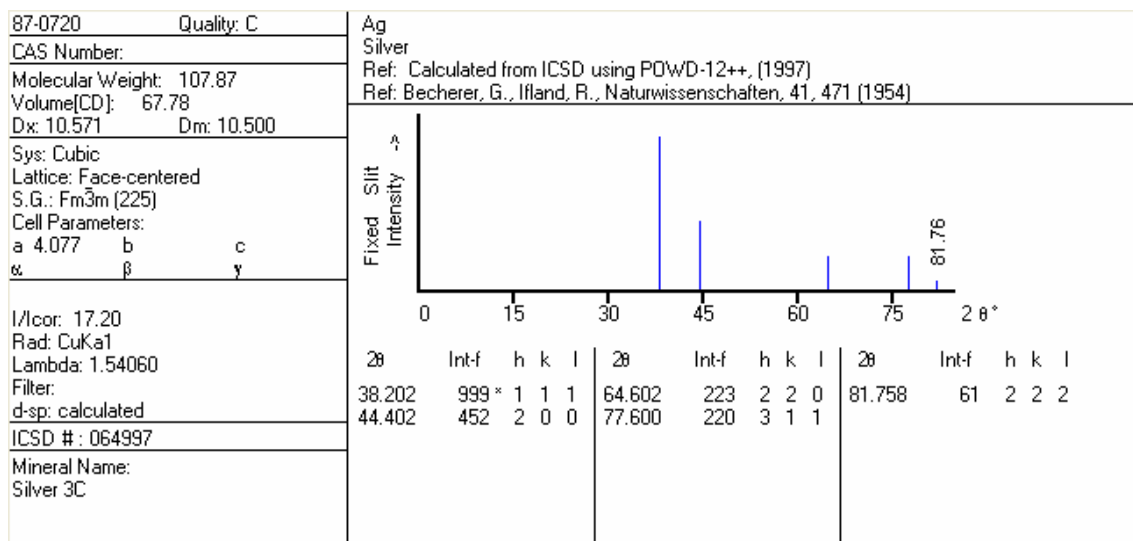


Figure A2. Powder diffraction data of Ag

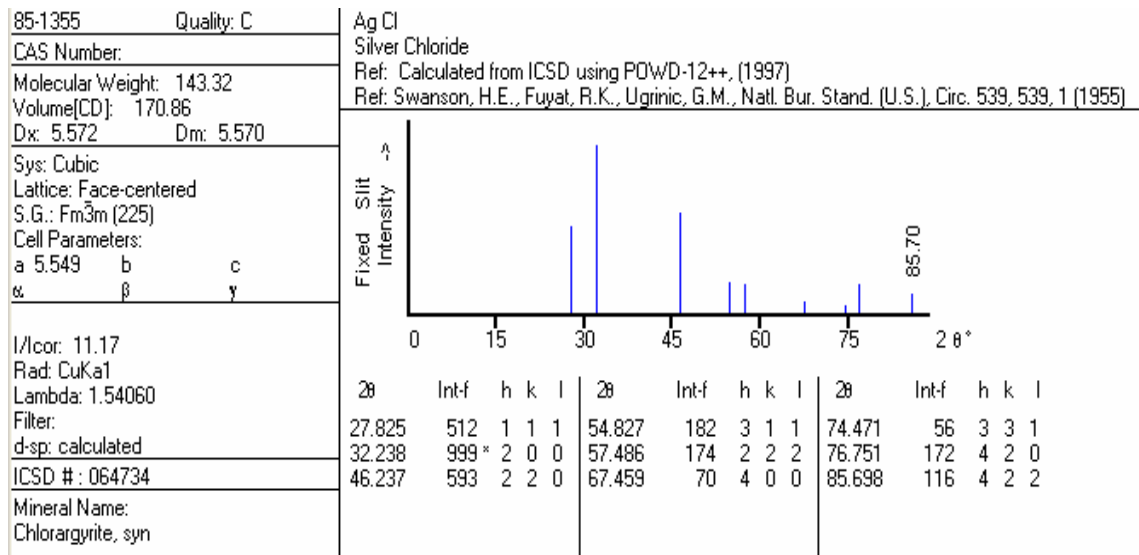


Figure A3. Powder diffraction data of AgCl

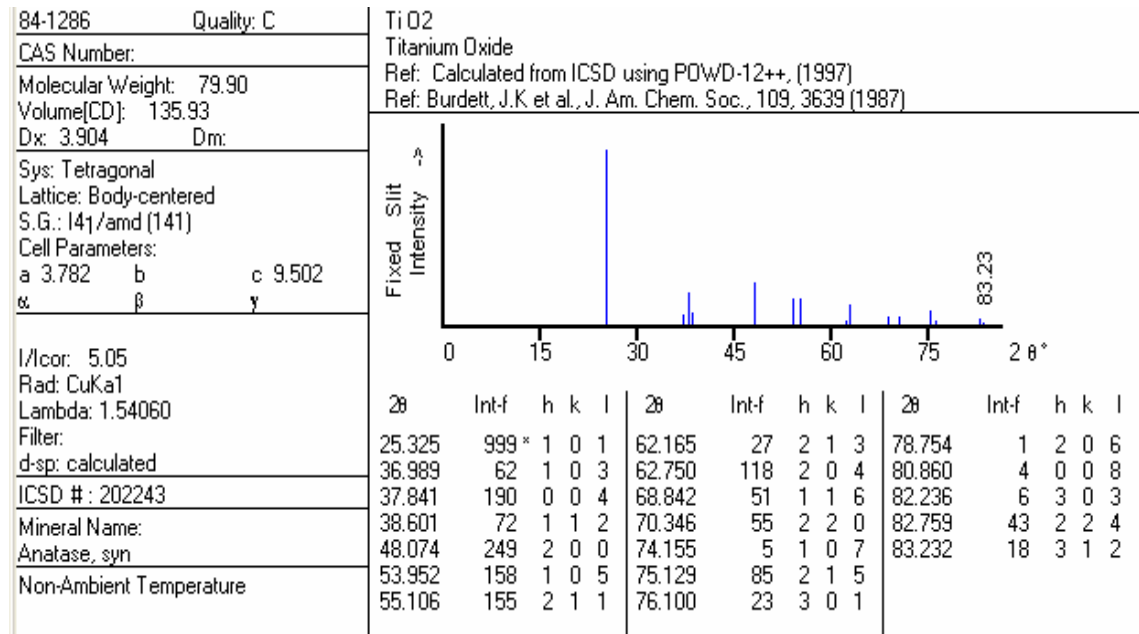


Figure A4. Powder diffraction data of TiO₂ with anatase phase

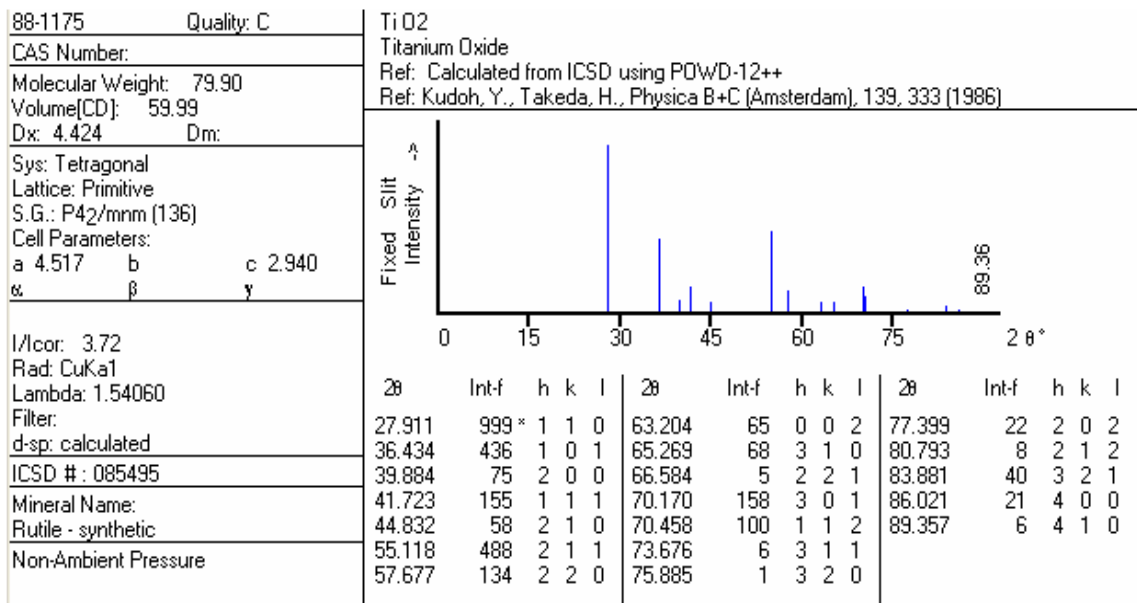


Figure A5. Powder diffraction data of TiO₂ with rutile phase

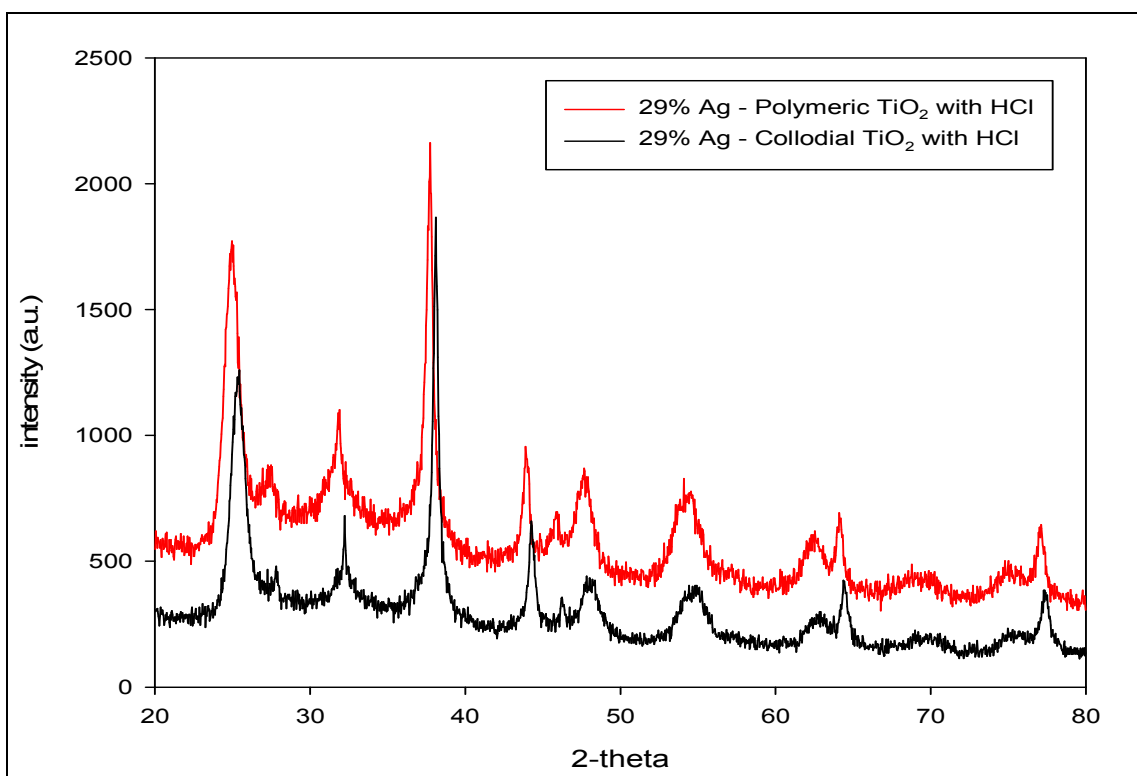


Figure A6. XRD pattern of colloidal and polymeric TiO₂ powders at Ag 29%

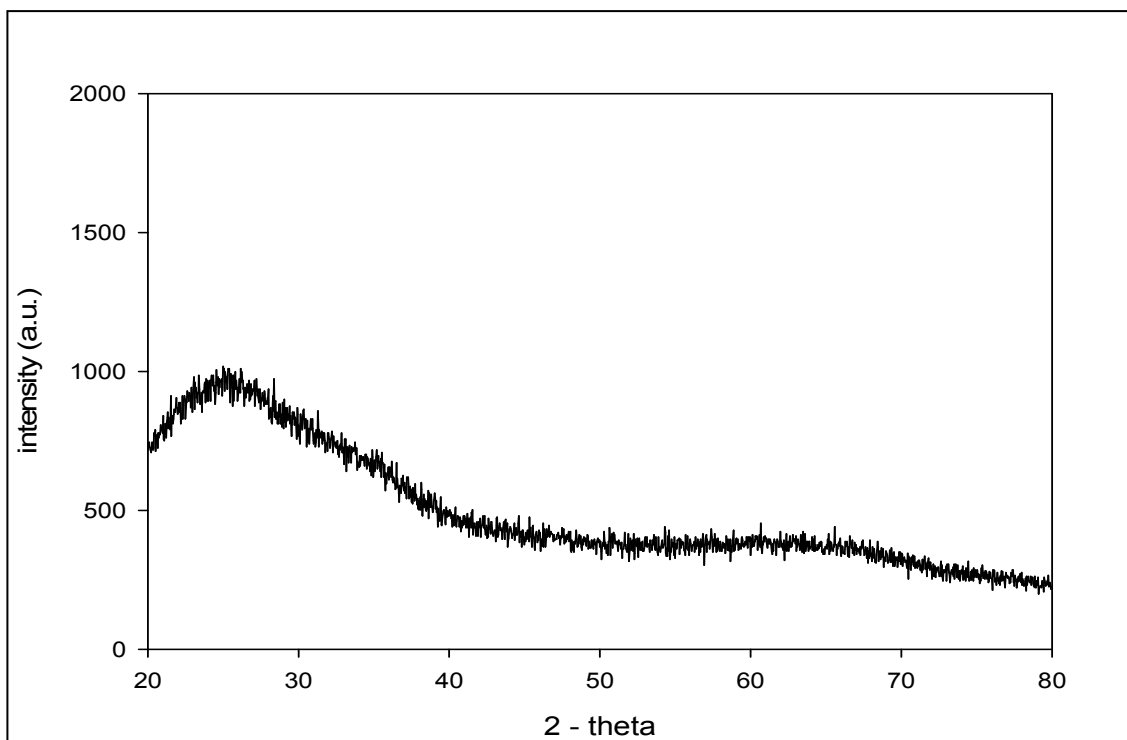


Figure A7. XRD pattern of Ag 12% - 30% ZnO – SiO₂

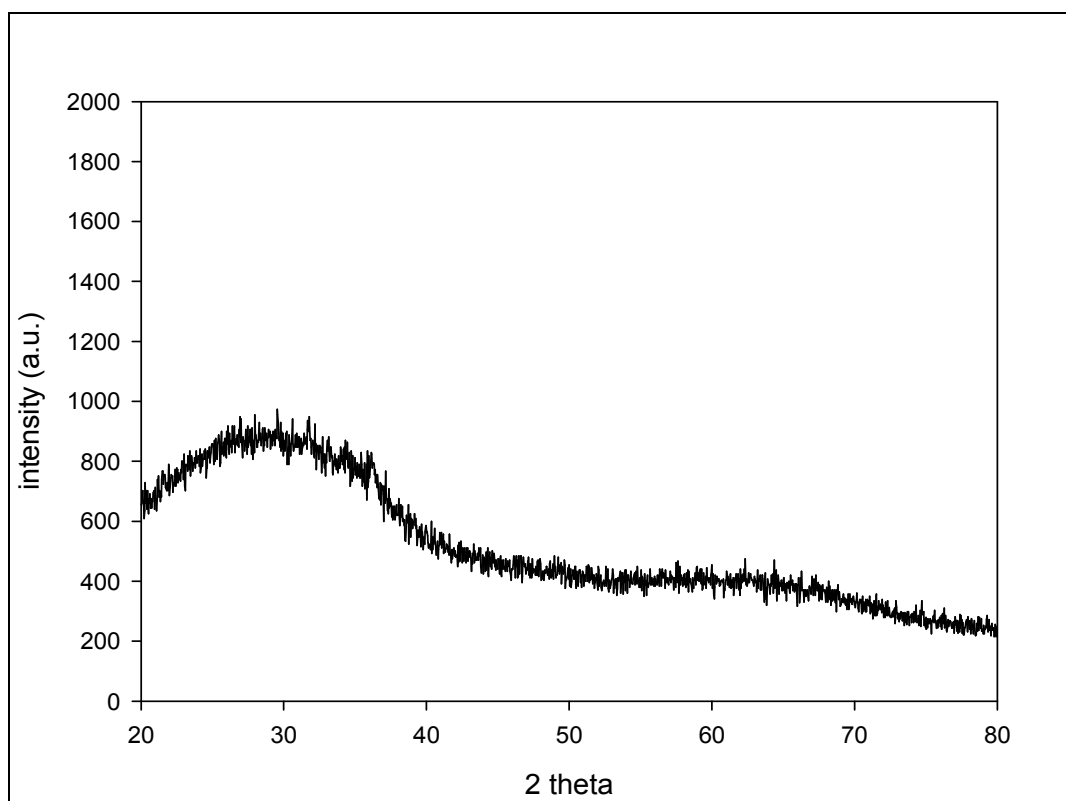


Figure A8. XRD pattern of Ag 12% - 50% ZnO – SiO₂

Table A1. Average Silver Crystalline Thicknesses of samples

Sample ID	Crystalline Thickness (nm)	
	AgCl	Ag
Polymeric 29% Ag	11.62	15.63
HCl treated Polymeric 29% Ag	29.31	-
Polymeric 29% Ag L3 (MH/n)*	25.81	15.54
Polymeric 29% Ag HNO3	-	16.39
Polymeric 29% Ag HNO3 (MH/n)*	24.54	16.4
Polymeric 29% Ag HNO3 (MH/a)**	14.75	16.36
Collodial 29% Ag	24.21	21.53
30% ZnO-SiO2 12% Ag – HCl treated	22.96	-

(MH/n)* - Samples used on Mueller Hinton / Nutrient agar

(MH/a)** - Samples used on Mueller Hinton / agar agar

Table A2. Antibacterial activity test results for the effect of silver concentration

Samples	Zone dia. (mm)
Polymeric 0% Ag	0
Polymeric 1.2% Ag	10.19 ± 0.008
Polymeric 12% Ag	11.20 ± 0.052
Polymeric 29% Ag	12.54 ± 0.133

Table A3. Antibacterial test results of ZnO– SiO₂ powders without silver.

Samples	Zone dia. (mm)
SiO ₂	0
30% ZnO – SiO ₂	13.08 ± 0.04
50% ZnO – SiO ₂	11.36 ± 0.01
70% ZnO – SiO ₂	12.22 ± 0.02
100% ZnO	13.68 ± 0.01

Table A4. Antibacterial test results of ZnO – SiO₂ powders with silver loading 12%

Samples	Zone dia. (mm)
30% ZnO – SiO ₂	18.10 ± 0.0
50% ZnO – SiO ₂	17.63 ± 0.10
70% ZnO – SiO ₂	15.68 ± 0.08