# Mechanical properties and antimicrobial performance evaluations for silicone rubber compounds

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In this work, nano-Ag colloids, Ag-based zeolite compound (Zeomic) and BIOCLEANACT™ were used as antimicrobial agents in silicone rubber compounds. The silicone rubber compounds were prepared using a two-roll mill followed by a hydraulic press to vulcanize the rubber compounds before their cure characteristics, mechanical properties and antibacterial performance were assessed. The antimicrobial performance for the silicone rubber compounds was examined through Plate-Count-Agar (PCA) and Drop-Plate-Agar methods, and Halo test. The results suggested that the additions of antibacterial agents increased the cure time except for the Zeomic. Zeomic appeared to give the silicone rubber compound with an improved mechanical properties whereas BIOCLEANACT™ exhibited a most effective antimicrobial agent, considered by the inhibition zone and % reductions of the *Staphylococcus aureus* and *Escherichia coli* for any given contact times. The lightness for the silicone vulcanizates appeared to decrease with increasing nano-Ag content, but to increase in the case of Zeomic and BIOCLEANACT™ agents.

*Keywords*: Silicone rubber, Nano-Ag colloids, Antibacterial activity, Halo test, Plate count agar method.

## Introduction

Bacteria are commonly found in the ground, water and in other living organisms and they can cause diseases and become harmful to the environment, animals and humans. Remarkable examples of such concerns include the bad consequences of food poisoning due to certain strains of *E. coli* being found within bathroom and kitchen. Most species of bacteria can be grouped into 2 categories based on their responses to a laboratory technique called gram staining. [1,2] Silicone rubber is the most widely used among biocompatible rubbers, especially for house-ware applications (children's toys, computer keyboards, phone keypad, ear plugs, ear phones and watch) that have to contact with human body. In such applications, the microbial contaminations are of main concern. The addition of antimicrobial agents into the rubber products is one of the widely referred methods to prevent the silicone rubber products from microbial contaminations.[3] However, existing literatures have clearly indicated that silicone rubber is widely used, but knowledge and understanding of its antibacterial properties are required, especially in relation with the mechanical property changes effected by addition of antibacterial agents. This present work was aimed to explore the mechanical and antibacterial properties of silicone rubber filled with a wider range of antibacterial agents, which included nano-Ag colloids, Ag-based zeolite compound (Zeomic) and BIOCLEANACT™, under a variety of testing conditions. The effects of dosages of antibacterial agents and contact time were of our main interests. The differences in the mechanical and antibacterial property results were discussed and explained in connection with morphological results.

## Experimental

**Materials and Chemicals.** Silicone Rubber (KE-951-U, Shin-Etsu Chemical Co., Ltd., Japan) was used as polymeric matrix, and 2,5-Dimethyl - 2 , 5 - di ( tert - butylperoxy ) hexane (designated as Trigonox®101-45s-ps, supplied by Akzo Noble Polymer Chemicals Ltd., Shanghai, China) was used as a vulcanizing agent. Nano-Ag colloids (supplied by Koventure Co., Ltd., Bangkok, Thailand), Silver

Substituted Zeolite (designated as Zeomic, supplied by Yamamoto Trading Co., Ltd., Thailand) and 2-Hydroxypropyl-3-Piperazinyl-Quinoline Carboxylic Acid Methacrylate (designated as BIOCLEANACT™, manufactured by Micro Science Tech Co., Ltd, South Korea ) were used as the antibacterial agents. *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as testing bacteria.

**Preparation of silicone rubber sample.** The formulation of the silicone rubber compounds was as follows: 100 phr silicone rubber and 0.5 phr 2,5- Dimethyl - 2,5 - di ( tert – butylperoxy ) hexane. The rubber samples were prepared through mastication and compounding processes. The rubber was first masticated on a laboratory two roll mill (Yong Fong Machinary Co., Ltd., Thailand) for 5 min and was then compounded with an antibacterial agents and 2,5-Dimethyl-2,5-di(tert-butylperoxy)hexane for 10 min, and the compounds were then compression-molded at a 90% cure using a hydraulic press (LAB TECH Co., Ltd., Bangkok, Thailand) at pressure of 170 kg/cm$^2$ with a cure temperature of 165$^o$C to produce vulcanized silicone rubber.

**Cure characteristics and crosslink density.** The cure characteristics of the silicone rubber compounds were assessed through delta torque (differences in maximum and minimum torques), cure time and crosslink density of the silicone rubber compounds with the three antimicrobial agents using an Oscillating Disk Rheometer (Model ODR GT 7070-S2, GOTECH Testing Machine, Inc., Taiwan) at a test temperature of 165$^o$C. The crosslink density determination for the vulcanized silicone rubber compounds was carried out with Flory-Rehner method given as Equation 1. [4,5]

$$v = \frac{-[\ln(1-v_2)+v_2+\chi v_2^2]}{2V_1(v_0^{2/3} v_2^{1/3}-v_2/2)} \qquad (1)$$

where $v$ is the moles of crosslinks per unit volume of polymer, $v_2$ is the volume fraction of polymer in the swollen sample, $V_1$ is the molar volume of the solvent and $v_0$ is the volume fraction of polymer at the time of crosslinking. The $v_0$ term is used to correct for material that is extracted by the solvent. $\chi$ is the rubber-solvent interaction parameter (0.499 in this case).

**Mechanical properties.** The tensile properties (tensile modulus at 200% elongation, tensile strength and elongation at break) of the silicone rubber vulcanizates followed ASTM D 412-92(1998) with dumbbell-shaped sample and tested by a universal testing machine (Autograph AG-I, Shimadzu, Tokyo, Japan). A hardness durometer (Shore A) Model 475, PTC instruments,(MA, USA) was used for hardness evaluation in accordance with ASTM D 2240-03 (2003).

**Antibacterial performance.** The antimicrobial performance for the silicone rubber compounds was examined through Halo test, Plate-Count-Agar (PCA) and Drop-Plate methods. The Halo test was initiated by mixing the nutrient agar and the nutrient broth in ratio 1:1 and incubated testing bacterial solution (OD$_{600}$ = 0.1) onto sterilized Petri dishes. The rubber samples (6mm in diameter), with and without antibacterial agents, were gently placed over solidified agar. The Petri dishes were then incubated at 37$^o$C for 24 hr for a zone of inhibition. Plate-Count-Agar (PCA) and Drop-Plate methods followed ASTM E 2149 (2001). Nutrient Broth was used as a growing medium of *E. coli* and *S. aureus* bacteria in overnight inoculums. After that it was diluted by peptone solution and cultivate bacteria 5 ml shaken on a reciprocal shaker at the speed of 100 rpm at 37$^o$C for contacted time of 30, 90, 150 and 210 min, respectively. 100 µl of bacterial solution was placed over the agar into sterilized Petri dishes. The inoculated plates were cultivated at 37°C for 24 h before counting the active bacteria and evaluating the antibacterial efficacies using Equation 2.

$$Reduction, \% \; (CFU/ml) = \frac{B-A}{B} \times 100 \qquad (2)$$

where A is CFU per milliliter for the flask containing the treated substrate after the specified contact time, and B is CFU per milliliter for the flask before the addition for the treated substrate.

**Discoloration testing.** UV-Vis Spectrophotometer was used to measure color changes of silicone rubber samples the CIELAB color system. L*a*b* coordinates of silicone rubber specimens were calculated based on a D65 light source. L* represents the lightness whereas a* and b* are the chromaticity coordinates. In this work, only lightness change of the silicone rubber with various loadings of nano-Ag colloid, Zeomic and BIOCLEANACT™ agents were of our interests. The higher the L* value the lighter the sample.

## Results and Discussion

**Antibacterial performance.** Fig. 1a and 1c show the results of inhibition zone by halo test for silicone rubber vulcanizates added with 15 phr of nano-Ag colloid, Zeomic and BIOCLEANACT™ agents against *E. coli* and *S. aureus*, respectively. It was found that silicone rubber vulcanizates with nano-Ag colloid and Zeomic exhibited no inhibition zone, only the vulcanizate with BIOCLEANACT™ sample possessed the inhibition zones for both *E. coli* and *S. aureus*. Fig. 1b and 1d show the effects of BIOCLEANACT™ concentration on the inhibition zone for the silicone rubber vulcanizates.

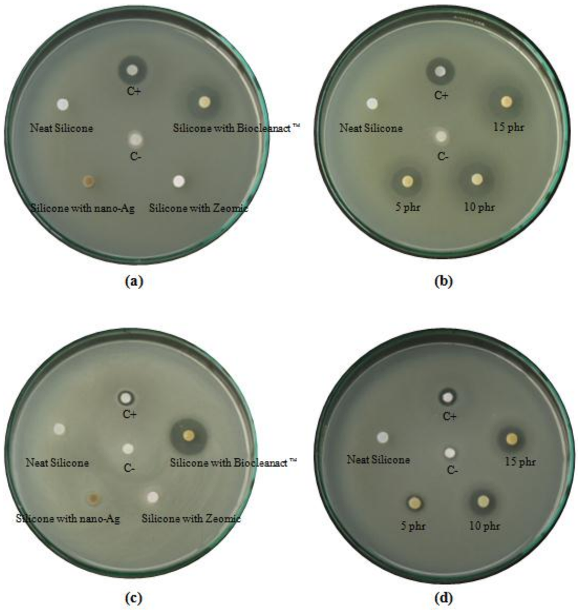

**Figure 1** Effect of type and loading of antibacterial agents on clear zone by Halo test. (a) Silicone rubber without and with various antibacterial agents at 15 phr loading for *E.coli* (b) Silicone rubber with BIOCLEANACT™ at 5, 10 and 15 phr loadings for *E.coli* (c) Silicone rubber without and with various antibacterial agents at 15 phr loading for *S. aureus* (d) Silicone rubber with BIOCLEANACT™ at 5, 10 and 15 phr loadings for *S. aureus.*

It was observed that the greater the BIOCLEANACT™ concentration the greater the radius of the inhibition zone, the most pronounced effect being observed at 10 phr of BIOCLEANACT™. The occurrence of the inhibition zone for BIOCLEANACT™ clearly suggested that the BIOCLEANACT™ was a diffusible antibacterial agent, and could inhibit the bacteria growth.

The quantitative results for antibacterial performance for these three agents are given in Fig. 2 and 3 which show the viable colony count for *E. coli* and *S. aureus*, respectively, under a wide range of concentrations and contact times. It was observed that the silicone rubber vulcanizates with nano-Ag colloid and Zeomic had very similar viable cell counts for any given concentrations and contact times. The *E. coli* and *S. aureus* growths appeared to increase with increasing contact times, indicating no bacteria killing. On the other hand, the *E. coli* and *S. aureus* growths in the rubber samples with BIOCLEANACT™ significantly decreased with the addition of BIOCLEANACT™ at 5 phr loading, and with increasing contact time. The results in Fig. 1-3 clearly suggested that the silicone rubber vulcanizates with BIOCLEANACT™ was the most effective antibacterial agent among the three agents used in this work. The reasons for this would be associated with that, BIOCLEANACT™ is an organic substance that is soluble in water, but nano-Ag colloid and Zeomic are inorganic substances that are non-soluble in water [3,4]. The BIOCLEANACT™ filled or trapped in the silicone vulcanizate sample during being shaken in peptone solution could be soluble, diffuse and eventually release to attack the cultivated bacteria in the peptone solution while the trapped nano-Ag colloid and Zeomic in the silicone rubber vulcanizates could not release from the sample to kill the bacteria. Besides, the SEM micrographs in Fig. 4 show that the nano-Ag colloid and Zeomic seemed to agglomerate and were trapped in the silicone rubber matrices whereas the BIOCLEANACT™ exhibited a well-dispersed characteristic around the silicone matrix. It has been well-documented [5,6] that agglomerations of the nano-particles, nano-Ag

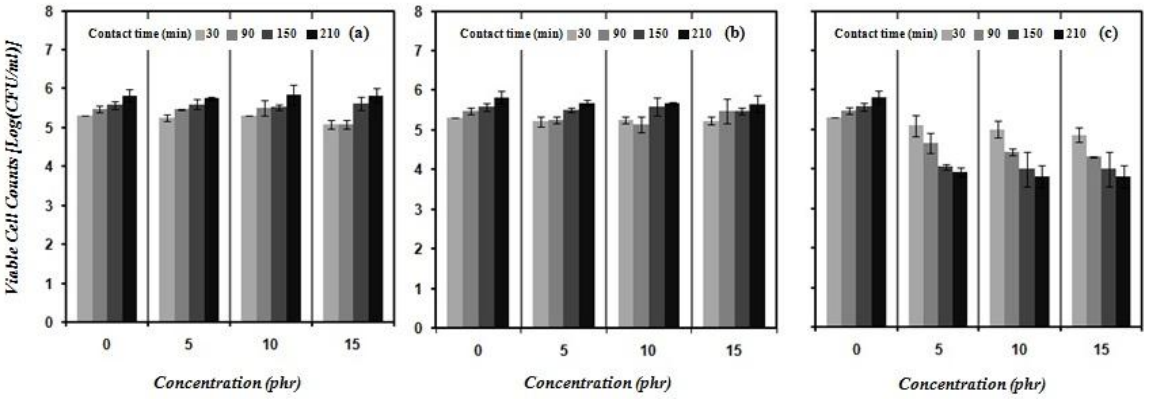

**Figure 2** Viable Cell Count for *E. coli* colonies as a function of antimicrobial loading. (a) Nano-Ag colloid, (b) Zeomic, and (c) BIOCLEANACT™.

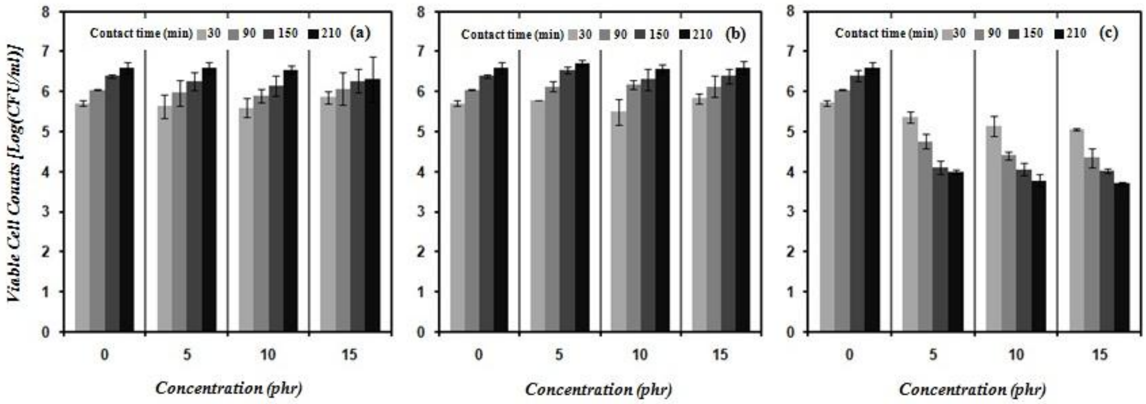

**Figure 3** Viable Cell Count for *S. aureus* colonies as a function of antimicrobial loading. (a) Nano-Ag colloid, (b) Zeomic, and (c) BIOCLEANACT™.

colloid and Zeomic in this case, would result in significant reductions of antibacterial performance for any antibacterial agents. The well dispersion character of the BIOCLEANACT™ would therefore ease the diffusion process of the BIOCLEANACT™ from the rubber sample to kill the bacteria in the peptone solution. In order to consider the antibacterial efficacies of the nano-Ag colloid, Zeomic and BIOCLEANACT™ agents added in the silicone vulcanizates in conjunction with the effects of loading and contact time, percentage reductions of bacteria colonies by PCA method would probably be useful, and the results are given in Tables 1 and 2 for *E. coli* and *S. aureus*, respectively. It was found that there were no definite trends for the percentage reductions of *E. coli* and *S. aureus* colonies for the silicone samples with the nano-Ag colloid, Zeomic agents, whereas increasing BIOCLEANACT™ yielded positive and progressive reductions of *E. coli* and *S. aureus* colonies to 99.9% when the loading had reached 15 phr at 150 min contact time. The maximum percentage reductions of *E. coli/S. aureus* colonies for the silicone samples with nano-Ag colloid and Zeomic were 46.3/59.7% and 39.7/53.27%, respectively. These percentage reductions were not acceptable as the residual bacteria could then grow for further period of times. Another aspect to consider was that for any given BIOCLEANACT™ loadings, *E.coli* appeared to be more sensitive to the BIOCLEANACT™ than *S.aureus*.This may be associated with the peptidoglycan thickness of *S. aureus* structure that is far wider than that of *E. coli* structure. This would then be more difficult for BIOCLEANACT™ to penetrate into the cell to kill the bacteria. [7].

**Mechanical properties.** Table 3 shows the effect of antibacterial agent contents on cure characteristics (cure time, delta torque and cross-link density for the silicone rubber vulcanizates with nano-Ag colloid, Zeomic and BIOCLEANACT™ agents for different loadings.

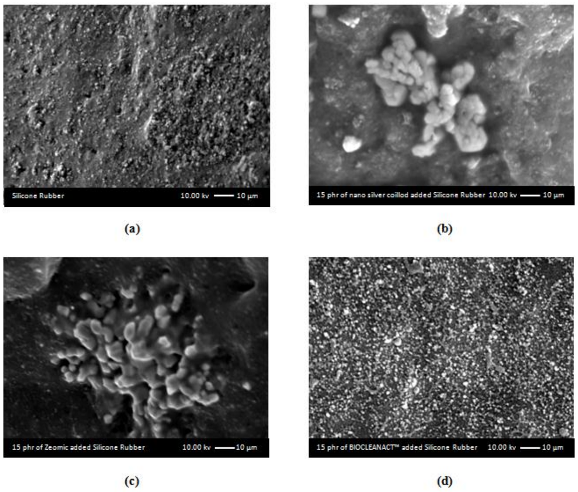

**Figure 4** SEM micrographs (×10,000) for silicone rubber vulcanizates added with antibacterial agents. (a) No antibacterial agent; (b) 15 phr nano-Ag colloid; (c) 15 phr Zeomic; (d) 15 phr BIOCLEANACT™.

**Table 1** Percentage reduction of *E. coli* colonies as a function of the concentration of nano-Ag colloid, Zeomic, BIOCLEANACT™ added in silicone rubber vulcanizates.

| Compound | | | Reduction of bacteria (%) | | | |
|---|---|---|---|---|---|---|
| | | | Contact time (min) | | | |
| | | | 30 | 90 | 150 | 210 |
| Silicone Rubber with nano-Ag colloid | content (phr) | 5 | 16.8 | 14.9 | 25.9 | 1.5 |
| | | 10 | 22.4 | 29.2 | 42.5 | 8.8 |
| | | 15 | 0.6 | 0.6 | 24.1 | 46.3 |
| Silicone Rubber with Zeomic | content (phr) | 5 | 0.6 | 0.6 | 0.6 | 0.6 |
| | | 10 | 39.7 | 0.6 | 18.3 | 5.6 |
| | | 15 | 0.6 | 0.6 | 0.6 | 0.6 |
| Silicone Rubber with BIOCLEANACT™ | content (phr) | 5 | 54.6 | 94.9 | 99.5 | 99.7 |
| | | 10 | 72.8 | 97.7 | 99.5 | 99.8 |
| | | 15 | 77.9 | 98.0 | 99.6 | 99.9 |

In general, it was found that additions of antibacterial agents increased the cure time except for the Zeomic. This suggested that the additions of nano-Ag colloid and BIOCLEANACT™ agents interfered with the vulcanizing reaction. The interference of the nano-Ag colloid caused a reduction in crosslink density whereas that of Zeomic and BIOCLEANACT™ agents led to an increase in crosslink density of the vulcanizates, the effect being more pronounced with the Zeomic agent. The crosslink density changes due to the presences of the three antibacterial agents were very important as they directly affected the mechanical properties of the silicone rubber vulcanizates.

**Table 2** Percentage reduction of *S. aureus* colonies as a function of the concentration of nano-Ag colloid, Zeomic, BIOCLEANACT™ added in silicone rubber vulcanizates.

| Compound | | | Reduction of bacteria (%) | | | |
|---|---|---|---|---|---|---|
| | | | Contact time (min) | | | |
| | | | 30 | 90 | 150 | 210 |
| Silicone Rubber with nano-Ag colloid | content (phr) | 5 | 12.9 | 2.3 | 0.6 | 15.9 |
| | | 10 | 0.6 | 0.6 | 10.9 | 0.6 |
| | | 15 | 39.7 | 59.7 | 0.6 | 1.1 |
| Silicone Rubber with Zeomic | content (phr) | 5 | 17.8 | 41.8 | 15.9 | 27.6 |
| | | 10 | 12.9 | 53.2 | 0.6 | 29.2 |
| | | 15 | 15.0 | 1.2 | 21.5 | 32.4 |
| Silicone Rubber with BIOCLEANACT™ | content (phr) | 5 | 36.9 | 62.8 | 97.0 | 98.7 |
| | | 10 | 49.3 | 84.5 | 97.4 | 99.1 |
| | | 15 | 49.3 | 93.1 | 97.4 | 99.1 |

The mechanical properties for the silicone rubber vulcanizates filled with nano-Ag colloid, Zeomic and BIOCLEANACT™ agents were expressed by tensile properties and hardness and the results are given in Fig. 5, showing the tensile modulus at 200% elongation and hardness, and tensile strength and elongation at break, respectively. It can be seen that the tensile modulus at 200% elongation and hardness of the rubber vulcanizates with Zeomic and BIOCLEANACT™ gradually increased with increasing antibacterial agent content. These results corresponded well to the crosslink density results given in Table 3. It has been widely known that the crosslink density and small deformation properties (modulus and hardness) are in a direct relationship [1,2] If this was the case, the decreases in the tensile modulus and hardness for the silicone rubber vulcanizates with nano-Ag colloid could then be explained by the decrease in crosslink density results in Table 3. Fig. 6 shows the results of ultimate tensile strength and elongation at break for the silicone rubber vulcanizates at different loadings of nano-Ag colloid, Zeomic and BIOCLEANACT™ agents. results in Table 3.

Fig. 6 shows the results of ultimate tensile strength and elongation at break for the silicone rubber vulcanizates at different loadings of nano-Ag colloid, Zeomic and BIOCLEANACT™ agents.

**Tables 3** Cure time, delta torque and crosslink density for silicone rubber vulcanizates with nano-Ag colloid, Zeomic and BIOCLEANACT™.

| Properties | Compound with anti-bacterial agent content (phr) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Neat Silicone Rubber | Nano Ag colloid | | | Zeomic | | | BIOCLEANACT™ | | |
| | | 5 | 10 | 15 | 5 | 10 | 15 | 5 | 10 | 15 |
| Cure time (min:sec) | 2:39 | 2:42 | 3:01 | 3:21 | 2:20 | 2:13 | 2:11 | 2:55 | 3:00 | 3:06 |
| Delta torque (dN/m) | 50.10 | 49.62 | 47.25 | 45.89 | 52.07 | 54.37 | 54.71 | 46.24 | 43.79 | 40.40 |
| Crosslink density ($\times 10^{-4}$ mole/cm$^3$) | 2.94 | 2.25 | 1.97 | 1.91 | 3.27 | 3.67 | 4.07 | 2.97 | 3.21 | 3.20 |

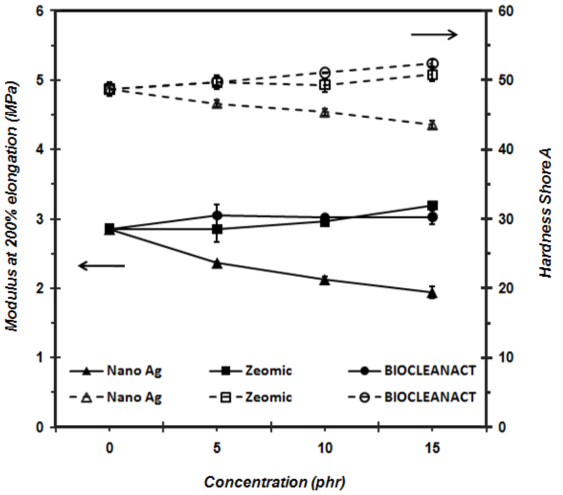

**Figure 5** Tensile modulus at 200% elongation and hardness Shore A for silicone rubber vulcanizates filled with nano-Ag colloid, Zeomic and BIOCLEANACT™.

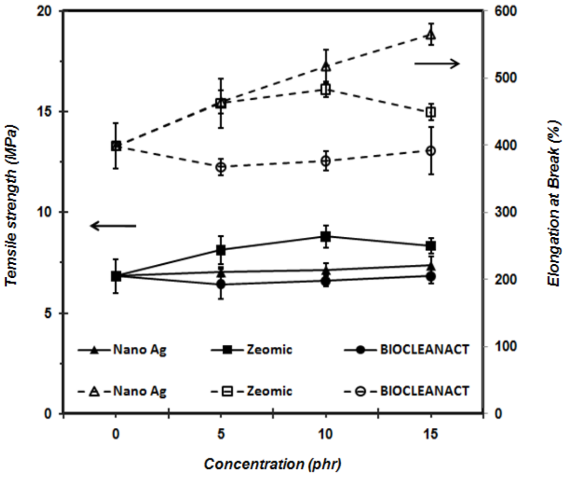

**Figure 6** Tensile strength and Elongation at break for silicone rubber vulcanizates filled with nano-Ag colloid, Zeomic and BIOCLEANACT™.

It was found that the additions of nano-Ag colloid and Zeomic improved the tensile strength and elongation at break, whereas those of BIOCLEANACT™ did not affect the ultimate mechanical properties. It should be noted that the decrease in elongation at break was observed after adding the Zeomic above 10 phr. This was because of too high crosslink density. The results in Fig. 6 clearly show that Zeomic agent gave the most improving effect on the mechanical strength of the silicone rubber vulcanizates although its antibacterial performance was inferior to the BIOCLEANACT™ agent. The increased mechanical properties for Zeomic were caused not only by the crosslink density increase, but also by the porous structure of Zeomic agent, The latter reason was that, the rubber molecules could penetrate and resided within the porous structure of the Zeomic and formed physical entanglements within the Zeomic structure, this improving resistances to the external forces during the tensile testing. It would be interesting to consider the physical appearances of the silicone rubber products if they were incorporated by the nano-Ag colloid, Zeomic and BIOCLEANACT™ agents. Table 4 shows the color changes for silicone rubber vulcanizates after incorporating with nano-Ag colloid, Zeomic and BIOCLEANACT™ agents by considering lightness (*L**) coordinate. It was found that the lightness (*L**) for the silicone rubber vulcanizates increased with increasing Zeomic and BIOCLEANACT™ contents, but decreased with increasing nano-Ag colloid. These color changes were due to the initial colors of all the antibacterial agents used.

**Table 4** Lightness changes for silicone rubber vulcanizates with nano-Ag colloid, Zeomic and BIOCLEANACT™.

| Compound | | | Lightness (L*) |
|---|---|---|---|
| Neat Silicone Rubber | | | 46.66 |
| Silicone Rubber with nano -Ag colloid | content (phr) | 5 | 37.94 |
| | | 10 | 32.24 |
| | | 15 | 30.29 |
| Silicone Rubber with Zeomic | content (phr) | 5 | 74.90 |
| | | 10 | 77.81 |
| | | 15 | 77.69 |
| Silicone Rubber with BIOCLEANACT™ | content (phr) | 5 | 54.25 |
| | | 10 | 65.61 |
| | | 15 | 66.73 |

## Conclusions

Nano-Ag colloids, Ag-based zeolite compound (Zeomic) and BIOCLEANACT™ were loaded at different amounts into silicone rubber compounds, and their antibacterial performance, cure characteristics and mechanical properties were monitored. The results suggested that for the nano-Ag colloids, the crosslink density decreased with increasing nano -Ag colloid content. The nano-Ag colloid of 15 phr gave a positive reduction of bacteria of 46.3% at the contact time of 210 min. Zeomic was found to enhance the overall mechanical properties of the silicone rubber compound whereas BIOCLEAN-ACT™ exhibited the most effective antimicrobial agent amongst the three antibacterial agents used in this work. This claim could be substantiated by obvious appearance of inhibition zone and higher percentage reductions of the *S. aureus* and *E. coli* for any given contact times. The additions of these three antibacterial agents were found to affect the color changes of the silicone rubber compounds. The nano-Ag colloid resulted in a decrease in vulcanizate lightness, but the opposite effect was seen for Zeomic and BIOCLEANACT™.

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