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MANUFACTURING OF ANTI-VIRAL INORGANIC MATERIALS FROM COLLOIDAL SILVER AND TITANIUM OXIDE

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In this study, the TiO_2/Ag colloid was produced by the sol-gel method, which is the hydrolysis and condensation of titanium (IV)-isopropoxide at room temperature. The product was compared with three others products (blending of TiO_2 and Ag colloid, Ag colloid, and TiO_2 sol) and their structure and morphology was determined by TEM and XRD. The formulated colloid is a composite of silver cluster in titanium dioxide with anatase structure whereas the blend was a mixture of silver and TiO_2. The anti-viral and anti-bacterial activities were investigated. It was shown that the composite product has an excellent anti-bacterial activity on the e-coli and the salmonella choleraesuis together with the anti-viral activity on the porcine epidemic diarrhea virus and the transmissible gastro enteritis virus.

INTRODUCTION

The unique physical properties of nanoparticles, due to surface or quantum-size effects, have recently been the subject of intense researches. Particular attention has been paid to the anti-virus or bactericidal application of colloidal titanium oxide (TiO_2) and silver. TiO_2 photocatalyst has attracted great attention as an alternative material to aid in the purification of water and air. TiO_2 photocatalyst generate strong oxidant power when illuminated with UV light with the wavelength of less than 385 nm. In many cases, the strong oxidizing power of TiO_2 photocatalyst has been frequently ascribed to OH radicals photogenerated on TiO_2 surface. The resulting free-radicals are very efficient oxidizers of organic matter.

Wastewater from hospitals, food factories, and contained sites contains microorganism, virus and organic compounds. One of the typical sterilization methods is the photocatalytic sterilization by illumination with UV light with the wavelength of 254 nm, which provided a high rate of sterilization at room temperature. Alternatively, it is well known that the TiO_2 in anatase form is capable of oxidizing and decomposing various kinds of compound. [1-5]

In 1985, Matsunaga and coworkers [6] reported that microbial cells in water could be killed by contact with a TiO_2-Pt catalyst upon illumination with near UV light for 60-120 minutes. Since then, research on TiO_2 photocatalytic killing has been intensively conducted on a wide spectrum of organisms including viruses, bacteria, fungi, algae, and cancer cells. However, TiO_2 has some weak points such as the slow decomposition rate, the confined material from treatment, and the slow treatment rate at atmosphere without UV. On the other hand, silver is widely known for its anti-bacterial properties. Recently, various inorganic anti-bacterial materials containing silver have been developed. Some of the chemically durable materials, which slowly release silver ion for a long period, are also desirable for medical applications such as composite for dental restoration. [7-10]

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TiO_2 containing silver is expected to be an important candidate for anti-bacterial and anti-viral materials for various applications such as the anti-viral mask equipped with a spray type vial and spray or aerosol to kill virus, bacteria and fungi, since it is assumed to show high chemical durability. However, it is difficult to prepare TiO_2-Ag composites by conventional synthesis methods. Although TiO_2-Ag blending can be achieved by simple mixing, segregation between silver colloid and TiO_2 sol often occurs due to their different pH range of stability. [11,12]

In this study, silver-containing TiO_2 sol was prepared by a sol-gel method in the presence of silver colloid. The samples were characterized by x-ray diffraction (XRD) and high-resolution transmission electron microscopy (TEM). These samples were investigated for anti-viral and anti-bacterial effects. The safety test on skin irritation and the oral doze toxicity were also conducted.

EXPERIMENTAL

Materials

In order to prepare silver colloid or titanium colloidal sol and their composite solution, silver nitrate ($AgNO_3$, Aldrich) and titanium (Ⅳ) isopropoxide (TTIP, Aldrich) were used as starting materials, respectively. For the reduction and stabilization of the silver colloid, ethanol (C_2H_5OH, Aldrich, 99%) and some stabilizer were used. On the other hand, isopropyl alcohol (IPA, $(CH_3)_2CHOH$, Junsei, 99.5%) and nitric acid (HNO_3, DC Chemical, 70%) were used for TiO_2 sol as the peptizing agent.

Experimental set-up

Fig. 1 illustrates the schematic diagram of the experimental apparatus for colloid preparation. The experimental set-up consists of the gas cylinder, the circulator, and the glass vessel. The reactor is the cylindrical-jacketed glass vessel with the dished bottom equipped with a propeller-type impeller, and the dropping funnel and condenser.

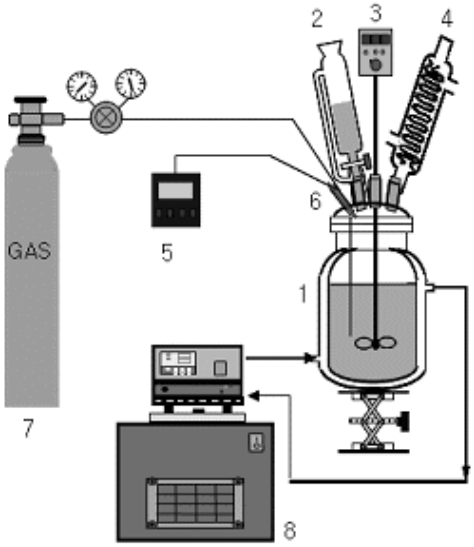


Fig. 1 – Schematic diagram of the experimental apparatus.
(1. Jacked glass reactor (250 ml), 2. Dropping funnel (100ml), 3. Motor & agitator,
4. Condenser, 5. Temperature controller, 6. Thermocouple,
7. Nitrogen, 8. Thermostatic bath and circulator)

Formulation of silver colloid

The formulation procedure of silver colloid is described in details elsewhere.[2] Silver colloid is prepared by the reduction of silver nitrate solution (0.05M) using ethanol in the presence of the stabilizer by the following reactions:

$$2AgNO_3 + C_2H_5OH \rightarrow 2Ag + 2HNO_3 + CH_3CHO, \quad \text{reaction step}$$
$$Ag + \text{Stabilizer} \rightarrow Ag \text{ with stabilizer}, \quad \text{stabilization step}$$

The prescribed amount of ethanol, stabilizer and water were charged into the reactor. The reactor was then flushed out with nitrogen. After stirring under the reaction temperature, the $AgNO_3$ powder was introduced into the reactor and reduced for 2 hrs. The reaction temperature was controlled with an accuracy of ± 0.1 ℃ by the circulating heating medium (silicone oil), which included the thermostatic bath and circulator (model Hakker 320). The sample prepared is stored in an amber glass bottle to prevent possible oxidation by light.

Preparation of TiO_2 sol and TiO_2/Ag composite sol

The preparation procedure of TiO_2 sol and TiO_2/Ag composite is described in details elsewhere.[3] The colloidal solution containing nano-sized titania and silver was prepared by the hydrolysis and condensation of TTIP at room temperature. TTIP was mixed with isopropyl alcohol in the glove box in the N_2 atmosphere to prevent contacting the moisture in the air. This mixture of metal alkoxide and alcohol was added into the ionized water including silver colloid prepared by above procedure with vigorous mixing. On adding the mixture, white hydroxide precipitates were formed. The peptizing treatment of the hydroxide precipitates using HNO_3 was then carried out as an important sol-gel step to control the physical properties. Finally, in order to remove all the additional alcohol and byproducts during the hydrolysis of metal alkoxide, the colloidal solution was aged at 80 ℃ for 6 hrs while stirring. The preparation procedure of TiO2/Ag composite sol is schematically shown in Fig. 2.

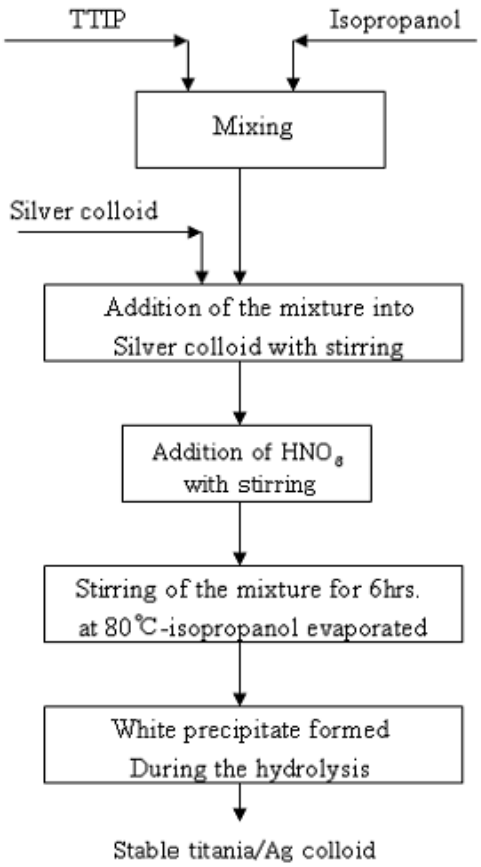


Fig. 2 – Procedure for manufacturing of the TiO_2/Ag formulation colloid.

Material Characterization

Transmission Electron Microscopy (TEM): The morphology of nano-size titania particles was observed by using TEM (Jeol Co., 1200 EX, U.K). A drop of the dispersed sample was placed on Cu grids (200 mesh) covered on a carbon film. Photomicrographs were obtained operating at 200 kV with a magnification order of 100,000.

X-Ray Diffraction (XRD): The crystalline phase of nano-size metal particles was determined by XRD (Rigaku, D-MAX, Japan) using CuKα as a radiant source.

UV-Visible spectroscopy: UV-visible spectra (Shimazu, Model-601, Japan) were also measured with a spectrophotometer.

Anti-virus property test: The porcine epidemic diarrhea virus (PEDV) and the transmissible gastro enteritis virus (TGEV) were tested in this study for the corona virus properties of samples. All tests were carried out in Korea Research Science Biomaterial and Biotechnology as in the following procedure. Vero cells as host cells for virus amplification were transferred to 96-well plates to the final concentration of $2x10^4$ cells/well and they were incubated for 16 hrs. The samples were serially diluted and then mixed with the virus-containing solution. After incubating at 4 °C for 30 min., each test solution was added to the 96-well plates overlaid with the monolayer of Vero cells. The Vero cells were treated with each test solution for 40 hrs, fixed with 70 % acetone solution and then dried. The SRB staining (0.4 % sulforhodamine B and 1 % acetic acid) was performed to assay the amount of cellular proteins, followed by measuring the absorbance at 600 nm with a microplate reader (96 well plate reader).

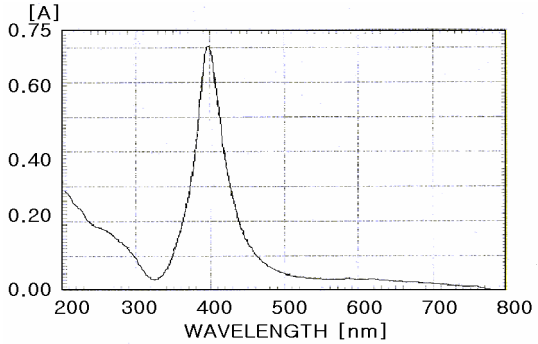
Anti-bacterial property test: The bacterium salmonella choleraesuis (KCTC 2933) and the Escherichia coli, which are known to be major bacterium inducing carriers, were used in this study to test the antibacterial property of the samples. All tests were carried out by the efficacy test method of the disinfectant of germs determined by Korea National Veterinary Research and Quarantine Service. The growth term of tested bacteria was 24 hrs. The final concentration of the bacterial cell was adjusted to $1x10^6$ cells/ml with a reduced transport fluid of pH 7.2.

Single dose toxicity and skin irritation test: The single dose toxicity and the skin irritation test were carried out by the method guided by Korea Food and Drug Administration. According to a test article (Number 1999-61), the single dose toxicity test was performed only one time with a dose of 5,000 mg/kg body weight of white rabbit. The clinical sign, the change in the body weight, and the necropsy findings were observed for 14 days.

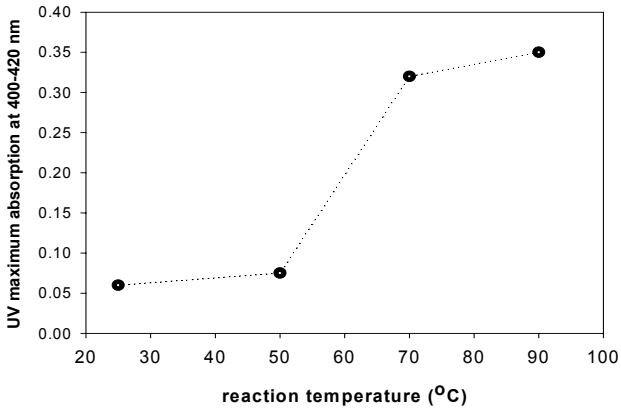
RESULTS AND DISCUSSION

Preparation of colloids including silver and TiO_2

Fig. 3 shows the typical UV absorption spectrum and the maximum UV absorption by silver colloid formed by the alcohol reduction of silver nitrate at various reaction temperatures in the presence of a stabilizer with 2 hrs of reaction time. For the sample containing pure Ag, only one absorption peak with the peak maxima at 400 nm was observed. With increasing the reaction temperature, the UV absorption band becomes stronger and appears as a clear peak attributed to the presence of smaller particle sizes. Silver nanoparticles have a strong absorption peak within the range of 400~450 nm of UV spectra. The formation of Ag nanoparticles can be tracked by the UV-visible spectra of solutions with the peak position and the shape strongly dependent on the physical characteristics of nanoparticles



(1) A typical UV absorption spectrum



(2) Maximum UV absorption of silver colloids prepared

Fig. 3 – The typical UV absorption spectrum and the maximum UV absorption by silver colloid formed by the alcohol reduction of silver nitrate at various reaction temperatures in the presence of stabilizer (reaction time, 2 hrs).

In order to investigate the crystalline behavior of each sample, XRD patterns were observed as in Fig. 4 such as silver colloid, TiO_2 sol, blending of silver colloid and TiO_2 sol (Ag/TiO_2 Blending), and formulation of TiO_2 sol in the presence of silver colloid (Ag/TiO_2 Formulation). TiO_2 particles were prepared as an anatase type and all the peaks were very broad owing to the very fine primary particles. On the other hand, it is clear that strong peaks corresponding to the (111) and (200) reflections of Ag are visible for samples having only Ag. Whereas in the case of blending of Ag colloid and TiO_2 sol, only Ag peak is observed owing to strong Ag peak intensity relative to TiO_2 peak. For the sample of formulation of TiO_2 in the presence of Ag colloid, all the peaks of Ag and TiO_2 corresponding to (111) and (200) reflections are obtained. The peaks intensity of Ag in formulation is, however, much smaller than that observed in Ag colloid only. It has been presumed that the formulation of Ag/TiO_2 leads to the formation of composite colloids due to the Ag cluster type instead of Ag nanoparticles, while the blending of Ag colloid and TiO_2 sol, in which both as nanoparticles, lead to the formation of phase separated Ag-TiO_2 mixed colloidal particles.

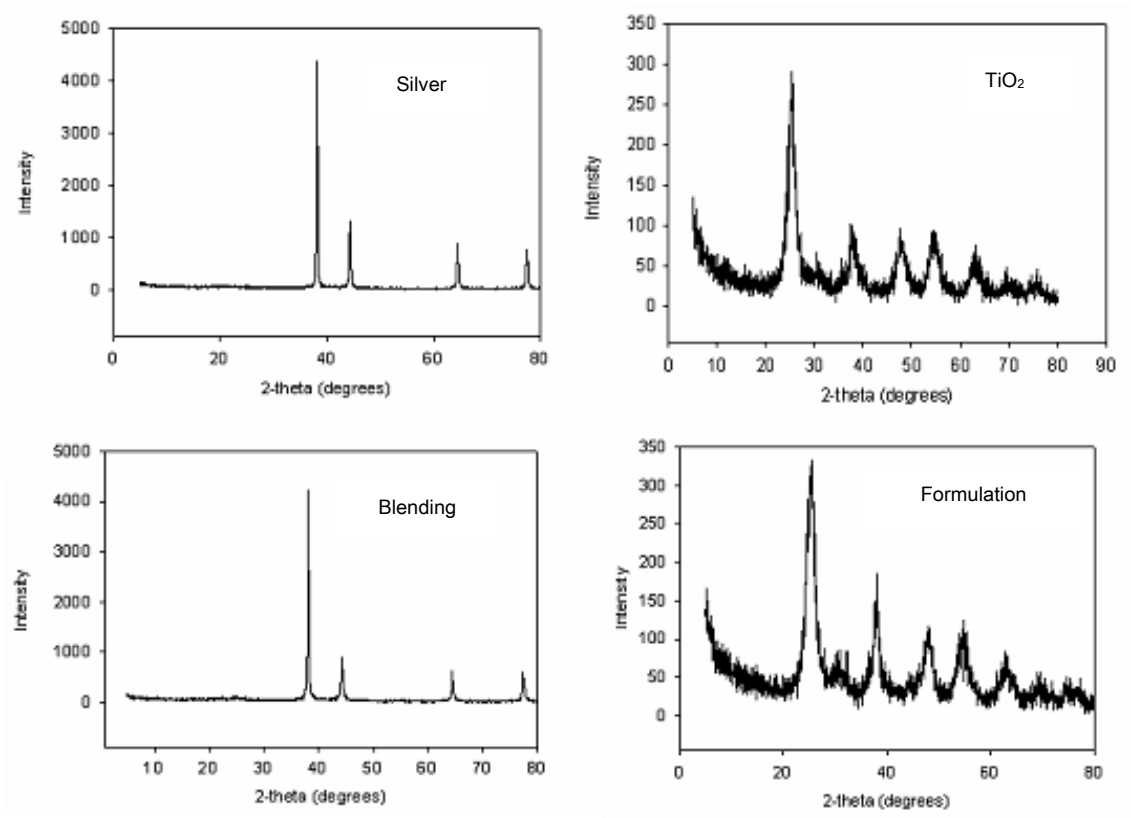


Fig. 4 – XRD patterns of nanoinorganic anti-viral samples.

To determine the size and shape of the sample obtained, HR-TEM studies were carried out. HR-TEM micrographs on same samples used in XRD analysis are shown in Fig. 5. As seen in the micrographs, the size of the nanoparticles is below 100 nm. TiO_2 particles have a rather narrow size distribution compared with Ag particles. Although Ag particles show clear particle contours, TiO_2 particles have unclear particle shapes due to the amorphous structure probed by the XRD. It was shown experimentally that the functioning of stabilizers during the formation of an Ag colloid indicates a means to control particle aggregation and morphology. A common stabilizer, PVP (poly(N-vinyl-2-pyrrolidine)) has been used to produce both the well-defined Ag nanoparticles and the Ag dendrite under various reaction conditions. This has been known to prevent practical applications due to the excessive amount of usage over two moles per molar Ag. In our case, the stabilizer is very effective with small amount (applied at 0.1 % per mole of $AgNO_3$) for preparation of Ag colloid. When the stabilizer molar ratio to Ag metal is too low or too high, the stability of the colloid decreases and the particles are precipitated due to aggregations.

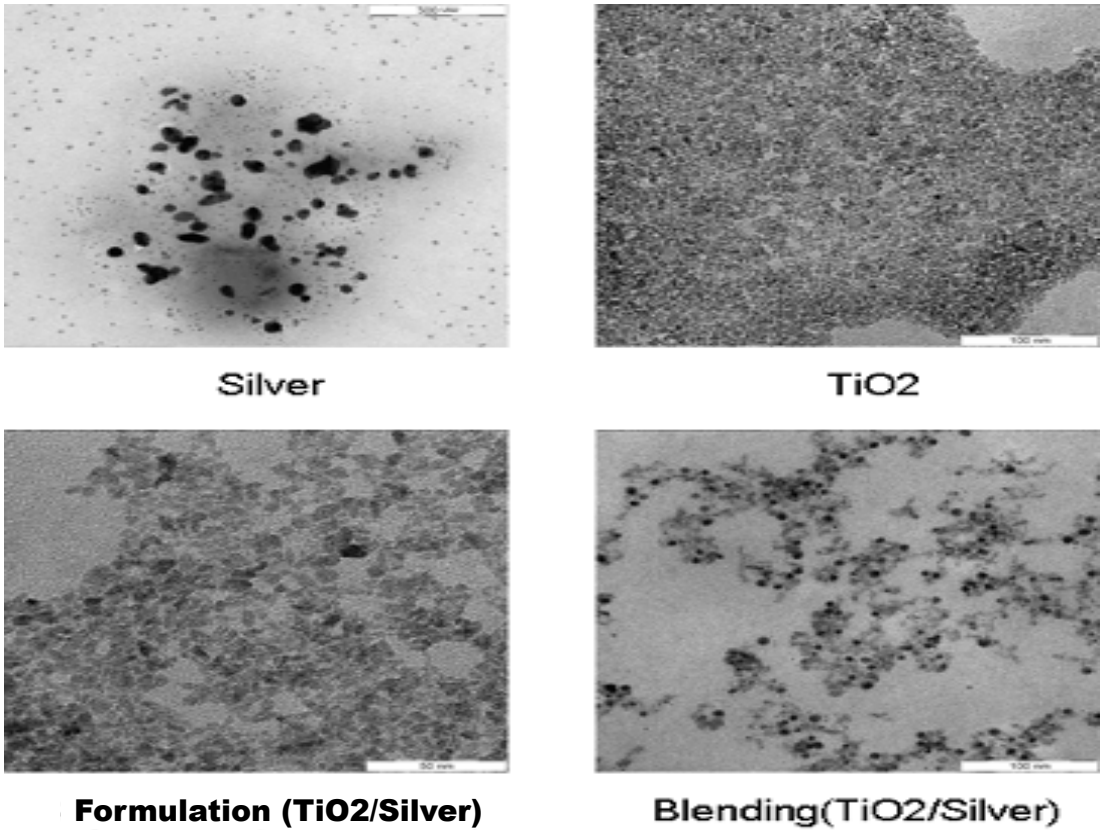


Fig. 5 – TEM pictures of nanoinorganic anti-viral samples.

Anti-bacterial and anti-viral properties

The anti-bacterial and anti-viral properties of colloid samples employed in this work were also examined for its effectiveness on the corona virus and anti-bacterial activity with the e-coli and the salmonella choleraesuis. Table 1 shows the anti-viral activity test with treatment time against salmonella choleraesuis in the case of two samples, i.e., the TiO_2/Ag formulation and the TiO_2/Ag blending. The anti-bacterial efficacy depends upon the properties of each material to be manufactured. It has been known that the TiO_2/Ag formulation has the high anti-bacterial activity. Corona virus is an RNA virus and assumed to cause SARS. It causes epidemic diseases in the respiratory and digestive organs of the mammals and birds. Table 2 shows the anti-viral activity test of Ag/TiO_2 against PEDV and TGEV, where it suggested nearly perfect anti-viral activities to the corona virus even when diluted by a factor of 10^2 or 10^3.

Table 1

Anti-bacterial activity test with treatment time against salmonella choleraesuis

Samples	Treatment Time		
	1hr	2hrs	3hrs
Ag/ TiO_2 Synthesis	1800	0	0
Ag/ TiO_2 Blending	2600	200	70
2% Ag colloid	3800	1750	580
2% TiO_2 Sol	4000	1900	620
Control	4000	4000	4000

Bacteria: salmonella choleraesuis (KCTC2933) (cfu/plate)

Table 2

Anti-viral activity test against PEDV and TGEV

Sample	Activity Test	Efficacy, dilution factor ($\times 10^2$)		Efficacy, dilution factor ($\times 10^3$)	
		PEDV [a]	TGEV [b]	PEDV [a]	TGEV [b]
G-Sol.	Antiviral Activity	Over 99.99 %	Over 99.9 %	Over 99.9 %	Over 93.0 %

[a] PEDV: porcine epidemic diarrhea virus
[b] TGEV: transmissible gastroenteritis virus

Fig. 6 displays the microscopic observation of host cells with applications of anti-virus materials using the Ag/TiO_2 formulation sample. The sample was observed as follows: the Vero cells were treated with each test solution for 40 hrs, and then fixed with 70 % acetone solution and dried. After this procedure, the SRB staining (0.4 % sulforhodamine B, and 1% acetic acid) was performed to assay the amount of cellular proteins, followed by measuring the absorbance at 600 nm with a micro plate reader (96 well plate reader). Fig. 6(A) shows the well surface is covered with entirely as host cells without any infection of virus. Fig. 6(B) shows the well without the application of Ag/TiO_2 formulated composite as anti-viral agent. It was detected that a large amount of host cells were disappeared in the well bottom by the destruction of cells that are infected by the virus.

On the other hand, Fig. 6(C) illustrates the application of anti viral agent, Ag/TiO_2 formulated composite diluted by a factor of 10^3. It has indicated very high anti-viral activity due to the increment of cell numbers by the suppression of the cell destruction ability by treatment with the Ag/TiO_2 formulated composite as anti-viral agent. Fig. 7 depicts the anti-viral/bacterial mechanism of silver/TiO_2 composite. The TiO_2/Ag composite sol is presumed to exert its anti-viral/bacterial effect through the following mechanism. When TiO_2/Ag composite sol is contacted with virus, bacteria and fungi, it could adversely affect the cellular metabolism and the inhibit cell growth. It suppresses the respiration, the basal metabolism of electron transfer system, and the transport of substrate in the microbial cell membrane. This inhibits the multiplication and growth of virus, bacteria and fungi. Finally, the nanocomposite of TiO_2/Ag penetrates into the microbial cell membrane combined with the functional groups (-SH, -COOH, -OH, etc.) of the microbe, and thus destroys the organic structure of the microbial cell, which results in the death of the microbe.

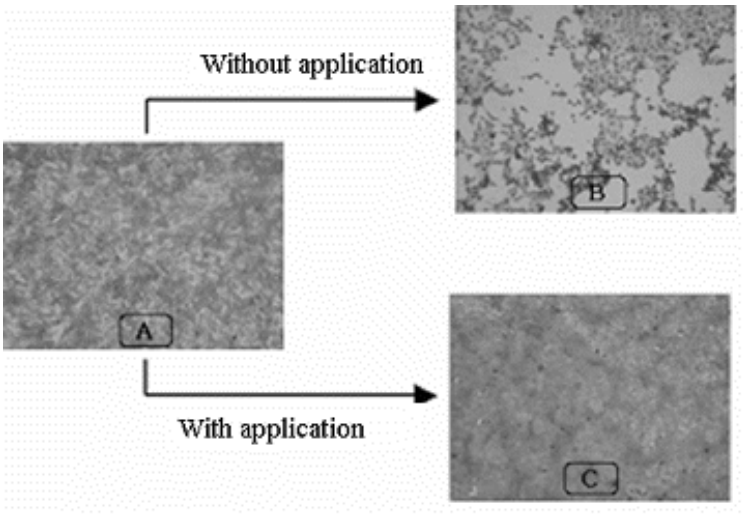


Fig. 6 – Comparison of anti-viral activity using formulated Silver/TiO_2.

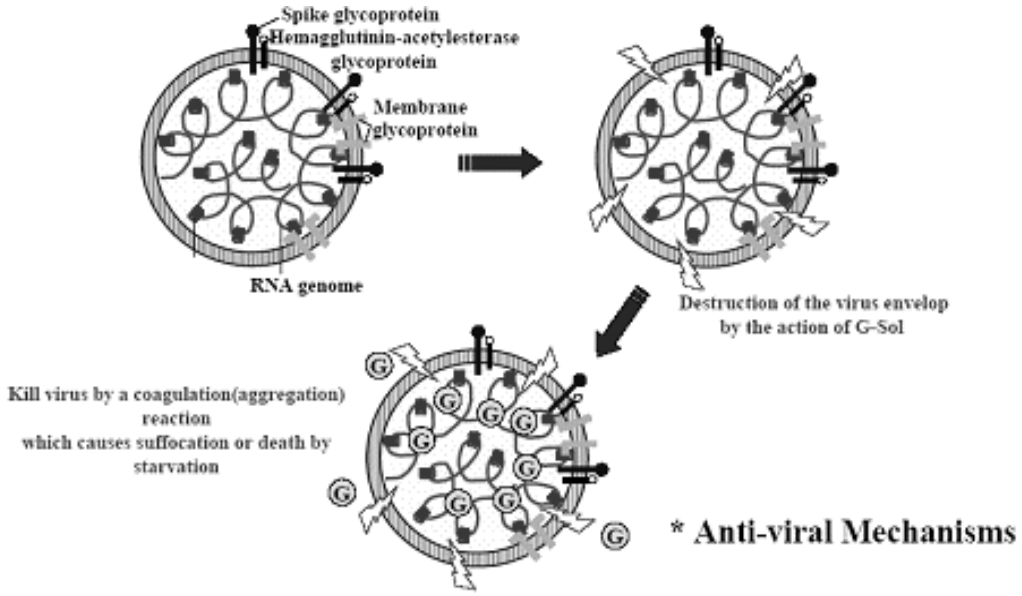


Fig. 7 – Anti-viral/bacterial mechanism of silver/TiO_2 composite.

Safety test on single dose (oral) toxicity and skin irritation test

A test article was orally administrated only one time with 5,000 mg/kg body weight. The clinical signs, the change of the body weight and the necropsy findings were observed for 14days. There were neither dead animals nor the significant change of the body weight. In addition, regardless of the amount of dosage, no clinical signs and lesions were observed. The LD50 value of nano-inorganic anti-viral agent was considered to be higher than 5,000 mg/kg body weight in rate. For the skin irritation test, the primary irritation index (PII) of the test article was scored as "0". This result suggests that the test material is non-irritant on the skin of rabbit.

Anti-bacterial activity of textiles

Tab. 3 exhibits the antibacterial activity of textiles in terms of the bacteriostatic reduction rate (%) with two test bacterias, staphylococcus aureus (ATCC 6538) and klebsiella pneumonia (ATCC 4352). The TiO_2/Ag formulated composite obtained by the method described in this work was doped in the surface of textiles. After 18 hr, the anti-bacterial activity drastically increased in both test bacteria, and the reduction rate was found to be 99.9 %.

Table 3

Antibacterial activity of textiles

Test bacteria		Blank	Sample
Staphylococcus aureus ATCC 6538	At beginning (Cells/ml)	1.8×10^4	1.8×10^4
	After 18hrs (Cells/ml)	4.2×10^6	〈10
	Bacteriostatic Reduction Rate (%)	---	99.9%
Klebsiella pheumoniae ATCC 4352	At beginning (Cells/ml)	2.1×10^4	2.1×10^4
	After 18hrs (Cells/ml)	7.6×10^6	〈10
	Bacteriostatic Reduction Rate (%)	---	99.9%

CONCLUSION

In this study, the silver-containing TiO_2 sol was prepared by the sol-gel method in presence of silver colloid. The samples were characterized by TEM and XRD. The manufactured products in this work were compared with three samples (blending of TiO_2 and Ag colloid, Ag colloid, and TiO_2 sol). The TiO_2/Ag formulated colloid is shown as a composite type formed by the silver cluster in anatase TiO2, whereas the blend is a mixture of silver and TiO_2. These samples were investigated for their anti-viral and anti-bacterial effects. The safety test on skin irritation and the oral doze toxicity were also conducted. It has shown to have an excellent anti-bacterial activity on the e-coli and salmonella choleraesuis as well as an anti-virus activity on the porcine epidemic diarrhea virus (PEDV) and the transmissible gastro enteritis virus (TGEV).

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