J Mater Sci (2007) 42:10085–10089
DOI 10.1007/s10853-007-2109-5

Preparation and antibacterial activities of Ag-doped SiO_2–TiO_2 composite films by liquid phase deposition (LPD) method

Bo Sun · Shu-qing Sun · Ting Li · Wen-qin Zhang

Received: 9 November 2006 / Accepted: 13 August 2007 / Published online: 22 September 2007

Abstract In the present study, Ag/SiO_2–TiO_2 thin films on ceramic tiles with glazed surface were successfully prepared by a liquid phase deposition method (LPD) at a low temperature. The Ag/SiO_2–TiO_2 thin films obtained were homogenous, well adhered and colored by interference of reflected light. The films were characterized by scanning electron microscopy and X-ray diffraction. From these analysis data, it was found that silver (Ag) nanoparticles were trapped in SiO_2–TiO_2 matrix. The antibacterial effects of Ag/SiO_2–TiO_2 thin films against *S. aureus* and *E. coli* were examined by the so-called antibacterial-drop test. The bactericidal activity for the above bacteria cells was estimated by relative number of bacteria survived calculated from the number of viable cells which form colonies on the nutrient agar plates. The Ag/SiO_2–TiO_2 thin films had an excellent antibacterial performance. Atomic absorption spectroscopy (AAS) was used for the quantitative determination of the Ag ion concentration releasing from the Ag/SiO_2–TiO_2 thin film. The releasing rate of Ag ions from the Ag/SiO_2–TiO_2 film is 0.123 μg/mL during 192 h. The antibacterial effect of Ag/SiO_2–TiO_2 thin film before and after aging in a weathering chamber for 48 h was compared and the results show that the antibacterial activity is not compromised after weathering.

B. Sun · S.-q. Sun (✉) · T. Li · W.-q. Zhang
Department of Chemistry, College of Science, Tianjin University, Tianjin, China
e-mail: s625827@yahoo.com.cn

Introduction

In the living environment, human beings are often infected by microorganisms such as bacterium, mold, yeast, virus, etc., especially under moist conditions. Therefore, the preparation of antibacterial products and investigating its antibacterial activity is of practical significance. The ceramic tiles with glazed surface possess excellent decorative and chemical durability, and are widely used in various areas such as the hospital environment and in every household. Unfortunately, ceramic tile itself does not have antibacterial activity and microorganisms easily breed on its surface, which results in biofilm formation in moist environment. Because biofilms provide a protected structure for bacteria growth, the bacteria become less susceptible to antibacterial agents than their free counterparts [1–3]. Several recent studies have focused on the preparation of antibacterial surfaces to inhibit the biofilm formation [4–9]. SiO_2–TiO_2 composite films possess deodorizing and self-cleaning function under ultraviolet light. However, its disadvantage is that the band-gap energy of TiO_2 is approximately 3.2 eV, therefore UV illumination is necessary to photoactivate this semiconductor materials.

Silver (Ag) or silver ions have long been known to be an excellent and broad-spectrum antibacterial agents [10, 11]. According to researches, it is believed that Ag reacts with proteins by combing the –SH groups of enzymes, which leads to the inactivation of the proteins. Feng et al. [12] studied the antibacterial mechanism of Ag^+ on bacteria. Ag^+ makes DNA molecules lose their replication abilities. Ag-doped materials are chemically durable and release Ag^+ for a long duration [13]. If Ag nanoparticles are immobilized in the SiO_2–TiO_2 composite films on glazed surface of ceramic tiles, the release time of Ag ions can be delayed

for a long time so that the ceramic tiles with this film will be of great potential for antibacterial application. This excellent antibacterial activity is not restricted by UV illumination.

Usually, SiO_2–TiO_2-based composite films on surface of ceramic tiles were prepared by techniques such as spray pyrolysis, sol-gel processing, and chemical vapor deposition (CVD). These methods usually require the cost-competitive techniques and expensive devices in those application areas for film deposition. Thus, SiO_2–TiO_2-based composite films on ceramic tiles were not easily achieved using these techniques.

The high level of bacterial resistance obtained by simple application techniques at low processing costs has results in commercial interest. In our work, a successful process to immobilize Ag nanoparticles in TiO_2–SiO_2 thin film on glazed surface of ceramic tiles can be achieved by a liquid phase deposition method (LPD) in a soft-solution at a low temperature. Deki et al. [14] reported on the synthesis of composite nanostructured thin films consisting of Au nanoparticles dispersed in TiO_2–SiO_2 matrix by LPD method and the correlations between preparation conditions and microstructures of obtained films. Here, we report the preparation of Ag-doped TiO_2–SiO_2 thin films on ceramic tiles and the investigation of microstructure of as-prepared films by XRD and SEM. Mainly, the antibacterial activity of Ag-doped TiO_2–SiO_2 thin films on glazed surface of ceramic tiles was also investigated.

Experiments

Preparation and characterization of Ag/SiO_2–TiO_2 films

The precursor aqueous solution was freshly prepared by mixing 200 mL ammonium hexafluorotitanate acid [$(NH_4)_2TiF_6$] solution (0.2 mol L^{-1}), 200 mL ammonium hexafluorotitanate acid [$(NH_4)_2SiF_6$] solution (0.1 mol L^{-1}), and 400 mL boric acid (0.45 mol L^{-1}) solution as well as 10 mL silver nitrate (1×10^{-2} mol L^{-1}) solution. The ceramic tiles with glazed surface were cut into pieces of $50 \times 50 \times 4$ mm^3 in size and washed ultrasonically with ethanol and deionized water. The as-cleaned specimens were immersed into the precursor aqueous solution and maintained at a constant temperature of 25–30 °C for 18–54 h under dark condition. Then, the specimens were rinsed with deionized water and dried at ambient temperature. Heat treatment for the deposited films was performed under air flow for 1 h at various temperatures.

The surface morphology measurements were performed using a scanning electron microscope (SEM, JEOL JSM-6300, Japan) equipped with an energy-dispersive X-ray analyzer, after the surfaces were coated with gold film. The crystal phase of the as-prepared Ag/SiO_2–TiO_2 thin film was identified using an X-ray diffractometer (XRD, Cu Kα, Rigaku, Japan, $\lambda = 0.1541$ nm). Data were collected from 10° to 80°, 2θ at a scan rate of 4°min^{-1}.

The determination of the antibacterial effect of deposited films

The antibacterial activities of films obtained against *S. aureus* and *E. coli* were studied using the so-called antibacterial drop-test. *S. aureus* ATCC6538 and *E. coli* O157 were used as the experimental bacteria and cultured on the culture medium at 37 °C for 18–24 h. Cultured bacteria were added in 10 mL saline solution to reach approximately the concentration of 10^8 CFU/mL. A portion of the saline solution containing the bacteria was diluted to 10^6 CFU/mL for the 'drop-method' antibacterial experiments. The samples were placed in sterilized Petri dishes. Then 100 μL saline solution with bacteria at a concentration of 10^6 CFU/mL was added dropwise onto the surface of each deposited film, and undeposited piece of ceramic tiles with glazed surface used as a blank. The surface of the samples was covered by an antistaling film. The samples were laid at ambient temperature for 4, 8, 12, 16, 20, and 24 h. After each time period the bacteria containing drops were washed from the surfaces of ceramic tiles using 5 mL phosphate buffer solution (PBS) in the sterilized Petri dish. Then 10 μL of each bacteria suspension was dispersed on the culture medium. The number of surviving bacteria on the Petri dishes were counted after incubation for 24 h at 37 °C.

AAS was used for the quantitative determination of the Ag ion concentration releasing from the Ag/SiO_2–TiO_2 films. The ceramic pieces with Ag/SiO_2–TiO_2 film, $50 \times 50 \times 4$ mm^3 in size, were immersed in 50 mL doubly distilled water. The liquids were taken out at designed time as the samples and the concentration of Ag^+ was measured by atomic absorption spectrophotometer (316 MC, Shanghai Analytical Instrument Factory, China). The stability of the Ag/SiO_2–TiO_2 films was tested in a weather chamber (Atlas Electric Device Co., Chicago, USA). During the test, a water spray was activated for 5 min in 30 min cycle. The simulated solar irradiation was directed at the film's surface with an intensity of 0.56 w/m^2 at 340 nm. After 48 h, the samples were removed and dried at ambient temperature before being subjected to an antibacterial test. The antibacterial activity of the film after weathering was then compared to the one before weathering.

Results and discussion

Deposition conditions and film properties

The deposition conditions for the preparation of Ag/SiO_2–TiO_2 films were examined using pieces of ceramic tiles with glazed surface as substrates. The thickness of Ag/SiO_2–TiO_2 films increased with the increasing of deposition time and it showed reflection-interference colors such as silver white, golden yellow, pink, blue, and green, successively .The films thicker than 300 nm were easily peeled off after heat treatment. These results were similar to ours in preparation of TiO_2 films described previously [15]. On the other hand, the films were dark brown in color below the 500 °C annealing conditions, this is because SiO_2–TiO_2 films were not completely densified and Ag^+ ions were not completely trapped in the SiO_2–TiO_2 matrix. Previous researcher reported that Ag is unstable at low annealing temperature [16]. The films treated over 650 °C had brown yellow. But the films treated at 550 °C showed reflection-interference colors mentioned above. 550 °C was selected as annealing temperature.

The surface morphologies of as-prepared Ag/SiO_2–TiO_2 films were examined by SEM (Fig. 1). The film was directly obtained on glazed surface of ceramic tiles using deposition process by heat treatment at 550 °C. In morphology of Ag/SiO_2–TiO_2 film, the metallic Ag agglomerates to small spheroids (Ag^+ to Ag formed during the annealing process) embedded in the SiO_2–TiO_2 matrix. The Ag/SiO_2–TiO_2 film is homogeneous and constructed of small particles showing no cracks.

Figure 2 shows the X-ray diffractogram in the 2θ range 10°–80°of the Ag-doped SiO_2–TiO_2 film calcined at 550°C. The diffraction peaks for the anatase phase of TiO_2 and Ag have been marked with 'A' and 'Ag', respectively. Two distinct diffraction peaks are clearly observed at ca. 2θ = 38.1° and 44.5°, being assigned to (111) and (200) reflections of Ag, respectively. The other remarkable peaks are observed at ca. 2θ = 25.3°, 48.12°, 53.94°, 55.04°, and 62.74°, being assigned to (101), (200), (105), (211), and (204) reflections of anatase phase of TiO_2, respectively. There is no remarkable diffraction peak of SiO_2 because it is amorphous. The mean size of Ag particles was estimated by analyzing the broadening of the (111) reflection. The mean particle sizes can be calculated by the Scherrer equation:

$$D = k\lambda/(\beta \cos\theta)$$

where K = 0.89, λ = 0.1541 nm, θ is the half-diffraction angle, β is the half-peak width, and D is the diameter of the crystalline particle. The estimated average crystal size of Ag particle embedded in SiO_2–TiO_2 matrix is 90 nm for the films heat treated at 550 °C for 1 h.

Antibacterial activity of the Ag/SiO_2–TiO_2 film and mechanism

Nanometer Ag particles show excellent bactericidal effects and do not cause adverse health effects [8]. Therefore, loading the biocompatible SiO_2–TiO_2 with a tiny amount of Ag in terms of economic importance and reusability, draws more and more attention [17, 18]. In the present study, we examined the antibacterial activity of the Ag/SiO_2–TiO_2 film without UV irradiation in comparison with SiO_2–TiO_2 film.

The antibacterial effects of SiO_2–TiO_2 and Ag/SiO_2–TiO_2 films against *E. coli*. and *S .aureus* were evaluated by antibacterial drop-test, and the results are shown in Figs. 3–5. In the case of the SiO_2–TiO_2 thin film, the growth inhibition effect is about 49% for *E. coli*, and 42% for *S. aureus* after 24 h. However, in the case of Ag/SiO_2–TiO_2 film, the growth inhibition effect is about 99% for *E. coli*, and 90% for *S. aureus* after 24 h. This reveals that Ag/SiO_2–TiO_2 films are more effective antibacterial material than SiO_2–TiO_2 films under conditions of without UV-ray illumination. The significant difference in antibacterial effects between SiO_2–TiO_2 and Ag/SiO_2–TiO_2 films may be attributed to the difference in antibacterial mechanisms

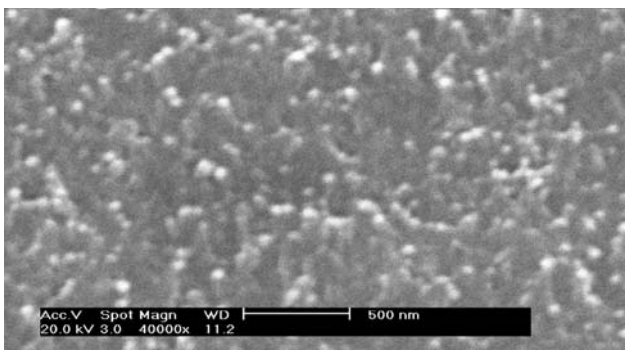

Fig. 1 SEM images for the as-deposited Ag/SiO_2–TiO_2 film

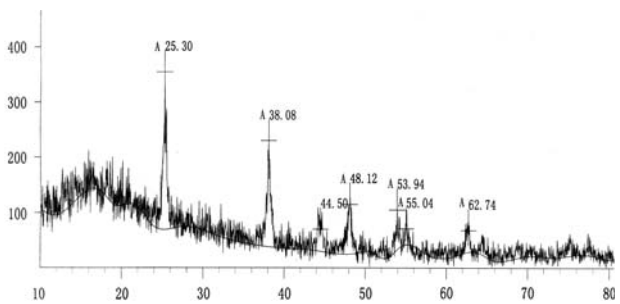

Fig. 2 X-ray diffraction patterns of Ag/SiO_2–TiO_2 film heat-treated at 550 °C

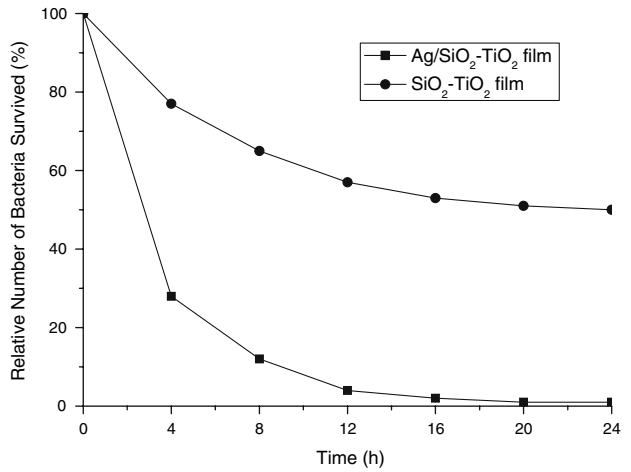

Fig. 3 In case of *E. coli*, relative number of bacteria survived for SiO_2–TiO_2 film and Ag/SiO_2–TiO_2 film

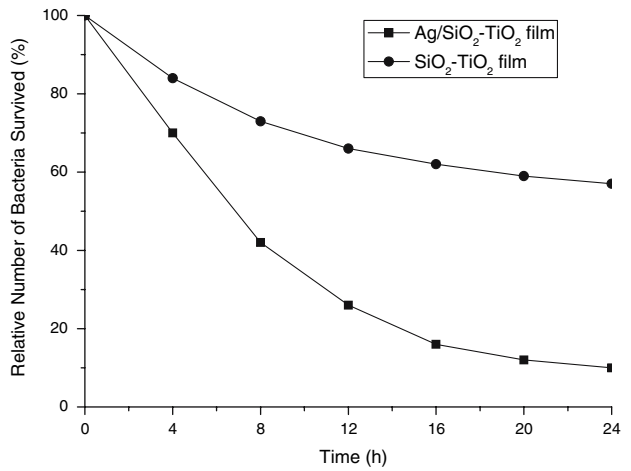

Fig. 4 In case of *S. aureus*, relative number of bacteria survived for SiO_2–TiO_2 film and Ag/SiO_2–TiO_2 film

between both films. It is the photo-catalytic property of the SiO_2–TiO_2 films that makes it to have a sterilizing capability. Under the illumination of the ultraviolet light from natural light, the TiO_2 can spontaneously decompose positive-negative, electron e^- and cavity h^+, which can form the electron-cavity, the cavities oxidize the OH^- and H_2O which are absorbed on the surfaces of the SiO_2–TiO_2 films to $^{\cdot}OH$, which can decompose the cell wall and the cell membrane of *S. sureus* and *E. coli* attached to the SiO_2–TiO_2 thin films, the leakage of intracellular molecules will result in a change in the cell viability. Of the total percentage of ultraviolet light from natural light only 5% results in the lower antibacterial activity.

The antibacterial activity of Ag/SiO_2–TiO_2 films is directly related to Ag nanoparticles. For the antibacterial mechanism of Ag, there are two kinds of theory. The one advocates that Ag can react with the oxygen dissolved in the water and generate activated oxygen O which can decompose the bacteria [19–22]. The other theory holds that metal silver can react with water and release Ag ions, and Ag ions combine with sulphydryl in bacteria, resulting in the blocking of breathing and finally the death of the bacteria [23]. We performed the Ag^+ ions-releasing experiment and AAS was used for the quantitative determination of the Ag ion concentration releasing from Ag/SiO_2–TiO_2 films in water. Figure 6 shows that the releasing concentration of Ag ions of the sample stably increases with the increase in sampling time. The releasing concentration of Ag ions from the Ag/SiO_2–TiO_2 film is 0.123 μg/mL during 192 h. These imply that Ag ions are released from the surface of Ag/SiO_2–TiO_2 films and these ions kill the microorganisms. This mechanism implies that the antibacterial material probably have a limited period of effectiveness. Therefore, the stability of Ag/SiO_2–TiO_2 films was carried out in this study.

The stability experiment of Ag/SiO_2–TiO_2 films was processed by exposing the antibacterial films to simulated weathering conditions. After processing simulated weathering with water spray and UV irradiation for 48 h, Ag/SiO_2–TiO_2 films were again tested for antibacterial activity and the viable cell number as a function of time was monitored. A comparison of antibacterial activity of Ag/SiO_2–TiO_2 films before and after weathering is given in Fig. 7. The results show that the antibacterial activity is not compromised after weathering.

In the release rate study and the weathering exposure experiments, the conditions were extreme, while application conditions are moderate, so these experiments accelerate to determine the antibacterial activity of materials. In fact, the time associated with the ultimate

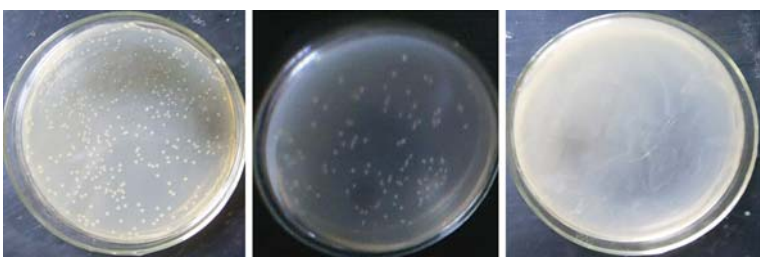

Fig. 5 Test results on *E. coli* after 24 h: (**a**) incubated with ceramics piece; (**b**) incubated with SiO_2–TiO_2 film; (**c**) incubated with Ag/SiO_2–TiO_2 film

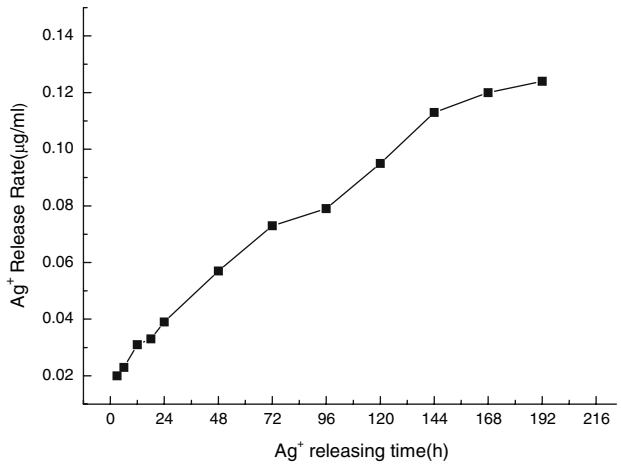

Fig. 6 Atomic absorption spectroscopy of Ag ion releasing rate from the Ag/SiO_2–TiO_2 film with the releasing time

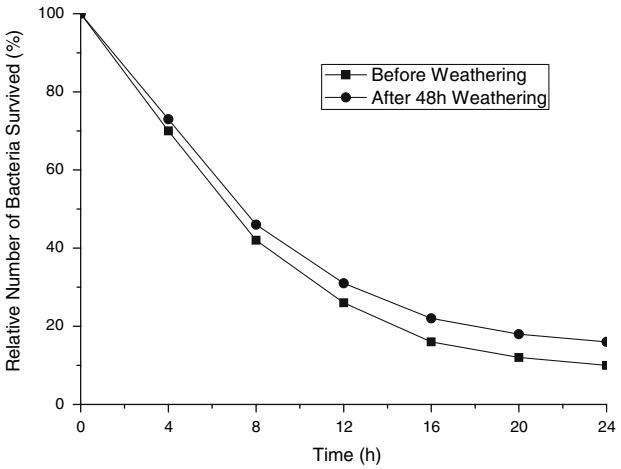

Fig. 7 Comparison of the antibacterial effect of the Ag/SiO_2–TiO_2 film before and after aging in a weathering chamber for 48 h

application of these materials far exceeds the relatively short duration of some of our tests. The Ag/SiO_2–TiO_2 film showed good growth inhibition effect for *E. coli* as much as 89% after 1 year.

Conclusions

Antibacterial Ag/SiO_2–TiO_2 thin films have been prepared on ceramic tiles by a liquid phase deposition method (LPD). The Ag/SiO_2–TiO_2 thin films obtained adhered well, and were homogenous and colored by interference of reflected light. XRD and SEM experiments indicated that Ag nanoparticles was completely trapped in SiO_2–TiO_2 matrix and reduction could be achieved at 550 °C annealing temperature. Ag/SiO_2–TiO_2 thin films show high antibacterial activity eliminating the *E. coli* and *S. aureus*. One reason is that the Ag ion is released from the Ag/SiO_2–TiO_2 thin films in a certain extent. The antibacterial functionality of Ag/SiO_2–TiO_2 thin films was not compromised even after aging in a weathering chamber. Ag/SiO_2–TiO_2 thin films are believed to be useful as an surface of antibacterial ceramic tile.

Acknowledgement The authors are indebted to the Chinese Natural Science Foundation for financial support.

References

1. Costerton JW, Stewart PS, Greenberg EP (1999) Science 284:1318
2. Parsek MR, Singh PK (2003) Annu Rev Microbiol 57:677
3. Brown MR, Barker J (1999) Trends Microbiol 7:46
4. Gray JE, Norton PR, Alnouno R, Marolda CL, Valvano MA, Griffiths K (2003) Biomaterials 24:2759
5. Ignatova M, Labaye D, Lenoir S, Strivay D, Jerome R, Jerome C (2003) Langmuir 19:8971
6. Klueh U, Wagner V, Kelly S, Johnson A, Bryers JD (2000) J Biomed Mater Res B 53:621
7. Dai JH, Bruenig ML (2002) Nano lett 2:497
8. Lee HJ, Yeo SY, Jeong SH (2003) J Mater Sci 38:2199
9. Jansen B, Kohnen WJ (1995) Ind Microbiol 15:391
10. Kang HY, Jung MJ, Jeong YK (2000) Korean J Biotechnol Bioeng 15:521
11. Kawashita M, Tsuneyama S, Miyaji F, Kokubo T, Kozuka H, Yamamoto K (2000) Biomaterials 21:393
12. Feng QL, Wu J, Chen GQ, Cui FZ, Kim IN, Kim JO (2000) J Biomed Mater Res 52:662
13. Toshikazu T (1999) Inorg Mater 6:505
14. Deki S, Yu KH, Fujita T, Akamatsu K, Kajinami A (2001) J Eur Phys D 16:325
15. Hu YT, Sun SQ, Xi ZY, Duan CY, Meng Y (2006) J Ceram Process Res 7:49
16. Ritzer B, Villegas MA, Fernandez Navarro JM (1997) J Sol-Gel Sci Technol 8:917
17. Zhang LZ, Yu JC, Ho YY, Li Q, Kwong KW, Xu AW, Wong PK (2003) Langmuir 19:10372
18. Sokmen M, Candan F, Suumer Z (2001) J Photochem Photobiol A 143:241
19. Peterwig HG (1996) Pharmacol Ther 69:127
20. Rahn RO, Landry LC (1973) Photochem Photobiol 6:29
21. Vallee BO, Ulmer DD (1972) Annu Res Biochem 41:91
22. Ritchie JA, Jones CL (1990) Lett Appl Microbiol 11:152
23. Zhang ZW (1996) Rare Metal Mater Eng 1:49