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Production of safe and antimicrobial ethylene propylene diene monomer (EPDM) rubber vulcanizates for toys and medical applications

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Abstract

The present work deals to prepare safety rubber goods for medical and kids toys applications. The rubber used in this study was ethylene propylene diene monomer (EPDM). The incorporated additives used to obtain promising results for safety rubber goods are ZnO of particle size 26 nm, potassium alum and tetracycline. Two fillers were used like $CaCO_3$ and bentonite. The rubber and its specified ingredients were mixed through the ordinary industrial mixer applied. The compounded rubber was vulcanized by a hydraulic press according to the time determined from the rheological test. The physico-mechanical properties and the cytotoxicity tests of the EPDM rubber vulcanizates were evaluated.

The results showed that rheological properties of the vulcanizates depend on the type of filler; $CaCO_3$ and bentonite used. The scorch time decreased with increasing filler concentrations. The minimum and maximum torque as well as the cure rate index were increased with increasing the filler concentration. The data obtained showed the improvement of the physicomechanical properties of the vulcanizates in presence of 10-20 phr fillers . Moreover, there is no great differences in the rheological and physicomechanical properties of the prepared vulcanizates in the presence of the bioactive materials such as ZnO, potassium alum, and tetracycline due to their good compatibility and distribution of these materials in the vulcanizates as clarified from the results of the scanning electron microscope (SEM). The results of cytotoxicity test on the prepared vulcanizates towards human normal cell lines showed good safety property therefore, the cytotoxicity activity of EPDM/Bentonite at 100 ppm was about -58.5 % , while, the antimicrobial activity of EPDM/Bentonite/2.5 ZnO towards Gram -ve or Gram +ve bacteria was good. These promising results push us to use EPDM rubber vulcanizates for producing safe and antimicrobial rubber goods in various industrial and medical applications.

Keywords: Antimicrobial, safety, ethylene propylene diene monomer (EPDM), cytotoxicity, medical applications.

Introduction

EPDM, is an elastomer, has unique mechanical and chemical properties. It can be used in many industrial applications. It may be utilized to prepare system structures of versatile properties. Microorganisms live creatures, as an example, microscopic organisms, growths, and parasites, that are the critical sources of contaminations [1]. Irresistible diseases result from morbid microorganisms hat murder many people worldwide [2]. Albeit varied antimicrobial medications are created to murder or restrain microbes, many irresistible infections keep onerous to treat [3,4]. Antimicrobial polymers represent a very promising class of therapeutics with unique characteristics for fighting microbial infections [5]. As the classic antibiotics exhibit an increasingly low capacity to effectively act on microorganisms, new solutions must be developed [6]. The importance of this class of materials emerged from the uncontrolled use of antibiotics, which led to the advent of multidrug-resistant microbes, being nowadays one of the most serious public health problems [7].

The rubber component of the inventive rubber formulation and cured articles is preferably selected from the group consisting of nitrile rubber [such as acrylonitrile-butadiene rubber (NBR), ethylene propylene diene monomer (EPDM) rubber, hydrogenated NBR, carboxylated NBR, and blends of them. It is important to consider the desired physical properties of the rubber article for the production of rubber goods. For example, high molecular weight EPDM polymers tend to exhibit higher green strength and tensile strength, lower compression set compared to lower molecular weight polymers.

Nowadays, the researchers are focused on the improvement of the antimicrobial activity of

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polymers, more particularly for those used for manufacturing of toys and medical products (e.g. vulcanized rubber compounds) [8] .Antimicrobial agents are the greatest contribution of the present century to create novel antibacterial rubber vulcanizates. ZnO in addition to its antimicrobial activity has influence on the improvement of rubber compound properties [9]. It enhances rubber vulcanizates efficiency, properties and reduces the vulcanization time. Therefore, the present work is cornered with using ZnO as an antimicrobial agent to reduce the toxicity of rubber goods [10].

Moreover, other workers studied the antibacterial activity of aluminum potassium sulfate (alum) against different types of pathogenic bacteria. It was found that alum exhibited antibacterial activity and inhibition growth of gram- positive and gram-negative bacteria isolated from different sites of infection [11,12].

The organic antimicrobial agent Tetracycline is a broad-spectrum antibiotic with a wide range of activity against both gram-positive and gram-negative bacteria. P.aeruginosa is less sensitive but is generally susceptible to tetracycline.Tetracycline inhibits protein synthesis on isolated 70S or 80S (Eukaryotic) ribosomes, bacteriostatic, and in both cases, their effect is on the small ribosomal subunit. However, most bacteria possess an active transport system for tetracycline that allow intracellular accumulation of the antibiotic at concentrations50 times as great as that in the medium. This enhances its antibacterial effectiveness and accounts for its specificity of action, since an efficiency concentration cannot be accumulated in animal cells.The combination of their broad- spectrum and low toxicity has led to their overuse and misuse by the medical community [13].

The organic antimicrobial agent is incorporated in rubber formulation in an amount from about 0.001 to 20 wt% of the total weight of the rubber formulation [8].

The aim of the present work is to prepare antimicrobial rubber vulcanizates for safety toys and medical application and to study the parameters affecting the properties of the investigated EPDM rubber vulcanizates as the type, concentrations, and the dispersions of the applied antimicrobial agents as well as evaluation of the antimicrobial activity and the cytotoxicity effect on human normal cell lines.

2. Materials

- Ethylene propylene diene monomer (EPDM), ethylene content 55%, the density is 0.86 g/cm3 is produced by Esso Chemi, Germany .
- Zinc oxide (ZnO) with specific gravity 5.6 at 15oC and particle size 26 nm.

Stearic acid with specific gravity 0.9-0.97 at 15oC.

- Elemental sulfur: fine pale yellow powder with a specific gravity 2.04-2.06 at room temperature (25 oC± 1).
- N-cyclohexyl-2-benzothiazole sulphenamide (CBS) with specific gravity 1.27- 1.31 at room temperature (25 oC± 1), and melting point is 95-100 oC.
- Polymerized 2,2,4-trimethyl-1,2dihydroquinoline (TMQ) was used as antioxidant.
- Calcium carbonate was chosen as a filler with specific gravity 2.6531 at room temperature (25 oC± 1),Particle size is 3.25µm, and surface area 3.6m2/g.
- Sodium bentonite clay with cation exchange capacity (CEC), 88 mequiv/100 g and surface area of 39 m2/g. (product code B3378; Munich, Germany).

- Antimicrobial agents: ZnO, potassium alum KAl(SO4)2•12H2O and Tetracycline.

- Buffer solutions: pH value was adjusted using hydrochloric acid (HCl) and sodium hydroxide (NaOH). Distilled water and analytical reagents were used for the preparation of all solutions. All experiments were performed at room temperature (□25°C) and all matrices were kept in a buffer solution at room temperature when not in use.

All these materials are supplied by Sigma–Aldrich, German

Preparation of the investigated rubber compounds:

The EPDM rubber mixes were prepared as illustrated in Table 1 by using a laboratory two-roll mill, where outside diameter= 470 mm, working distance= 300 mm,speed of slow roll= 24 rpm with a gear ratio of 1:1.4. The formulations of the EPDM rubber mixes are illustrated in Table 1. The ingredients were added in the same order as listed in Table 1, and mixed at room temperature.

Vulcanization process: The prepared EPDM rubber mixes were left overnight before vulcanization. Then they were compressed in a stainless steel mold under 4 MPa pressure using a hydraulic press at 152 oC (Mackey Bowley, C1136199) according to their respective cure time (e.g. Tc90) that was determined with TA instruments, MDR one [Moving Die Rheometer], USA.

Characterization:

Morphology Study:

The surface morphology of the prepared rubber mixes were investigated using scanning electron microscopy (SEM, (JEOL JX 840, Japan). To prepare the SEM sample, a thin layer of Au was coated onto the specimens. Prior to observation, all the fracture surfaces for the selected samples were obtained by breaking the specimens.

Mechanical properties:

Tensile properties of the EPDM rubber vulcanizates were determined using Zwick tensile testing machine (Z 010) Germany according to ASTM D 412 at a crosshead speed of 500 mm/min. Dumbell shape tensile specimens with 1-mm thickness were cut from the molded sheets with a Wallace die cutter, S6/1/6.A. The tests were performed at room temperature.

Cytotoxic activity test

The rubber vulcanizates loaded with different ingredients were tested towards the human tumor cell line: RPE1 [normal retina cell line]using MTT assay as follow:

Cytotoxicity Test:

The examined samples were prepared via the following steps:

a. Washing:

Strips from different rubber samples were washed with distilled water to remove soil effectively as well as leachable materials.

b. Drying and sterilization:

Washed strips were dried and sterilized by suspending in 70% ethanol for 10 min before the experiment.

c. Cell culture:

Human normal cell line: RPE1[normal retina cell line] was cultivated and maintained in RPMI1640 medium (Sigma-Aldrich, St Louis, MO) supplemented with 10% fetal calf serum (FCS), 100 U mL21penicillin, 100 IL mL21 streptomycin, and 2 mmol L21L-glutamine (Cambrex Bio Science, Verviers, Belgium) at 37oC in a humidified atmosphere of 95% air and 5% CO2as was described [14 Farajnia et al 2008]. Sub-cultivation was performed with cells from confluent cultures treated with 0.2 g L21 ethylene diaminetetraacetic acid (EDTA) in phosphate buffered saline (PBS).

d. Cell cytotoxicity MTT assay:

The rubber samples strips (10 mg rubber weight) were immersed in RPMI1640 media and agitated for 48 and 72 h at 37□C according to ISO standard 10993-12 [15]. The control samples (negative control)which contained only medium, were similarly treated.

Human normal cell line: RPE1[normal retina]:cells were diluted in a fresh medium containing 2%,5%, and 10% of FCS and seeded into 96-well plates (104cells well 21). After incubation for 24 h, the medium was aspirated from all wells and replaced by EPDM vulcanizates or control medium and incubated for another 24 h before checked cytotoxicity. The colorimetric MTT assay developed by Mosmann [16] and modified by Edmondsonet al. [17] was used as a test for cell proliferation and survival assay. 20 mL MTT dye (5 mg mL 21 in PBS) was added to each well and incubated at 37□C, in air containing 5% CO2 and at 95% relative humidity for 4 h in the dark. After incubation, the MTT was aspirated and the formazan product was dissolved in 100 mL of the acidified isopropanol (0.04 N HCI in isopropanol). The plates were shaken before the optical densities (OD) were measured at 570 nm wavelength. Three tests for each rubber sample and control were performed in each experiment. All assays were repeated at least twice to ensure reproducibility.

The cytotoxic activity test (In vitro bioassay on human normal cell lines) was conducted and determined by the Bioassay-Cell Culture Laboratory, National Research Centre, 33 El-Behouth St., Dokki, Giza 12622, Egypt.

Antimicrobial activity

Toxicity test

Rubber vulcanizates loaded with different ingredients and antibiotics were individually tested against a panel of gram-positive and gram-positive bacterial pathogens and yeast. Bacillus subtillus ATCC6633 and Staphelococcusaureu, are G+ve, while E. coli ATCC 25922 and Pseudomonas. aeroginosa ATCC27953 are G-ve pathogenic bacteria, Candida albicansNRRL Y-477and Saccharomyces cervesiATCC 29213 are the tested pathogenic yeast strains used for detection the toxicity of the investigated rubber vulcanizates . Antimicrobial tests were carried out by the agar well diffusion method [18-19] using 100 µL of suspension containing 1 x108 CFU/mL of pathological tested bacteria ,1 x106 CFU/ml of yeast spread on nutrient agar (NA) and Sabourand dextrose agar (SDA) respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and each well was loaded with a piece of different rubber vulcanizates (5 mm2) and placed on the solidified agar. The inculcated plates were then incubated for 1week at 37 °C. At the end of incubation time, the lethal effect of the tested rubber vulcanizates towards bacteria was evaluated by measuring the inhibition zone around the rubber vulcanizates pieces. Toxicity effect was expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Results and Discussion

Fillers are powder particles added to rubber material to lower the consumption, cost and to improve some properties as density, hardness, tensile strength, impact strength, chemical resistance, heat distortion temperature, processability and even the appearance of the final products.

Processability studies:

The processability studies of the prepared EPDM rubber composites containing fillers are important since the final vulcanized product is to be molded. The processability of composites was determined by evaluating the rheological characteristics such as

minimum torqueML, maximum torque MH, scorch time ts2, and optimum cure time tc90. Determination of the curing time is a prerequisite for molding the rubber compounds. The variations in cure time for different rubber loading fillers were studied. The curing or vulcanization time was determined for each prepared formulation. The formulations and their ingredients as well as results of rheometric characteristics are illustrated in Table (1).

Minimum torque is an indirect measure of the viscosity of the compounded rubber, or it can be generally treated as the measure of the stiffness of the unvulcanized rubber compound at the lowest point of the curve. From the table it is shown that, the minimum torque increases with increasing loadings of the fillers. As the torque is directly proportional to the stiffness, the results indicated that the increasing of fillers in rubber matrix would increase the stiffness of the vulcanizates. Therefore the incorporation of the filler in rubber matrix could have reduced the mobility of the macromolecular chains of the compounded rubber under investigation.

Also maximum torque (MH), increases with increasing filler contents, where MH gives an idea about the shear modulus of the fully vulcanized rubber at the specified vulcanization temperature [20]. These observations confirm that the addition of fillers to EPDM rubber affect the processability as a reinforcement agent for the matrix.

On the other hand, scorch time ts2 of rubber mixes decreased with increasing fillers content. The decrease in scorch time ts2 as the fillers content increased may be due to the greater thermal history of these compounds during mixing as a result of their higher viscosities. It is known that, the shear heating during mixing increases when fillers loading are increased due to the increase of viscosity [21]. On the other hand, Cure Rate Index CRI increases due to the presence of fillers that supports the activation of the curing reaction. [22].

Physicomechanical properties of EPDM vulcanizates

Figures (1,2) show the effect of filler concentrations, $CaCO_3$ and Bentonite, on tensile strength and elongation at break of the EPDM vulcanizates. $CaCO_3$ and Bentonite were chosen due to their safety and good dispersion properties. It was noticed that, the tensile strength increased with increasing the concentration of fillers.

The tensile strength increased in the presence of fillers up to (10-20)phr then decreased by increasing their concentrations.

The increasing of tensile strength may be due to better filler dispersion which reflect good filler-rubber interaction. Strong rubber-filler interaction would increase the effectiveness of the stress transferred from rubber matrix to filler particles dispersed in the rubber matrix. On the other hand, the decrease of the tensile strength at higher filler content can be related to the poor dispersion of the filler as a result of agglomeration of the particulate filler .

Fig. (2) shows the influence of fillers, CaCO3 and Bentonite, on the elongation at break of the EPDM vulcanizates. It is clear that the values of elongation at break increased until (10-20) phr then decayed.

The previous results showed the improvement of the physico mechanical properties of EPDM rubber vulcanizates at a filler concentration up to 10-20 phr, and can be used as binding matrix for the antibacterial for producing safe rubber products as toys, packaging products, ..etc.

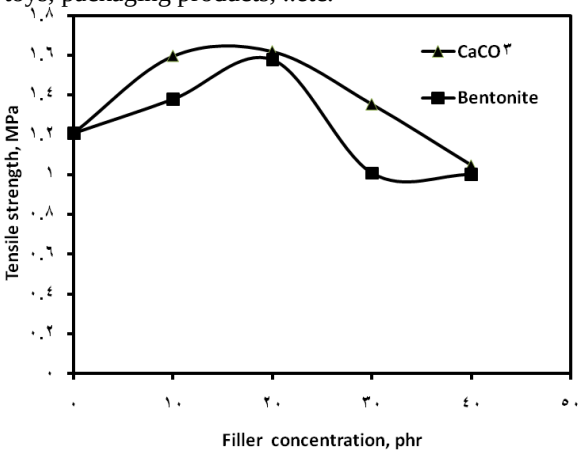


Fig. (1): Effect of filler type and concentration ($CaCO_3$ and Bentonite) on the tensile strength of EPDM vulcanizates

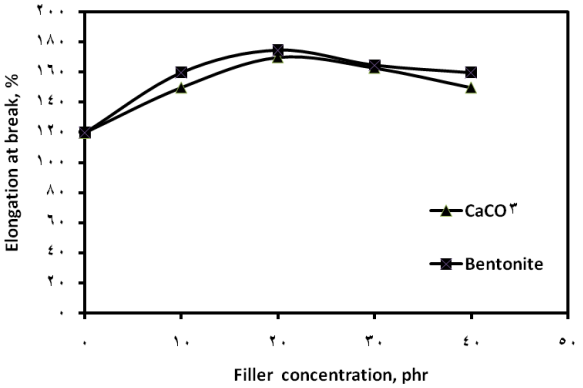


Fig. (2): Effect of filler type and concentration (CaCO3 and Bentonite) on the elongation at break (%) of EPDM vulcanizates

EPDM vulcanizates loaded with different fillers and antimicrobial agents

The investigated rubber (EPDM) were compounded with the essential ingredients and the chosen fillers (20 phr) as well as the different concentrations of antimicrobial materials as ZnO, potassium alum $KAl(SO_4)_2.12H_2O$ and tetracycline.

The rheological properties of EPDM rubber formulations reinforced with $CaCO_3$ and bentonite

fillers and containing the antimicrobial agents are illustrated in Tables (2-7).

It was clear that increasing the concentration of ZnO as antimicrobial agent has a slight (negligible) effect on the rheological properties of EPDM vulcanizates.

On the other hand, the incorporation and increasing the concentration of potassium alum and tetracycline had moderate change in the rheological properties of EPDM vulcanizates.

The physicomechanical properties

The physico-mechanical properties of the investigated vulcanizates were tested and the obtained results are presented in tables (8- 10).

The physico-mechanical properties of the prepared EPDM rubber vulcanizates were good and no great change was observed by incorporation of different concentrations of ZnO as shown from the summarized results in Table (8) .

The obtained results illustrated there is no great differences in the physico mechanical properties due to the presence of different concentrations of ZnO. Therefore, all these results were good.

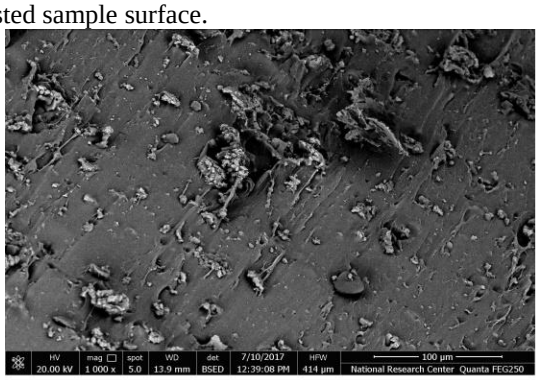
It was found from the data in Tables (9& 10) that, the presence of potassium alum and tetracycline not change the physicomechanical properties of the tested vulcanizates. The physicomechanical properties of the prepared EPDM rubber vulcanizates were good and no great change was observed by incorporation of different concentrations of ZnO, potassium alum and Tetracycline.

The morphological characteristic of the investigated EPDM rubber vulcanizates:

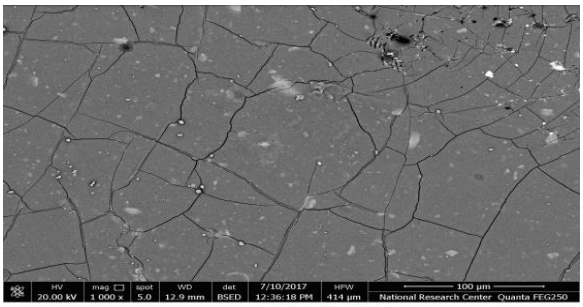
SEM was used to study the morphological characteristic of the investigated EPDM vulcanizates filled with 20 phr fillers, CaCo3 and bentonite, and containing different types of investigated active antimicrobial agents such as ZnO , potassium alum and Tetracycline.

Fig.(3&4) illustrate the obtained SEM images of the examined samples.

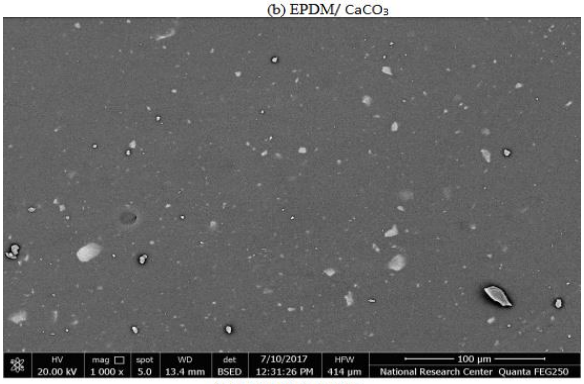
The micrographs depict the good dispersion and distribution of all ingredients. The rough surfaces indicate good miscibility for all EPDM rubber with their additives and the incorporated fillers through the tested sample surface.



(a) EPDM

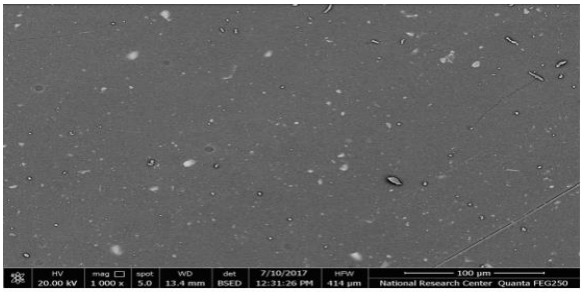


(b) EPDM/ $CaCO_3$

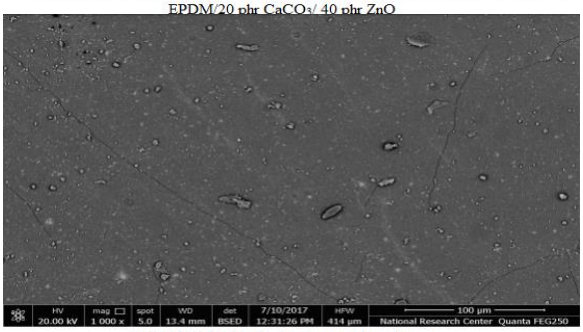


(c) EPDM/ Bentonite

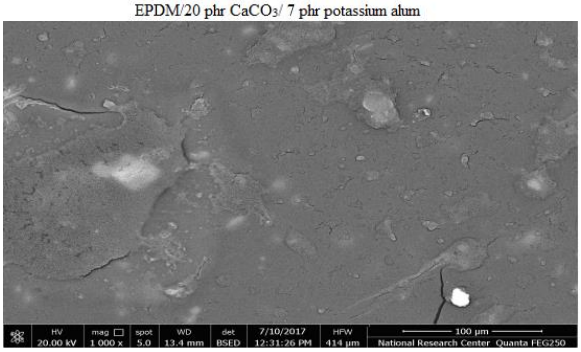
Fig. (3): SEM photographs of (a) EPDM, (b) EPDM/20 phr $CaCO_3$ and (c) EPDM/20 phr Bentonite



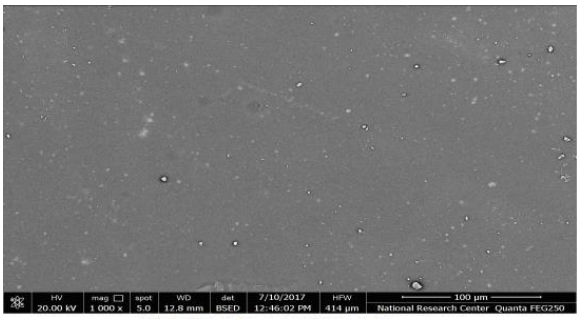
EPDM/20 phr $CaCO_3$/ 40 phr ZnO



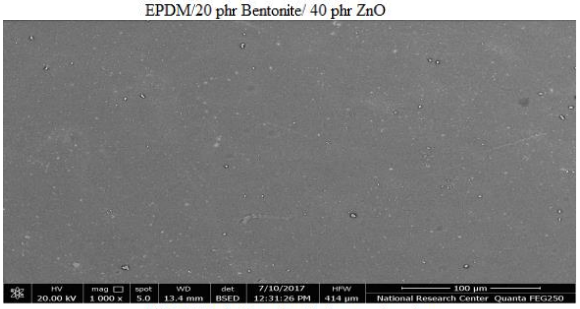
EPDM/20 phr $CaCO_3$/ 7 phr potassium alum



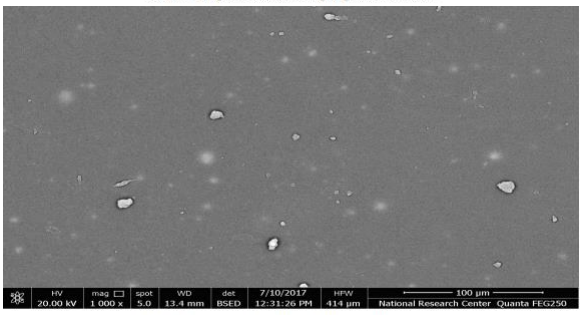
EPDM/20 phr $CaCO_3$/ 0.7 phr Tetracycline



EPDM/20 phr Bentonite/ 40 phr ZnO



EPDM/ 20 phr Bentonite / 7 phr potassium alum



DM/20 phr Bentonite / 0.7 phr Tetracycline

Fig.(4): SEM for the investigated rubber (EPDM) vulcanizates containing various antimicrobial agents

Results

Cytotoxic activity test

The results of the cytotoxic effect of rubber vulcanizates loaded with different ingredients towards the human normal cell line: RPE1 [normal retina cell line]are illustrated in Table (11). The investigated rubber vulcanizates without antimicrobial agents were tested against normal cell line RPE1, and it was found that they are safe and nil in cytotoxic effect as shown in Table (11).

The results of the tested EPDM rubber vulcanizates loaded with different ingredients and the antimicrobial agents towards the tested bacteria and fungi are presented in table (12). Each experiment was carried out in triplicate and the average zone diameter of inhibition was calculated.

Results in table 12 showed that EPDM rubber vulcanizates containing antimicrobial agents gave a promising results. So, the potassium alum and tetracycline antibiotic showed higher effects towards the investigated bacteria and fungi than the other EPDM rubber vulcanizates loaded with other additives. The antibacterial activity of the tested additives can be arranged in the following order:

Tetracycline > P alum >ZnO

Table (1): EPDM rubber formulations (filled with $CaCO_3$ and bentonite fillers) and the rheometric characteristic of EPDM mixes

	E0	EC1	EC2	EC3	EC4	EB1	EB2	EB3	EB4
$CaCO_3$	---	10	20	30	40	---	---	---	---
Bentonite	---	---	---	---	---	10	20	30	40
M_H	11	15	22	27	30	17	27	31	33
M_L	3	4	6	6	7	6	6	7	7
t_{C90}	12	10	8.5	7	6.5	9	8	7	6
t_{S2}	3.12	2	1.5	1	1	2.5	1.5	1	1
CRI	11.26	12.5	14.29	16.67	18.18	15.38	15.39	16.67	20

EPDM 100, ZnO 5, Stearic acid 2, CBS 0.8, S 1.5, Processing Oil 2, TMQ antioxidant 1.5

M_L, minimum torque; M_H, maximum torque; tc_{90}, optimum cure time; ts_2, scorch time; CRI, cure rate index.

Table (2): EPDM rubber formulations (filled with $CaCO_3$) containing different concentrations of ZnO as antimicrobial agent, and the rheometric characteristics of EPDM mixes

	EC1	EC2	EC3	EC4	EC5	EC6
$CaCO_3$	20	20	20	20	20	20
ZnO	2.5	5	10	20	30	40
M_H	22.06	22.82	23.93	23.44	23.67	24.63
M_L	6.84	6.92	7.46	7.7	8.22	8.5
t_{C90}	8	8	7.5	7	6.5	6.5
t_{S2}	1.5	1.5	1.1	1	1	1
CRI	15.38	15.38	15.63	16.67	18.18	18.18

Table (3): EPDM formulations (filled with bentonite) containing different concentrations of ZnO as antimicrobial agent, and the rheometric characteristics EPDM mixes

	EB1	EB2	EB3	EB4	EB5	EB6
Bentonite	20	20	20	20	20	20
ZnO	2.5	5	10	20	30	40
M_H	27.36	27.45	28.13	28.35	28.35	29
M_L	6.22	6.59.81	6.81	6.99	7.66	7.85
t_{C90}	7.8	7.44	6.75	6.46	6.46	6.5
t_{S2}	1.23	1.13	1.04	1.00	1.00	1.00
CRI	15.22	15.84	17.51	18.32	18.32	18.18

Table (4): EPDM formulations (filled with $CaCO_3$) containing different concentrations of potassium alum as antimicrobial agent, and the rheometric characteristics of EPDM mixes

	EC1	EC2	EC3	EC4	EC5
$CaCO_3$	20	20	20	20	20
Potassium alum	0.07	0.35	0.7	3.5	7
M_H	22.27	23.12	23.76	24	24.8
M_L	6.05	6.31	7.11	7.55	8
t_{C90}	8	7.5	7.5	6.15	6.00
t_{S2}	1.31	1.30	1.14	1.00	1.00
CRI	14.94	16.13	15.72	19.4	20

Table (5): EPDM formulations (filled with bentonite) containing different concentrations of potassium alum as antimicrobial agent, and the rheometric characteristics of EPDM mixes

	EB1	EB2	EB3	EB4	EB5
Bentonite	20	20	20	20	20
Potassium alum	0.07	0.35	0.7	3.5	7
M_H	27.46	27.46	28.24	28.56	28.9
M_L	6.3	6.88	7.11	7.85	8.3
t_{C90}	7.25	7.00	6.74	6.26	5.96
t_{S2}	1.27	1.07	1.00	1.00	0.82
CRI	16.72	16.86	17.42	19.01	19.46

Table (6): EPDM formulations (filled with $CaCO_3$) containing different concentrations of Tetracycline as antimicrobial agent, and the rheometric characteristics of EPDM mixes

	EC1	EC2	EC3	EC4	EC5
$CaCO_3$	20	20	20	20	20
Tetracycline	0.07	0.35	0.7	3.5	7
M_H	22.68	22.99	23.51	23.8	24.11
M_L	6.14	6.28	6.94	7.33	7.95
t_{C90}	8.2	7.5	7.1	7.1	7.00
t_{S2}	1.44	1.13	1.06	1.06	1.00
CRI	14.79	15.7	16.56	16.56	16.67

Table (7): EPDM formulations (filled with bentonite) containing different concentrations of Tetracycline as antimicrobial agent, and the rheometric characteristics of EPDM mixes

	EB1	EB2	EB3	EB4	EB5
Bentonite	20	20	20	20	20
Tetracycline	0.07	0.35	0.7	3.5	7
M_H	27.05	27.21	27.82	28.11	28.65
M_L	6.1	6.17	6.38	6.93	7.2
t_{C90}	7.89	7.31	7.03	6.55	5.71
t_{S2}	1.3	1.2	1.2	1.00	1.00
CRI	15.76	16.36	17.15	18.02	21.23

Table (8): Physico- mechanical properties of the EPDM vulcanizates (filled with $CaCO_3$ and Bentonite) containing different concentrations of ZnO as antimicrobial agent

	EC1	EC2	EC3	EC4	EC5	EC6	EB1	EB2	EB3	EB4	EB5	EB6
$CaCO_3$	20	20	20	20	20	20	------	------	------	------	------	------
Bentonite	------	------	------	------	------	------	20	20	20	20	20	20
ZnO	2.5	5	10	20	30	40	2.5	5	10	20	30	40
Tensile Strength (Ts), MPa	1.43	1.68	1.92	2.09	2.2	2.42	1.75	1.85	1.9	2.06	2.33	2.5
Elongation at break, (E), %	189	195	220	250	275	276	175	183	190	197	216	223

Table (9): Physico-mechanical properties of the EPDM vulcanizates (filled with $CaCO_3$ and Bentonite) containing different concentrations of potassium alum as antimicrobial agent

	EC1	EC2	EC3	EC4	EC5	EB1	EB2	EB3	EB4	EB5
$CaCO_3$	20	20	20	20	20	20	------	------	------	------
Bentonite	------	------	------	------	------	------	20	20	20	20
potassium alum	0.07	0.35	0.7	3.5	7	0.07	0.35	0.7	3.5	7
Tensile Strength (Ts), MPa	1.96	2.05	2.44	2.9	3.49	1.86	2.22	3.44	3.67	3.49
Elongation at break, (E), %	273	285	290	295	343	283	295	300	330	340

Table (10): Physico-mechanical properties of the EPDM vulcanizates (filled with $CaCO_3$ and Bentonite) containing different concentrations of Tetracycline as antimicrobial agent

	EC1	EC2	EC3	EC4	EC5	EB1	EB2	EB3	EB4	EB5
$CaCO_3$	20	20	20	20	20	20	---	---	---	---
Bentonite	---	---	---	---	---	---	20	20	20	20
Tetracycline	0.07	0.35	0.7	3.5	7	0.07	0.35	0.7	3.5	7
Tensile Strength (Ts), MPa	2.08	2.56	3.05	3.65	3.94	2.26	2.76	3.09	3.26	3.5
Elongation at break, (E), %	360	386	390	465	530	237	250	280	295	320

Table (11): Cytotoxic activity of different EPDM rubber vulcanizates towards human normal retina cell line: RPE1

Rubber type	**Cytotoxic effect at 100 ppm**
EPDM	-52 %
EPDM/Bentonite	-58.5 %
EPDM/$CaCO_3$	-13.1 %
DMSO	5 %
Negative control	0 %

Table (12): Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of different EPDM rubber vulcanizates towards the pathological strains based on diffusion assay.

	Gram-positive bacteria		***Gram-negative bacteria***		***Yeast***	***Fungi***
Rubber formula	*Staphelococcus aureus* ATCC 29213	*B. subtilis ATCC6 633*	*Pseudomonas . aeroginosa* ATCC27953	*E. coli ATCC 25922*	*Saccharomyces cerevisiae*	*Aspergillus niger Local isolate*
EPDM/$CaCO_3$/2.5 ZnO	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
EPDM/$CaCO_3$/40 ZnO	13	15	13	12	N.A.	25
EPDM/Bentonite/2.5 ZnO	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
EPDM/Bentonite/40 ZnO	20	14	15	15	13	20
EPDM/Bentonite/0.7 Tetracycline	17	20	18	15	20	18
EPDM/Bentonite/3.5 Tetracycline	30	30	27	28	30	32
EPDM/$CaCO_3$/0.7 Tetracycline	22	20	20	19	18	20
EPDM/$CaCO_3$/7 Tetracycline	30	32	29	27	30	32
EPDM/Bentonite/0.7 potassium alum	15	17	14	14	19	16
EPDM/Bentonite/7 potassium alum	22	25	23	22	23	26
EPDM/$CaCO_3$/0.7 potassium alum	14	18	15	16	12	11
EPDM/$CaCO_3$/7 potassium alum	27	21	22	24	26	24
Vancomycin	20	20	25	29	25	22
Cefodizime	18	27	28	26	28	25

N.A. : Not active

Conclusions:

1- Ethylene propylene diene monomer rubber (EPDM) vulcanizates containing the bioactive materials (Tetracycline, potassium alum and ZnO) gave safety effect property with good mechanical properties (Tensile and elongation at break), and can be applied for production of safety rubber goods.

2- The cytotoxicity of the investigated EPDM vulcanizates gave negative results to normal human cell liver (-52% and -58.5 %).

3- Based on the tested antimicrobial additives, the antibacterial activity of the EPDM vulcanizates can be arranged in the following order:

Tetracycline > P alum >ZnO

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References

[1] Yehia A A, Khalaf A I and El Nashar D E **2017** KGK Kautschuk Gummi Kunststoffe **70** 42

[2] Lin Y S, Lee M Y, Yang C H and Huang K S **2014** Curr. Proteom. **11** 116

[3] Chan C F, Huang K S, Lee M Y, Yang C H, Wang C Y and Lin Y S **2014** Curr. Org. Chem. **18** 204

[4] Sun D, Shahzad M B, Li M, Wang G and Xu D **2015** Mater. Technol. **30** B90

[5] Santos MRE, Fonseca AC, Mendonça PV, Branco R, Serra A C, Morais P V and Coelho J F J **2016** Materials **9** 599

[6] Jain A, Duvvuri L S, Farah S, Beyth N, Domb A J and Khan W **2014** Adv. Healthc. Mater. **3** 1969

[7] Huang K S, Yang C H, Huang S L, Chen C Y, Lu Y Y and Lin Y S **2016** Int. J. Mol. Sci. **17** 1578

[8] Haas G R, Spartanburg S C, Kerr R C and Moore SC **2010** Patent No.: US 7,858,674 B2. Dec. 28

[9] Dobrucka R, Dlugaszewska J and Kaczmarek M 2018 Biomed. Microdevices **20** 5

[10] Nazoori E S and Kariminik A **2018** J. Appl. Biotechnol. Rep. **5** 162

[11] Amadi L O and Ngerebara N N **2017** Int. J. Curr. Microbiol. App. Sci. **6** 941

[12] Ali Z M **2018** Sci. J. Med. Res. **2** 47

[13] Mahmoud H A, Melake N A and El-Semary M T **2012** Biofilms. Pharmaceut. Anal. Acta, **S15** 1

[14] Farajnia S, Darbani B, Babaei H, Alimohammadian M H, Mahboudi F and Gavgani A M **2008** Parasitology **135** 1035

[15] International Organization for Standardization (ISO). Biological Evaluation of Medical Devices (Standard No. ISO10993-12:**1996**). ISO, Geneva, Switzerland.

[16] Mosmann T **1983** J. Immunol. Methods **65** 55 [17] Edmondson J M, Armstrong L S and Martinez A O **1988** J. Tissue Cult. Methods **11** 15
[18] Al-Salahia R, Marzouka M, Awad G, Al-Omara M and Ezzeldin E **2013** J. Pharm. Pharmacol. **56** 7
[19] Mady M F, Awad G and Jørgensen K B **2014** Europ. J. Medicinal Chemist. **84** 433
[20] Mohamed R M, EL-Nemr K F and Raslan H A **2019** J. Macromol. Sci. **56** 429
[21] Khalaf A I, Helaly F M, El Sawy S Egypt. J. Chem. **63** (1), 129
[22] MohdNor N A and Othman N **2016** Proced. Chemist. **19** 351