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METAL OXIDE DOPED ANTIBACTERIAL POLYMERIC COATED TEXTILE MATERIALS AND ASSESSEMENT OF ANTIBACTERIAL ACTIVITY WITH ELECTRON SPIN RESONANCE

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Abstract: *Antibacterial activity of a food conveyor belt is an essential property in some cases. However, every antibacterial chemical is not suitable to contact with food materials. Many metal oxides are suitable option for this purpose. The aim of this study was to investigate antibacterial properties of zinc oxide doped PVC polymer coated with electron spin resonance technique. Therefore, optimum zinc oxide containing PVC paste was prepared and applied to textile surface. Coating construction was designed as double layered, first layer did not contain antibacterial agent, thin second layer contained zinc oxide at 10-35% concentration. Oxygen radicals released from zinc oxide containing polymeric coated surface were spin trapped with DMPO (dimethylpyrroline-N-oxide) spin trap and measured with Electron Spin Resonance (ESR). Besides conveyor belt samples, oxygen radical release from zinc oxide surface was measured with ESR under UV light and dark conditions. Oxygen radical release was determined even at dark conditions. Antibacterial properties were tested with ISO 22196 standard using Listeria innocua species. Measured antibacterial properties were related with ESR results. Higher concentration of zinc oxide resulted in higher antibacterial efficiency. DCFH-DA flourometric assay was carried out to determine oxidative stress insidebacteria. It is tought that, this technique will lead to decrease on the labour and time needed for conventional antibacterial tests.*

Keywords**:** *Antibacterial, conveyor belt, Electron Spin Resonance, zinc oxide, spin trapping*

1. INTRODUCTION

A conveyor system is a very important part of an industrial facility with regard to continuous production. A conveyor belt has to fulfil some special expectations of the industry branch that it is used. From this point of view, food carrying conveyor belts are generally produced with antibacterial properties. Since those are in direct contact with food products, the antibacterial agents should be chosen form a very narrow list. Metal oxides, such as zinc oxide, magnesium oxide, calcium oxide, are very suitable option for an antibacterial addition for food conveyor belts due to non-toxic and durable properties [1-3]. Besides, with their low prices, metal oxides will be feasible alternative.

Though there are still some different thoughts about the antibacterial mechanisms of metal oxides, current researches concentrated on antibacterial effect with oxygen radical releasing of these chemicals. The electronic structure of metal oxides allows them to produce oxygen radicals such as $^{\bullet}OH$, $O_2^{\bullet-}$, $^1O_2$ [4-10]. Especially with UV light photons, the excitation and motion of electrons from valance band to conduction band leads to hole and electron couple formation. Electrons react with molecular

oxygen to form superoxide anion, holes take electrons from hydroxyl ions or water to form hydroxyl radical and superoxide anion reacts with water and singlet oxygen occurs [11].

Since oxygen radicals are highly unstable, it is difficult to detect these radicals under normal environmental conditions. Therefore, oxygen radicals must be reacted with special chemicals to obtain more stable and detectable compounds. Electron Spin Resonance (ESR) is one of the most important techniques to study with these oxygen containing compounds that have one or more unpaired electrons. The physical concept of ESR technique depends on the orientation of unpaired electron spins in the magnetic field. Under specific conditions, radicals have characteristic ESR spectrum. Signal intensities change with the radical concentration, thus, it is possible to predict about radical concentration [12, 13].

Spin traps are used in ESR to detect oxygen radicals. Spin traps react with oxygen radicals to form a stable radical which have a lifetime that at least let to take an ESR measurement. DMPO (dimethylpyrroline-N-oxide) is a suitable spin trap to detect $^{\bullet}OH$ radical [13]. The reaction between $^{\bullet}OH$ radical and DMPO spin trap is shown on Figure 1.

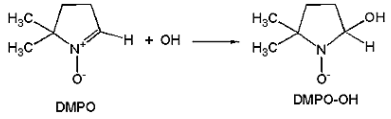

Fig. 1. *The reaction of $^{\bullet}OH$ radical and DMPO*

DCFH-DA is not a fluorescent compound, it can be pass through cell membrane and it is decomposed to DCFH inside the cell by intercellular esterases. When DCFH molecule reacts with oxygen radicals, it turns into DCF which is highly fluorescent. By this way, oxidative stress inside the cell can be detected [14-19].

The aim of this study is to confirm a relation between the radical formation that is detected by ESR measurements and antibacterial activity of textile materials. By this way, the labour and time (at least four days) which is spent for antibacterial test will be eliminated. Also, it will be possible to predict the antibacterial activity without pausing the production of the food producing facility.

2. EXPERIMENTAL

Zinc oxide (Sigma-Aldrich) was used as the antibacterial agent. Aqueous suspensions of 1, 10 and 100 g/L zinc oxide concentrations were investigated. Conveyor belts were produced with %10 and %35 zinc oxide concentration. Knife coating method was carried out and PVC polymer was used. Two layer of coating was carried out, top layer (0,05-0,1 mm) was the antibacterial layer. Oxygen radical releasing was observed under UV light and in dark conditions for zinc oxide powders and under UV light for conveyor belt samples. UV light exposure was 15 minutes.

ISO 22196-2011-*Measurement of antibacterial activity on plastics surfaces and other non-porous surfaces* standart was performed for antibacterial tests and *Listeria innocua* was used as the test bacteria.

ESR measurements were performed with Bruker e-scan model X-band spectrometer with following conditions: Microwave frequency, 9.80 GHz; scan width 65G; receiver gain $1.26x10^2$; resolution 512; conversion time, 81.92 msec; time constant 655 msec; scans, 4; modulation frequency, 86 kHz. 0.02 M DMPO (Sigma-Aldrich) was used as a spin trap. 360nm UV light was used for UV excitation of zinc oxide.

The concentration of released oxygen radical was calculated by the comparison of spin-adduct signals with signals of the known concentrations (1-30 µM) of stable TEMPO radical (Sigma-Aldrich) [20-22]. A calibration curve was built with the double integration of the ESR spectrum (signals only) of TEMPO ((2,2,6,6-Tetramethylpiperidin-1-yl)oxyl) in different concentrations. The bottom point of each signal was set to zero for area calculation.

Oxidative stress inside the bacterial cells was monitored under florescent microscobe using 100 µM DCFH-DA (Fluka). Bacteria culture in soy broth was incubated with 100g/L zinc oxide at 37ºC for 3 hours. After 3 hours of incubation, DCFH-DA solution was added to the culture media and incubation was continued for 1 hour. After 4 hours of total incubation, samples were analized under fluorescent microscobe. Excitation and emission wavelengths were λex= 498 nm and λem=522 nm, respectively

3. RESULTS AND DISCUSSION

The antibacterial efficiency is a very complicated phenomenon since there are countless parameters to be taken into account depending on the used antibacterial agent. As for metal oxides, such as zinc oxide, it is important to understand the mechanism of antibacterial activity in order to predict possible antibacterial effects and design a zinc oxide containing antibacterial product considering these properties. In this study, oxygen release from zinc oxide surface and the relation between antibacterial activity was studied and possible usage of zinc oxide in food conveyor belts was evaluated.

The ESR spectra of 100 g/L zinc oxide under UV light and in dark conditions are, shown on Figure 3. Specific signals for DMPO-OH adduct were obtained for ESR measurement of zinc oxide – DMPO solution interaction. As seen in Figure 2, even in dark conditions, the existence of oxygen radicals was seen in Fig.2B and C.

Effect of UV light on zinc oxide is clearly seen in Fig.2D. Electron-hole pairs created by electron excitation led to high amount of oxygen radical formation.

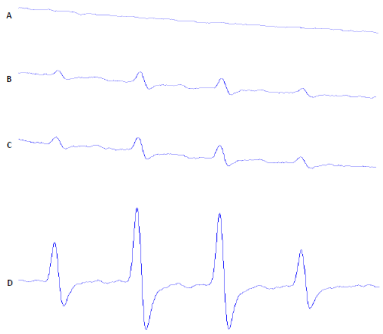

Fig 2: *ESR spectra of 100 g/l zinc oxide samples (A: Only DMPO solution, B: DMPO + zinc oxide under dark conditions, C: Pre-exposure of zinc oxide than mixing with DMPO solution, D: UV exposure of DMPO + zinc oxide suspension)*

As mentioned above, oxygen radical concentrations were determined by the comparison of DMPO-OH spin adduct ESR signals with the stable radical TEMPO's signal. The calibration curve obtained with signal area and concentration of TEMPO is shown in Figure 3.

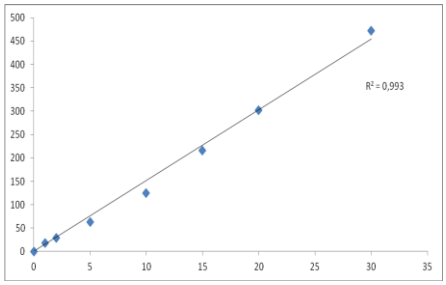

Fig. 3: *Calibration curve obtained with known concentrations of TEMPO stable radical*

Oxygen radical concentrations indicate that there is no dramatic change on oxygen radical release depending on the zinc oxide concentration. The lowest oxygen radical concentration (0.3 µM) was detected for 1 g/L zinc oxide concentration without UV light. Oxygen radical amounts detected with DMPO spin trap under dark conditions were 1.15 and 0.82 µM for 10 g/L and 100 g/L zinc oxide concentrations, respectively. On the other hand, zinc oxide exhibited antibacterial activity at much lower concentrations in dark conditions. Small amount of oxygen radical could be adequate for the antibacterial effect. Thus, increasing the zinc oxide concentration is not necessary for antibacterial efficiency due to slight changes of oxygen radical amount depending on concentration.

During UV exposure, oxygen radical releasing increases dramatically. Under UV light, there is almost no difference on oxygen radical concentration between 1, 10 and 100 g/L zinc oxide concentrations (7.78 µM, 7.67 µM, 7.82 µM, respectively). There are two situations conceivable; since zinc oxide release oxygen radicals via catalytic reaction, the potential of the medium for catalytic reaction might be limited or since zinc oxide is not water soluble, the UV exposure area of zinc oxide powder could be limited.

0.03 µM oxygen radical releasing was detected in 15 minutes of DMPO solution interaction with %10 zinc oxide containing conveyor belt material. On the other hand, 0,72 µM oxygen radical releasing was determined for 35% ZnO containing belt material. ESR signals of conveyor belt samples are shown on Figure 5.

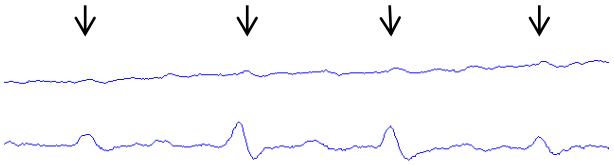

Fig. 4: *ESR signals of DMPO interaction with zinc oxide containing conveyor belts (10% ZnO: above, 35% ZnO: below)*

Very low concentration of oxygen radical was released from the surface of %10 ZnO containing belt material. The antibacterial activity of this belt material was 86.84%. 35% ZnO containing conveyor belt material exhibited % 99.99 antibacterial activity. These results are consistent with the calculated oxygen radical concentrations by using ESR measurements. The oxidative stress related ZnO inside the cells is shown in Fig.5. ZnO related oxidative stress inside the cells is seen on Figure 5.

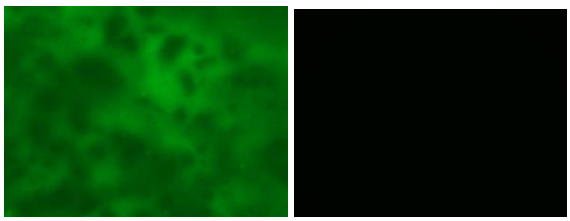

Fig. 5: *Fluorescent microscobe images of DCFH-DA treated bacteria (ZnO treated bacteria on the left, only bacteria and DCFH-DA on the right)*

Fluorescent microscope images belong to DCFH-DA assay was confirmed that ZnO caused an oxidative stress inside *Listeria innocua.*

4. CONCLUSION

Since the antibacterial activity arises from the oxygen radicals, determination of the radical quantity through the signal area of DMPO-OH spin adduct is a suitable way for the assessment of antibacterial activity. Even at low oxygen radical concentrations, antibacterial activity occurred.

In light of the information derived from this study, the concentration of the zinc oxide does not have a dramatic effect on antibacterial activity in the powder form. The released oxygen radical amounts for different concentrations were close to each other. UV light exposure created a significant effect on oxygen radical formation.

It can be told that, if a metal oxide (zinc oxide) will be used as an antibacterial agent in a conveyor belt, small amount of metal oxide usage will be effective. The important point is the contact of metal oxide powders with bacteria, otherwise, since the oxygen radicals have a very short time and might be fade and cause a decrease of antibacterial activity. At this point, the construction of the conveyor belt has an important role. Metal oxides should not be buried inside the polymer coating. Double layered coating with a higher concentration of ZnO on top layer would be an appliciable construction.

UV light usage is an important factor to increase antibacterial activity of zinc oxide. Oxygen radical amount increased nearly 20 times for 1 g/L ZnO concentration with UV light exposure. A facility that uses a zinc oxide containing antibacterial conveyor belt can use UV light in a point of the production and by this way, the antibacterial activity will be raised.

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5. REFERENCES

[1] Ivask, A., George, S., Bondarenko, O., Kahru, A. Metal-containing nano-antimicrobials: differentiating the impact of solubilized metals and particles. In: Nano-antimicrobials - Progress and Prospects., N. Cioffi and M. Rai (eds). Springer. 2012 p. 253-290.

[2] Gedik, G., Aksit, A., Engin, B., Paksu, U., Kutlu, B., Albayrak, A., Assessment of antibacterial activity of metal oxide containing conveyor belts with Electron Spin Resonance technique, ıv International Conference on Antimicrobial Research Malaga, Spain 29 June-1July 2016

[3] Akşit, A., Kutlu, B., Albayrak, A., Gedik, G., Özerdoğan, M.A., Mızrak, B., 13th AUTEX World Textile Conference, Dresden, Germany 2013

[4] Sawai, J, Igarashi, H., Hashimoto, A., Kokugan, T., Shimizu, M., Evaluation Of Growth Inhibitory Effect Of Ceramics Powder Slurry On Bacteria By Conductance Method, Journal Of Chemical Engineering Of Japan 1995 28(3):288-293

[5] Sawai, J., Shiga, H., Kojima, H., Kinetic Analysis Of Death Of Bacteria In CaO Powder Slurry, International Biodeterioration & Biodegradation, 2001 47, 23-26

[6] Sawai, J., Kawada, E., Kanou, F., Igarashi, H., Hashimoto, A., Kokugan, T., Shimizu M., Detection Of Active Oxygen Generated From Ceramic Powders Having Antibacterial Activity, Journal Of Chemical Engineering Of Japan, 1996 29, 627-629

[7] Sawai, J., Kajima, H., Hashimoto, A., Shoji, S., Sawaki, T., Hakoda, A., Kawada, E., Kokugan, T., Shimizu, M., Antibacterial Characteristics Of Magnesium Oxide Powder, World Journal of Microbiology and Biotechnology, 2000, 16, 187-194

[8] Makhluf, S., Dror, R., Nitzan, Y., Abramovich, Y., Jelinek, R., Gedanken, A., Microwave-Assisted Synthesis of Nanocrystalline MgO and Its Use as a Bacteriocide, 2005, 15, 1708-1715

[9] Cho, M., Chung, H., Wonyong, C., Yoon, J., Linear Correlation Between Inactivation Of *E. Coli* And OH Radical Concentration In Tio_2 Photocatalytic Disinfection, Water Research, 2004, 38, 1069-1077

[10] Xie, Y., He, Y., Irwin, P..L, Jin, T., Shi, X., Antibacterial Activity And Mechanism Of Action Of Zinc Oxide Nanoparticles Against *Campylobacter jejuni*, Appl Environ Microbiol. 2011, 77, 2325-31

[11] Li, Y., Zhang, W., Niu, J., Chen, Y., Mechanism of Photogenerated Reactive Oxygen Species and Correlation with the Antibacterial Properties of Engineered Metal Oxide Nanoparticles, ACS Nano, 2012, 6, 5164-5173

[12] Yin JJ., Liu J., Ehrenshaft M., Roberts J.E., Fu PP, Mason RP, Zhao B. Phototoxicity of Nano Titanium Dioxides In Hacat Keratinocytesd Generation Of Reactive Oxygen Species And Cell Damage. Toxicol Appl Pharmacol, 2012 263, 81-8

[13] He, W., Liu, Y., Wamer, W.G., Yin, J., Electron Spin Resonance Spectroscopy For The Study Of Nanomaterial-Mediated Generation Of Reactive Oxygen Species, Journal of Food and Drug Analysis 2014, 22, 49-63

[14] Armstrong, D., Advanced protocols in Oxidative Stress II, Humana Press - New York 2010

[15] Dikalov, S., Griendling, K.K., Harrison, D.G., Measurement of Reactive Oxygen Species in Cardiovascular Studies. Hypertension, Nisan 717-727, 2007

[16] Hempel, S.L., Buetner, G.R., O'Malley, Y.Q, Wessels D., Flaherty, D.M., Dihydrofluorescein diacetate is superior for detecting intracellular oxidants: comparison with 2',7'-dichlorodihydrofluorescein diacetate, 5(and 6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate, and dihydrorhodamine 123. Free Radical Biology & Medicine, Vol. 27, Nos. 1/2, pp. 146–159, 1999

[17] Wang, H., Joseph, J.A., Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. Free Radical Biology & Medicine, 27, 612–616, 1999

[18] Gomes A., Fernandes, E., Lima, J.L.F.C., Fluorescence probes used for detection of reactive oxygen species. J. Biochem. Biophys. Methods, 65, 45–80, 2005

[19] Hoet, P.H., Nemery, B., Napierska, D., Intracellular oxidative stress caused by nanoparticles: What do we measure with the dichlorofluorescein assay? Nano Today 8, 223-227, 2013

[20] Bauma, S.L., Anderson, I.G.M., Baker, R.R., Murphya, D.M., Rowlands, C.C., Electron Spin Resonance And Spin Trap Investigation Of Free Radicals In Cigarette Smoke: Development Of A Quantification Procedure, Analytica Chimica Acta, 2003, 481, 1–13

[21] Kuppusamy, P., Zweier, J. L., Identification and Quantitation of Free Radicals and Paramagnetic Centers from Complex Multi-Component EPR Spectra, Appl. Radiat. Isotopes. 1992, 44, 367-372

[22] Roubaud, V., Sankarapandi, S., Kuppusamy, P., Tordo, P., Zweier J. L., Quantitative Measurement of Superoxide Generation Using the Spin Trap 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide, Analytical Biochemistry 1997, 247, 404–411