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Impact of $BaSO_4$ Particles on the Viability of Eukaryotic and Prokaryotic Cells

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Abstract: The process of car stopping is inevitable on a daily basis when driving a car and is related to the $BaSO_4$ particles emission because of friction of brake pads. Barium was determined by EDS (energy dispersive spectroscopy) as the third highest quantity of metals after iron and magnesium in the samples of organic brake pads. Elemental mapping SEM (scanning electron microscope) analysis confirmed the presence of $BaSO_4$. A stable $BaSO_4$ NPs (nanoparticles) suspension has been prepared and described by PI (polydispersity index) and Zeta potential. For the increased stability of suspension Tween80 was used. The impact on viability of prokaryotic cells (gram-positive *S. aureus* and gram-negative *E. coli*, *P. aeruginosa* wild-type bacteria) and eukaryotic cells (CHO (Chinese hamster ovary) cells) was determined by usage of $BaSO_4$ NPs with Tween80 or without. *S. aureus* bacteria were more sensitive to the exposure of $BaSO_4$ NPs and reached MIC50 when affected with 3.32 mg/mL $BaSO_4$ NPs coated with Tween80. Zeta potential value increases in all examined bacteria and NPs suspensions. The highest increase was observed for the *S. aureus*, *P. aeruginosa* bacteria and $BaSO_4$ NPs with Tween80 suspensions. Minimal concentration of $BaSO_4$ NPs with Tween80, which had negative effect on viability of CHO cells, was 0.1 mg/mL.

Key words: $BaSO_4$ NPs, Zeta potential, viability, eukaryotic and prokaryotic cells.

1. Introduction

NPs (Nanoparticles), ranging from 1 to 100 nm [1], are abundant in (1) nature, as they are produced in many natural processes, such as volcanic eruptions and erosion as well as due to (2) human activities: cars usage, industry and (3) engineered nanomaterials [2].

According to studies, in urban zones brake wear can contribute up to 55% by mass to total non-exhaust traffic-related particles [3]. Particulate matter pollution is very harmful for human health, it was designated as Group I carcinogen by the IARC (International Agency for Research on Cancer) [4]. It was determined that the main elements that can be found due to friction of brake pads of vehicle braking system are: iron [5-7], copper [5, 7, 8], zinc [6, 8], titanium [6], barium [5, 6] and minerals: ferrite [7, 9], pyrite [9], graphite [9], barite [7, 9], corundum [9], calcite [9], mullite [9]. Only some of the studies refer to the impact of these particles that include mentioned elements or minerals on viability of eukaryotic and prokaryotic cells [10, 11].

NPs properties (size, shape, chemical composition, surface area, etc.) [12], their changes (agglomeration accompanied by free surface energy release and an increase of dispercity degree) [13, 14] and environmental impact are closely related.

NPs impact on the viability of eukaryotic and prokaryotic cells depends on physical and chemical properties of NPs and their suspensions, type of cells as well. The biological membrane is a covering layer of eukaryotic and prokaryotic cell which regulates the

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molecular flows inside a cell and from it. The lipid bilayer provides a dynamic but stable barrier between extracellular and intracellular compartments of a biological cell [15]. Gram-positive bacteria are covered by one membrane and have a peptidoglycan layer, while gram-negative bacteria membrane structure is bilayer and has outer and inner parts [16]. The most important derivatives in the biomembrane structure are proteins; they selectively pass material from and to the cell [17].

There are three main pathways that NPs can enter into cells and exert their toxic effects: mechanically breaking the cell membrane, endocytosis (phagocytosis and pinocytosis), diffuse through the membrane [18]. Non-polar materials, such as O_2 and CO_2, readily diffuse into the lipid bilayer, but polar molecules such as ions or polar NPs (usually coated) can only enter the cell through special protein channels or endocytosis [19]. In the case of pinocytosis, the endocytosed substance enters the plasma membrane (inner) in the membrane, and during phagocytosis, the particles are encapsulated in the membrane folds. During phagocytosis, the particle size can be significantly higher (> 1 μm) than pinocytes and phagocytes themselves are specialized cells. Pinocytosis is divided into macropinocytosis, which causes the pocket-type membrane to bend and thus envelop (> 1 μm) absorbed material. The second type of pinocytosis mediates clathrin receptors. They form a deflection and incorporate transferable materials of about 120 nm in size. The third way of deflection in the membrane is formed by certain lipid calveolin rafts. In this way, the cells do not exceed 60 nm [19].

The antimicrobial mechanism of action of NPs is generally described as adhering to one of the three models: oxidative stress induction, metal ion release, or non-oxidative mechanisms [20].

Oxidative stress induced by ROS (reactive oxygen species) is an important mechanism of NPs on bacteria. Different NPs activate different forms of ROS: superoxide radical ($O_2^{\bullet-}$), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2) and singular oxygen ($^1O_2$). Oxidative stress is a major factor leading to changes in the membrane permeability [21] and irregularities due to ROS significant effect on DNA-bacterial interactions. It increases the expression of oxidative protein genes [22, 23]. The antibacterial effect of metal oxides NPs is related with their dissociation and metals ions release, their absorption through the bacterial cell membrane, direct interaction with the functional groups of proteins and amino acids, impairement enzyme activity and alteration cellular structure, disruption transmembrane electron transfer or act as antimicrobial transporters [24].

NPs of barium sulfate ($BaSO_4$) are described as very low solubility NPs, but addition to catheters or endotracheal tubes has been shown to exhibit some antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria [25]. The negative effect on the rat lungs was observed under the exposure of $BaSO_4$ NPs aerosol in four weeks [10]. Konduru with coworkers showed that there is negligible contribution from fur deposition and ingestion during inhalation exposure and that barium detected in extrapulmonary organs after inhalation translocates from the lungs to the blood. Short-term inhalation studies of investigating comparable SiO_2, ZrO_2 and $BaSO_4$ NPs showed that neither of them elicited any effects upon 28-day oral exposure to rats and the tested substances do not elicit systemic toxicity under the reported experimental conditions [11].

As mentioned above one of the factors affecting the low solubility metal oxides NPs action on the viability of cells depends on release of metal ions to medium [26-29] but also depends on size, coating and the culture medium pH can act in different ways [30]. It was demonstrated that Ba^{2+} ions blocked a flux of K^+ ions out of the cells [31] and caused the effect of hyperpolarization [32, 33]. Another very important factor affecting the interaction of NPs and surface of

cells is Zeta potential [34, 35], which indicates the properties of outer layer of particles. The net charge of the most bacteria is negative, and the Zeta potential value depends on the composition of double electric layer which varies depending on a lot of factors including the measuring medium. Considering the medium, the value of the Zeta potential varies from -44.2 ±0.50 mV for *E. coli* and -35.6 ±0.54 mV for *S. aureus* [16] when measurements were performed in 0.5 mM potassium phosphate buffer solution (pH 7.4), -23.6 mV *E. coli* 1× phosphate buffer saline and for *B. subtilis* -18 mV respectively [36]. It was demonstrated that cationic NPs, or NPs coated with positively charged compounds, are more related to membranes, making them easier to pass through the membrane of cancer cells [37] compared to negatively charged NPs and do not utilize the clathrin-mediated endocytosis pathway [38].

The goal of this study consists of two parts: first is the elemental and the minerogical analysis of brake pads with the idea to choose the most stable particles. The second part is the evaluation of the effect of chosen particles on the viability of prokaryotic and eukaryotic cells.

2. Method and Materials

2.1 Estimation of Elemental and Mineralogical Composition of Brake Pads

The most used in the Lithuanian market new organic and ceramic brake pads were purchased and used in this study.

The elemental composition of brake pads was studied using SEM (scanning electron microscope, HITACH S-3400N) and EDS (Energy Dispersive Spectroscope, BRUKER Quantax), while XRD (X-Ray diffraction) spectrometer (Bruker D8 Discover) was used for the identification of mineralogical composition.

For this research, both solid brake pads and samples of powder got by rubbing same brake pads one to another were used. All presented data were calculated from three independent experiments.

2.2 Estimation of Particle Size and PI (Polydispersity Index)

The particle size of NPs suspensions was measured according to the cumulative distribution of intensity by using Delsa™Nano C particle size meter (Beckman Coulter). The particle size meter uses the photon correlation spectroscopy which determines particle size by measuring the rate of fluctuations in the laser light intensity scattered by particles. The NNLS (non-negative least-squares) algorithm was used to analyze dynamic scattering data for the particle size distribution. The size of particles of suspensions was measured in triplicate and averaged.

2.3 Estimation of Zeta Potential

Zeta potential (ζpotential) measurements were performed by using Delsa™Nano C particle size meter (Beckman Coulter). The Zeta potential of suspensions was measured in triplicate and averaged.

2.4 Cultivation of Prokaryotic Cells

Prokaryotic cells—gram-negative *Escherichia coli* KMY, *Pseudomonas aeruginosa* and gram-positive *Staphylococcus aureus* wild type bacteria were used in experiments. Standard overnight culture was grown in shaker at 220 rpm at 37 ℃ with aeration in Luria-Bertani broth (LB; Roth, Germany) for 16 to 18 h. For the measurements of MIC and Zeta potential 2% fresh LB medium was prepared. And 20-50 mL of fresh medium was inoculated with 1/20 diluted overnight culture and bacteria were grown under the same conditions as the overnight culture until mid-exponential phase (OD_{600} of 1, measurements performed using UV-Vis spectrophotometer HAMO DB-30, as control was grown medium).

2.5 Measurements of Prokaryotic Cells Viability

For the evaluation of viability of bacteria in all cases bacteria suspensions were pre-grown for about 18 h. In each experiment volume of bacterial suspension was calculated to adjust 0.3 OD in 200 μL for the each

well of the 96 well-plates. All the wells were filled with $BaSO_4$ NPs or nonionic surfactant Tween80, or $BaSO_4$ NPs coated with Tween80 by applying twice dilution method. Viability of bacteria was evaluated by measuring suspensions absorption using spectrometer (TECAN GeniosPro) at 610 nm after 18 h of incubation (37 ℃) with mentioned reactants.

2.6 Cultivation of Eukaryotic Cells

Eukaryotic cells—CHO (Chinese hamster ovary) cells were cultivated in sterile DMEM (Dulbecco's Modified Eagle's cell culture medium) with 10% FBS (fetal bovine serum), 1% penicillin/streptomycin (P/S) and 1% amphotericin B (AmphB). Temperature in the incubator was 37 ℃, concentration CO_2 5%, humidity 80%. Cells were grown for 4-5 days.

2.7 Measurements of Eukaryotic Cells Viability

Viability of CHO cells was evaluated by rapid colorimetric assay based on the cleavage of the tetrazolium ring of 3-(4,5-dimethylthazolk-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by dehydrogenases in active mitochondria of living cells. CHO cells were plated in 96-well culture dishes (10^4 cells/0.2 mL) and grown for 24 h. Then different amounts of $BaSO_4$ NPs or nonionic surfactant Tween80, or $BaSO_4$ NPs coated with Tween80 were added and incubated for 3 h at 37 ℃. Untreated cells were used as control. After incubation, the medium was removed and added to 100 μL of the prepared 0.5 mg/mL MTT solution in growth medium, then incubated for 1 h at 37 ℃. At the end of the time, the MTT dye medium is removed, the cells are washed twice with PBS, buffer removed and 50 μL of isopropanol is added, which dissolves the formazan formed after the reduction process. Absorption is measured using a TECAN GeniosPro multifunction microplate reader at 535 nm.

2.8 Statistical Analysis

Statistical analysis was performed with one-way ANOVA (analysis of variance). Statistical significance was defined as $p < 0.05$ for all tests.

2.9 Chemicals

All chemicals were at least of analytical grade. $BaSO_4$ NPs (particles diameter 100 nm) from Nanoshel (USA), Luria-Bertani broth (LB; Roth, Germany), nonionic surfactant Tween80 from Sigma (St. Louis, MO), Dulbecco's Modified Eagle's medium (DMEM), fetal bovine serum (FBS) from Roth (Karlsruhe, Germany), penicillin/streptomycin (P/S) (10 kU/mL/10 mg/mL) solution, amphotericin B (AmphB), 3-(4,5-dimethylthazolk-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were from Biochrom AG (Berlin, Germany).

NPs of 100 nm can be separated by the centrifugation at 3,000 rpm for 30 min [39], although Ref. [40] stated that 110 nm could be separated at 8,000 rpm for 15 min. Taking the literature data into consideration and after making experiments, the stable NPs suspension was prepared by the centrifugation at 7,000 rpm for 10 min. The stock solution of $BaSO_4$ NPs 0.125 g/mL suspension was prepared in autoclovated medium and was affected by ultrasound for 2 h before 10 min centrifugation at 7,000 rpm. For the coating with Tween80 firstly $BaSO_4$ (0.125 g/mL) NPs suspension was ultrasounded for 1.5 h, then added Tween80 (0.013 mg/ml) and left for 45 min. After that time suspension was ultrasaunded again for 30 min and centrifuged for 10 min at 7,000 rpm. The stock solutions were stored in the dark at +4 ℃ and were vortexed before each use.

3. Results and Discussions

3.1 Elemental and Mineralogical Composition of Brake Pads

Automotive brake pads depending on the materials from which they are composed could be organic, semi-metallic or ceramic. Additives, such as abrasives, friction modifiers, fillers and reinforcements, binder

materials are used in brake pad material to enhance proper functions of braking system. Some additives are potentially toxic. After the ban of asbestos fibers for brakes manufactured in mid-90s, composition of organic non-asbestos brake pads has rapidly changed but there is still a few possible toxics used (heavy metals, their sulphides, PAHs etc.) [41, 42]. Detailed information about materials used for creating brake pads is not provided by manufacturers. However, it is known that some of the main components of brake pads are transition metals, organic compounds, minerals and biological origin substances. The aim of this part of study was to investigate elemental and mineral composition of organic and ceramic brake pads including most popular for cars in the country. The information acquired through research is important for emission inventories and toxicological studies.

The elemental composition of powder of organic and ceramic brake pads is given in Table 1. The analysis data (gained by EDS) confirmed that the brake pads are heterogeneous due to their nature.

Carbon was found at higher levels in the organic brake pads compared to ceramic, with a ratio of 1.16. It was determined that the largest amount of metal is iron in the examined organic brake pads samples, while in the ceramic samples it is calcium. Both of the samples contained magnesium and titanium that are representatives of friction materials [43]. Research data are in correlation with Ref. [8] that zinc levels are higher than copper. It was determined that in the samples of organic brake pads the third highest quantity of metals after iron and magnesium is barium. For further research organic brake pads mostly used in passenger cars were chosen.

Research shows that the quantitative analysis data are highly dependent on the area from which the sample of powder was prepared. Hence, the elemental analysis of different areas of solid brake pad was done using EDS. The scheme of brake pads with named areas of the sampling is given in the Fig. 1.

The elemental composition of four different parts of solid organic brake pad is given in Table 2 and SEM images of the particular areas are shown in Fig. 2.

The data presented in the Table 2 revealed that the composition of deeper layer of left side (B) differs from the other investigated parts of the brake pad with the highest amount of Al and Ba. Al oxide is one of the typical abrasives with the hardness around 7-8 by the Mohs, $BaSO_4$ is one of the commonly used fillers, whose melting point is 1,350 °C [44].

Elemental mapping SEM analysis was performed to confirm the hypothesis that the inner layers of the brake pads are described by the higher concentrations of resistant to high temperature filler barium sulphate. Mapping photograph (Fig. 3) confirms the close distribution of Ba, S and O, that is the identification of existing $BaSO_4$ in the brake pad.

As a model for the evaluation of impact on eukaryotic and prokaryotic cells viability, NPs of low solubility barium sulphate ($BaSO_4$ solubility in 20 °C water is 2.3 mg/L) were chosen, because the normal brake temperature does not exceed 300 °C [45] when the melting point of the compound is much more higher.

Barite, calcite, mullite determined by X-Ray diffraction spectrometer are the main components of mineral composition (and small amounts of CuO, Fe_2O_3, ZnO, TiO_2).

3.2 Evaluation of NPs Suspension Properties

In the suspension NPs tend to aggregate due to the excess of surface energy. Therefore, NPs suspension polydispersity studies were performed before the study of NPs impact on the viability of cells. The value of PI is an indicator of particle size distribution in the system. If it is closer to zero it denotes the monodisperse system, if values are greater than 0.7, it indicates that the system has a very broad size particles distribution (ISO standards 13321:1996 E and ISO 22412:2008). Polydisperse system has a greater tendency to aggregation than monodisperse, because

Table 1 The elemental analysis of powder of organic and ceramic brake pads.

Element	Concentration of elements of brake pads powder samples, % (wt)	
	Organic	Ceramic
C	41.830 ±0.200	36.025 ±0.575
O	15.185 ±0.115	35.510 ±0.580
Na	-	0.170 ±0.040
Mg	4.775 ±0.003	3.430 ±0.120
Al	0.465 ±0.025	1.275 ±0.105
Si	0.560 ±0.010	3.330 ±0.040
S	1.440 ±0.010	0.095 ±0.035
Cl	0.195 ±0.015	0.135 ±0.045
K	-	1.015 ±0.075
Ca	0.860 ±0.030	9.910 ±0.080
Ti	0.240 ±0.060	0.320 ±0.010
Fe	29.23 ±0.540	8.785 ±0.925
Zn	1.035 ±0.085	-
Ba	4.105 ±0.045	-

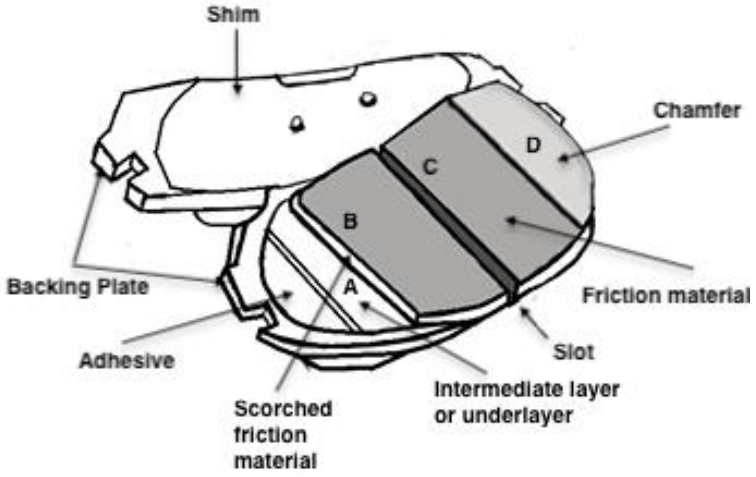
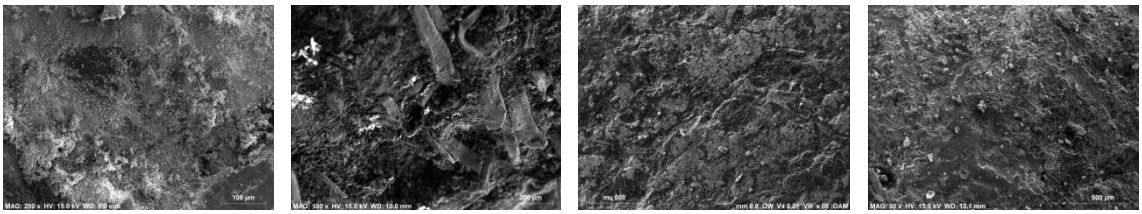


Fig. 1 Brake pad scheme. The corresponding parts of the organic brake pad: A – surface of the left side; B – the deeper layer of left side; C – the surface of central area; D – the surface of right side which were analyzed.

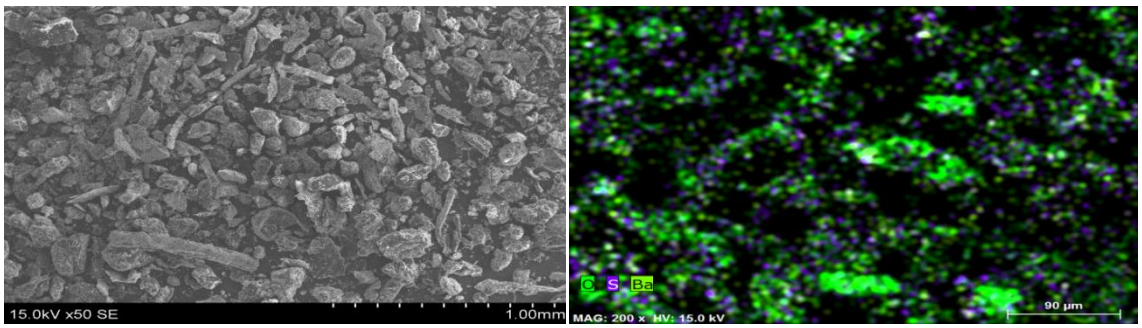
Table 2 The elemental analysis of solid organic break pad A, B, C, D areas.

Element	Concentration, % (wt)			
	A	B	C	D
C	32.86	31.65	34.06	37.88
O	14.39	10.88	19.05	9.77
Na	-	0.21	0.24	0.13
Mg	2.14	3.25	3.40	1.49
Al	0.10	0.22	0.09	0.13
Si	0.28	0.50	0.24	0.33
S	0.91	1.18	1.09	0.86
Cl	0.29	0.30	0.23	0.09
K	0.13	0.18	0.14	0.11
Ca	0.61	0.79	0.54	0.32
Ti	0.23	0.29	0.26	0.30
Fe	44.34	44.98	36.27	44.67
Zn	1.30	1.41	1.36	1.03
Ba	2.42	4.17	3.03	2.88



(a) (b) (c) (d)

Fig. 2 SEM images of corresponding parts of organic brake pad on which elemental analysis was performed by EDS: (a) surface of the left side; (b) the deeper layer of left side; (c) the surface of central area; (d) the surface of right side.



(a) (b)

Fig. 3 Brake pad surface (1 mm, 50×) by SEM (a) and SEM mapping photograph of Ba, S and O (b).

of that; PI could be one of the factors when evaluating the stability of NPs suspension. Another factor for the indirect evaluation of disperse system stability is governed by electrostatic interaction that could be measured by Zeta potential (ζ). Zeta potential measurements are the parameters by which evaluation of the effect of NPs on cell viability could be performed.

The measurements of particles size show that the $BaSO_4$ NPs suspension in the medium mainly consists of 100 nm and lower size particles who number was 4 times higher than 200 nm size particles.

Because the goal of this study is to evaluate an impact of $BaSO_4$ NPs on the viability of eukaryotic and prokaryotyc cells, the properties of NPs suspensions in Luria-Bertani broth (LB) and Dulbecco's Modified Eagle's medium (DMEM) were examined (Table 3). $BaSO_4$ NPs suspensions in a base of LB or DMEM are polydisperse with the PI 0.35 and 0.38 respectively and can be used for the studies of life sciences [46]. When $BaSO_4$ NPs suspension in LB was prepared with nonionic Tween80, the PI decreases 2 times indicating decreased polydispersity and increased stability of suspension. The results of the Zeta potential studies (Table 3) correlate with the (more negative) increase in the potential value reported by Refs. [47, 48] as a factor in assessing the stability of the NPs suspension. Addition of nonionic surfactant Tween80 to LB and DMEM medium decreases Zeta potential value about 1.3 and 1.2 times respectively.

3.3 Impact of $BaSO_4$ NPs Suspension on the Viability of Prokaryotic Cells

This section provides the results of $BaSO_4$ NPs suspension on the viability of gram-positive *S. aureus* (Fig. 4) and gram-negative *E. coli*, *P. aeruginosa* (Fig. 5) wild-type bacteria. In order to determine the effect of nonionic surfactant Tween80 (which was used to stabilize the NPs suspension) on viability of bacteria, control studies were carried out regardless of the type of bacteria. The results presented in Figs. 4 and 5 show that Tween80 does not affect the growth of *S. aureus*, *E. coli* and *P. aeruginosa* in the range of concentration 0.0004-0.013 mg/mL. This surfactant stabilizes the suspension of $BaSO_4$ NPs, which was proved by the measurements of PI and Zeta potential.

$BaSO_4$ NPs and $BaSO_4$ NPs coated with nonionic Tween80 had negative effect on the growth of *S. aureus* bacteria when the concentration of NPs suspension was 0.1 mg/mL.

An increase of the concentration of NPs in the suspension highlighted the effect of coating with nonionic surfactant on the viability of *S. aureus* bacteria. When the bacteria were affected with 3.32 mg/mL $BaSO_4$ NPs coated with Tween80 suspension and reached MIC50, half of the cells died. Without surfactant the affinity of NPs surface was lower than outer surface (which is characterized by the reactivity of peptidoglycan or teichoic acid [49]) of bacteria and their viability was about 30% higher compared to viability of bacteria affected with NPs coated with Tween80. Statistically, reliable effect of $BaSO_4$ NPs on the viability of gram-negative *E. coli* was observed when the concentration of NPs suspension was 0.42 mg/mL independently whether $BaSO_4$ NPs were coated with Tween80 or not. Increasing the concentration of $BaSO_4$ NPs increases the impact of NPs coating onto bacteria viability. The effect of

Table 3 $BaSO_4$ NPs suspension properties.

NPs suspension composition	Property	
	PI	ζ (mV)
$BaSO_4$ in LB	0.35 ± 0.01	-27.71 ± 1.22
$BaSO_4$ and Tween80 in LB	0.17 ± 0.01	-36.81 ± 3.04
$BaSO_4$ in DMEM	0.38 ± 0.07	-13.93 ± 1.53
$BaSO_4$ and Tween80 in DMEM	0.49 ± 0.02	-16.55 ± 2.64

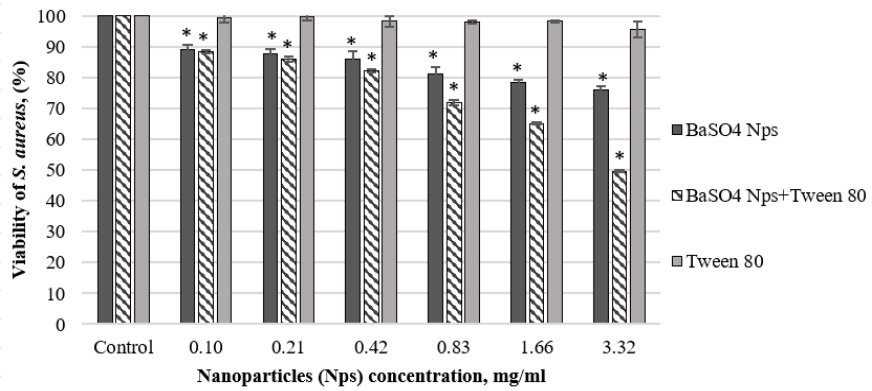
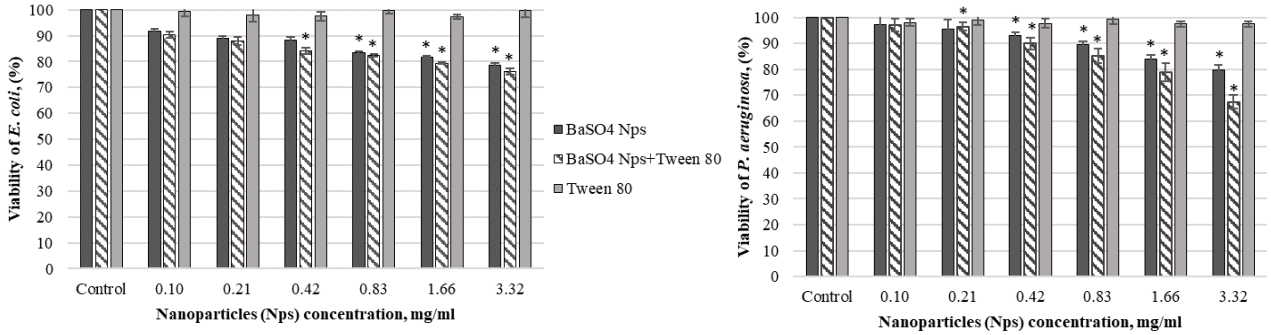


Fig. 4 Viability of *S. aureus* bacteria in the presence of $BaSO_4$ NPs, $BaSO_4$ NPs with Tween80 and Tween80.
*: statistical reliability of control compared with bacteria affected NPs (ANOVA, $p < 0.05$).



(a) (b)

Fig. 5 Viability of *E. coli* (a) and *P. aeruginosa* (b) bacteria in the presence of $BaSO_4$ NPs, $BaSO_4$ NPs with Tween80 and Tween80.
*: statistical reliability of control compared with bacteria affected NPs (ANOVA, $p < 0.05$).

nonionic surfactant Tween80 could be related to NPs dispersion effect preventing the agglomeration process, facilitated the release of NPs through the bacteria membrane or viability as well as affinity to gram-negative bacteria membrane. This surfactant reduces the polydispersity and stabilizes the suspension of $BaSO_4$ NPs. Thus, reduced viability of bacteria could be explained by the higher affinity of coated NPs to gram-negative bacteria lipopolysaccharides and phospholipids located in outer membrane of bilayer membrane, or different pathways through the membrane. It is known that in water $BaSO_4$ is insoluble (0.00285 mg/mL at 30 ℃) and release of Ba^{2+} due to dissociation is very negligible ($K = 1.1 \times 10^{-10}$). Therefore, the effect of $BaSO_4$ NPs on viability of bacteria is related to mechanisms of undissociated form of $BaSO_4$ NPs.

For the comparative studies gram-negative bacteria *P. aeruginosa* (B) (Fig. 5) were chosen due to proved very low permeability of a wide range of solutes compared to *E. coli* [50, 51]. Results show that only under effect of highest studied concentrations $BaSO_4$ NPs suspension with nonionic surfactant Tween80, it was observed about 9% lower viability of *P. aeruginosa* than *E. coli*.

Considering the importance of membranes as a barrier for NPs entering bacteria managed by size of particles and electrostatic interaction, the measurements of Zeta potential were performed.

The value of Zeta potential measurements depends on the dispersing medium, temperature, the phase of bacteria growth, the concentration of the bacteria in the culture [52]. In the methodological point of view all mentioned parameters were kept the same in all measurements to get the comparable data. As mentioned above Zeta potential is one of the parameters indicating the stability of the suspension and bacteria viability. Zeta potential of the $BaSO_4$ NPs coated with Tween80 becomes about 9 mV negative proving the stabilizing effect of this nonionic surfactant (Table 4) related to hydroxyl groups in its molecular structure. The value of bacteria surface charge depends on bacteria type and in general, for most bacteria, the net surface charge is negative. The value of Zeta potential of bacteria depends on acidic and basic functional groups for gram-positive *S. aureus* bacteria mainly on structural elements of peptidoglycan and teichoic acid, as well as lipopolysaccharides, phospholipids for gram-negative *E. coli* and *P. aeruginosa* bacteria [16, 53] and is balanced by oppositely charged counter ions present in the LB growth media. Research data presented in the Table 4 show the negative charge of the surface bacteria independently on their type.

The net charge of bacteria surface becomes less negative under the effect of the $BaSO_4$ NPs suspension: about 35% in the case of *S. aureus*, 15% *E. coli* and 38% *P. aeruginosa*. Statistically reliable effect of nonionic surfactant on the increase of Zeta potential was observed in all cases: twice higher value comparing Zeta potential of *S. aureus* and *P. aeruginosa* bacteria surface, and about 1.4 time of *E. coli*.

3.4 Impact of $BaSO_4$ NPs Suspension on the Viability of Eukaryotic Cells

For the comparative analysis of the $BaSO_4$ NPs impact on the viability of eukaryotic cells as a model CHO cells were chosen. The concentrations of $BaSO_4$ NPs and $BaSO_4$ NPs coated with nonionic Tween80 were the same as in the study with procaryotic cells. The tetrazolium salt thiazolyl blue (MTT) is widely used for assessment of cell viability and proliferation studies in the cell biology [54]. While experiments were done with low solubility $BaSO_4$ NPs, there is no threat of reducing activity of used reagent to cause the unsuitability of MTT method [55]. MTT gives a yellowish aqueous solution which, on reduction by dehydrogenases and reducing agents present in metabolically active cells, yields a water insoluble violet-blue formazan. The lipid soluble formazan product was extracted with isopropanol and estimated

Table 4 Zeta potential of NPs suspensions.

Type of suspension	Zeta potential (mV)
$BaSO_4$ NPs	-27.71 ±2.71
$BaSO_4$ NPs coated with Tween80	-36.81 ±4.09
Gram-positive bacteria	
S. aureus bacteria	-14.08 ±1.22
S. aureus bacteria $BaSO_4$ NPs	-9.16 ±0.43*
S. aureus bacteria and $BaSO_4$ NPs coated with Tween80	-7.10 ±0.38**
Gram-negative bacteria	
E. coli bacteria	-24.09 ±1.93
E. coli bacteria $BaSO_4$ NPs	-20.53 ±0.51*
E. coli bacteria and $BaSO_4$ NPs coated with Tween80	-17.36 ±0.82**
P. aeruginosa bacteria	-2.75 ±1.11
P. aeruginosa bacteria $BaSO_4$ NPs	-1.70 ±0.25*
P. aeruginosa bacteria and $BaSO_4$ NPs coated with Tween80	-1.38 ±0.94**

*: statistical reliability of bacteria compared with bacteria affected by NPs (ANOVA, $p < 0.05$);

**: statistical reliability of bacteria affected by NPs compared with bacteria treated with NPs coated with Tween80 (ANOVA, $p < 0.05$).

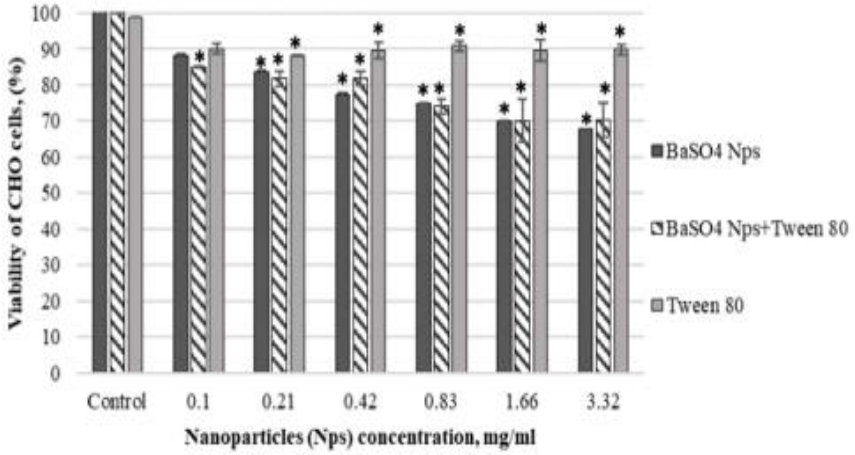


Fig. 6 Viability of CHO cells in the presence of $BaSO_4$ NPs, $BaSO_4$ NPs with Tween80 and Tween80.

*: statistical reliability of cells compared with cells affected NPs (ANOVA, $p < 0.05$).

by spectrophotometer at 535 nm wavelength. The amount of MTT formazan is directly proportional to the number of living cells.

Nonionic surfactant Tween80 affects the integrity of the CHO cells biology membrane, when its concentration in the cells growth medium was 0.013 mg/mL, the viability decreases by about 10%.

In the presence of $BaSO_4$ NPs starting from 0.1 until 3.32 mg/mL in the cells growth medium, CHO cells viability determined by the MTT test decreases (Fig. 6).

4. Conclusions

It was determined that in the samples of organic brake pads carbon is at higher levels when largest amount of metal is iron, while in the ceramic samples it is calcium. Friction materials magnesium and titanium were found in the samples of both types of brake pads. Barium was determined to be the third highest quantity of metals after iron and magnesium in the samples of organic brake pads. Elemental mapping SEM analysis confirmed the presence of Ba sulphate (one of the commonly used fillers in the organic brake pads) and NPs suspensions were chosen for the evaluation of impact on eukaryotic and prokaryotic cells viability.

PI and Zeta potential of $BaSO_4$ NPs suspensions prepared in LB and DMEM were examined. Both NPs

systems were polydisperse, consequently adding Tween80 lowered the PI about two times. Positive effect of this nonionic surfactant for the stabilization of $BaSO_4$ NPs suspensions was appointed by Zeta potential measurements. Addition of nonionic surfactant Tween80 to LB and DMEM medium decreases Zeta potential value about 30% and 20% respectively.

The results of $BaSO_4$ NPs suspension on the viability of gram-positive *S. aureus* and gram-negative *E. coli*, *P. aeruginosa* wild-type bacteria show, that it has the negative effect on bacteria growth. The importance of the membrane for the entry of NPs into bacteria can be seen: *S. aureus* bacteria which have a one layer membrane were more sensitive to the exposure of $BaSO_4$ NPs and reached MIC50 when affected with 3.32 mg/mL $BaSO_4$ NPs coated with Tween80 while this value was not reached for *E. coli*, *P. aeruginosa* having two layers of membrane bacteria independently whether $BaSO_4$ NPs were coated with Tween80 or not. Zeta potential measurement data show the negative charge of the surface bacteria independently on their type: -14.08 ± 1.22 mV for *S. aureus*, -24.09 ± 1.93 mV for *E. coli* and -2.75 ± 1.11 for *P. aeruginosa*. Zeta potential value increases in all examined bacteria and NPs suspensions. The highest increase about 2 times was observed for the *S. aureus*, *P. aeruginosa* bacteria and $BaSO_4$ NPs with Tween80 suspensions and about 1.4 time for *E. coli* and $BaSO_4$ NPs with Tween80 suspension.

Assessing the experiment results of bacteria viability and Zeta potential measurements it is possible to see the correlation between increased value of Zeta potential and the death of bacterial cells.

The negative effect of $BaSO_4$ NPs was observed on viability of eukaryotic cells. Minimal concentration of $BaSO_4$ NPs with Tween80, which had influence on viability of CHO cells, was 0.1 mg/mL. If we increase the concentration of $BaSO_4$ NPs with Tween80 or without it, the viability of cells decreases.

References

[1] Khan, I., Saeed, K., and Khan, I. 2017. "Nanoparticles: Properties, Applications and Toxicities." *Arabian Journal of Chemistry* 5: 1-24.

[2] Buzea, C., Pacheco, I. I., and Robbie, K. 2007. "Nanomaterials and Nanoparticles: Sources and Toxicity." *Biointerphases* 2 (4): MR17-71.

[3] Grigoratos, T., and Martini, G. 2015. "Brake Wear Particle Emissions: A Review." *Environmental science and Pollution Research* 22: 2491-504.

[4] Hamra, G. B., Guha, N., Cohen, A., Laden, F., Raaschou-Nielsen, O., Samet, J. M., Vineis, P., Forastiere, F., Saldiva, P., Yorifuji, T., and Loomis, D. 2014. "Outdoor Particulate Matter Exposure and Lung Cancer: A Systematic Review and Meta-Analysis." *Environmental Health Perspectives* 122: 906-11.

[5] McKenzie, E. R., Money, J. E., Green, P. G., and Young, T. M. 2009. "Metals Associated with Stormwater-Relevant Brake and Tire Samples." *The Science of the Total Environment* 1: 407-22.

[6] Von Uexkü ll, O., Skerfving, S., Doyle, R., and Braungart, M. 2005. "Antimony in Brake Pads—A Carcinogenic Component?" *Journal of Cleaner Production* 13: 19-31.

[7] Blau, P. J. 2001. *Compositions, Functions, and Testing of Friction Brake Materials and Their Additives*. Report for U.S. Department of Energy, Assistant Secretary for Energy Efficiency and Renewable Energy Office of Transportation Technologies.

[8] Davis, A. P., Shokouhian, M., and Ni, S. 2001. "Loading Estimates of Lead, Copper, Cadmium, and Zinc in Urban Runoff from Specific Sources." *Chemosphere* 44: 997-1002.

[9] Švabenska, E., Roupcova, P., and Schneeweis, O. 2015. "Analysis of Nanoparticles Released from the Car Brakes." In *Proceedings of the 15th Nanocon*, 1-5.

[10] Konduru, N., Keller, J., Ma-Hock, L., Gr öters, S., Landsiedel, R., Donaghey, T. C., Brain, J. D., Wohlleben, W., and Molina, R. M. 2014. "Biokinetics and Effects of Barium Sulfate Nanoparticles." *Particle and Fibre Toxicology* 11: 55.

[11] Buesen, R., Landsiedel, R., Sauer, U. G., Wohlleben, W., Groeters, S., Strauss, V., Kamp, H., and van Ravenzwaay, B. 2014. "Effects of SiO_2, ZrO_2, and $BaSO_4$ Nanomaterials with or without Surface Functionalization upon 28-Day Oral Exposure to Rats." *Archives of Toxicology* 88 (10): 1881-906.

[12] Gutwein, L. G., and Webster, T. J. 2002. "Osteoblast and Chrondrocyte Proliferation in the Presence of Alumina and Titania Nanoparticles." *Journal of Nanoparticle Research* 4: 231-8.

[13] Powers, K. W., Palazuelos, M., Moudgil, B. M., and

Roberts, S. M. 2009. "Characterization of the Size, Shape, and State of Dispersion of Nanoparticles for Toxicological Studies." *Nanotoxicology* 10: 42-51.

[14] Chen L., Wang, J., Wang, H., Zheng, Y., Qi, Z., Chang G., Xu, S., Li, R., Wu, T., and Xu, W. 2016. "Green Synthesis of Barium Sulfate Particles Using Plant Extracts." *Proceedings of the MATEC Web of Conferences* 67: 1-7.

[15] Shen, Y., Saboe, P. O., Sines, I. T., Erbaka, M., and Kumar, M. 2014. "Biomimetic Membranes." *Journal of Membrane Science* 454: 359-81.

[16] Halder, S., Yadav, K. K., Sarkar, R., Mukherjee, S., Saha, P., Haldar, S., Karmakar, S., and Sen, T. 2015. "Alteration of Zeta Potential and Membrane Permeability in Bacteria: A Study with Cationic Agents." *Springer Plus*, 4: 672 (November): 1-14.

[17] Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. 2002. *Molecular Biology of the Cell.* New York: Garland Science.

[18] Foroonzadeh, P., and Aziz, A. A. 2018. "Insight into Cellular Uptake and Intracellular Trafficking of Nanoparticles." *Nanoscale Research Letters* 13: 339.

[19] Conner, S. D., and Schmid, S. L. 2003. "Regulated Portals of Entry into the Cell." *Nature* 422: 37-44.

[20] Wang, L., Hu, C., and Shao, L. 2017. "The Antimicrobial Activity of Nanoparticles: Present Situation and Prospects for the Future." *International Journal of Nanomedicine* 12: 1227-49.

[21] Ansari, M. A., Khan, H. M., Alzohairy, M. A., Jalal, M., Ali, S. G., Pal, R., and Musarrat, J. 2015. "Green Synthesis of Al_2O_3 Nanoparticles and Their Bactericidal Potential against Clinical Isolates of Multi-drug Resistant *Pseudomonas aeruginosa*." *World Journal of Microbiology and Biotechnology* 31: 153-64.

[22] Wu, B., Zhuang, W. Q., Sahu, M., Biswas, P., and Tang, Y. J. 2011. "Cu-Doped TiO_2 Nanoparticles Enhance Survival of *Shewanella oneidensis* MR-1 under Ultraviolet (UV) Light Exposure." *Science of the Total Environment* 21: 4635-9.

[23] Matai, I., Sachdev, A., Dubey, P., Kumar, S. U., Bhushan, B., and Gopinath, P. 2014. "Antibacterial Activity and Mechanism of Ag-ZnO Nanocomposite on *S. aureus* and GFP-Expressing Antibiotic Resistant *E. coli*." *Colloids and Surfaces B: Biointerfaces* 115: 359-67.

[24] Hussein-Al-Ali, S. H., El Zowalaty, M. E., Hussein, M. Z., Geilich, B. M., and Webster, T. J. 2014. "Synthesis, Characterization, and Antimicrobial Activity of an Ampicillin-Conjugated Magnetic Nanoantibiotic for Medical Applications." *International Journal of Nanomedicine* 9: 3801-14.

[25] Aninwene II, G. E., Stout, D., Yang, Z., and Webster, T. J. 2013. "Nano-$BaSO_4$: A Novel Antimicrobial Additive to Pellethane." *International Journal of Nanomedicine* 8: 1197-205.

[26] Isani, G., Falcioni, M. L., Barucca, G., Sekar, D., Andreani, G., Carpene, E., and Falcioni, G. 2013. "Comparative Toxicity of CuO Nanoparticles and $CuSO_4$ in Rainbow Trout." *Ecotoxicology and Environmental Safety* 97: 40-6.

[27] Midander, K., Wallinder, I., O., and Leygraf, C. 2006. "*In Vitro* Studies of Copper Release from Powder Particles in Synthetic Biological Media." *Environmental Pollution* 145: 51-9.

[28] Bondarenko, O., Sihtmäe, M., Kuzmičiova, J., Ragelienė, L., Kahru, A., and Daugelavičius R. 2018. "Plasma Membrane Is the Target of Rapid Antibacterial Action of Silver Nanoparticles in *Escherichia coli* and *Pseudomonas aeruginosa*." *International Journal of Nanomedicine* 13: 6779-90.

[29] Horie, M., Fujita, K., Kato, H., Endoh, S., Nishio, K., Komaba, K. L., Nakamura, A., Miyauchi, A., Kinugasa, S., Haginasa, Y., Niki, E., Yoshida, Y., and Iwahash, H. 2012. "Association of the Physical and Chemical Properties and the Cytoxicity of Metal Oxide Nanoparticles: Metal Ions Release, Adsorption Ability and Specific Surface Area." *Metallaomics* 4: 350-60.

[30] Saliani, M., Jalal, R., and Kafshdare Goharshadi, E. 2015. "Effects of pH and Temperature on Antibacterial Activity of Zinc Oxide Nanofluid against *Escherichia coli* O157:H7 and *Staphylococcus aureus*." *Jundishapur Journal Microbiology* 8 (2): 1-6.

[31] Erdogan, A., Schaefer, C. A., Most, A. K., Schaefer, M. B., Mayer, K., Tillmanns, H., and Kuhlmann, C. R. 2006. "Lipopolysaccharide-Induced Proliferation and Adhesion of U937 Cells to Endothelial Cells Involves Barium Chloride Sensitive Hyperpolarization." *Journal of Endotoxin Research* 4: 224-30.

[32] Hardin, J., Bertoni, G., and Kleinsmith, L. J. 2010. *Becker's World of the Cell.* San Francisco: Benjamin-Cummings Publishing Company.

[33] Yellen, G. 1999. "The Bacterial K^+ Channel Structure and Its Implications for Neuronal Channels." *Current Opinion in Neurobiology* 3: 267-73.

[34] Kozelskaya, A. I., Panin, A. V., Khlusov, I. A., Mokrushnikov, P. V., Zaitsev, B. N., Kuzmenko, D. I., and Vasyukov, G. Y. 2016. "Morphological Changes of the Red Blood Cells Treated with Metal Oxide Nanoparticles." *Toxicology in Vitro* 37: 34-40.

[35] Pan, X., Wang, Y., Chen, Z., Pan, D., Cheng, Y., Liu, Z., Lin, Z., and Guang, X. 2013. "Investigation of Antibacterial Activity and Related Mechanism of a Series of Nano-Mg $(OH)_2$." *ACS Applied Materials & Interfaces* 5 (3): 1137-42.

[36] Arakha, M., Saleem, M., Mallick, B. C., and Jha, S. 2015. "The Effects of Interfacial Potential on Antimicrobial

Propensity of ZnO Nanoparticle." *Scientific Reports* 5: 1-10.

[37] Cho, E. C., Xie, J., Wurm, P. A., and Xia, Y. 2009. "Understanding the Role of Surface Charges in Cellular Adsorption Versus Internalization by Selectively Removing Gold Nanoparticles on the Cell Surface with a I2/KI Etchant." *Nano Letters* 3: 1080-4.

[38] Harush-Frenkel, O., Debotton, N., Benita, S., and Altschuler, Y. 2007. "Targeting of Nanoparticles to the Clathrin-Mediated Endocytic Pathway." *Biochemical and Biophysical Research Communications* 353 (1): 26-32.

[39] Yahaya, M. Z., Abdullah, M. Z., and Mohamad, A. A. 2015. "Centrifuge and Storage Precipitation of TiO_2 Nanoparticles by the Sol-Gel Method." *Journal of Alloys and Compounds* 651: 557-64.

[40] McCall, R. L., and Sirianni, R. W. 2013. "PLGA Nanoparticles Formed by Single- or Double-Emulsion with Vitamin E-TPGS." *Journal of Visualized Experiments* 82: 1-8.

[41] Kukutschová, J., Moravec, P., Tomášek, V., Matejka, V., Smolík, J., Schwarz, J., Seidlerová, J., Safářová, K., and Filip, P. 2011. "On Airborne Nano/Micro-sized Wear Particles Released from Low-Metallic Automotive Brakes." *Environmental Pollution* 159: 998-1006.

[42] Siti Nur Afiqah, M. M., Lehb, O. L. H., Omarb, D., and Karuppannan, S. 2015. "Theoretical Review on Environmental Health in Relation to Neighbourhood Planning and Human Physical Activity." *Procedia—Social and Behavioral Sciences* 201: 325-32.

[43] Federal-Mogul Products, Inc. Southfield, MI 48033 (US). 2018. *Friction Material for Brakes.* European Patent EP 2 491 267 B1, Filed October 23, 2009, and issued August 29, 2012.

[44] Kim, H.-C., and Chung, Y. S. 2013. "Preparation and Radiopaque Properties of Chitosan/$BaSO_4$ Composite Fibers." *Fibers and Polymers* 2: 292-7.

[45] Hagino, H., Oyama, M., and Sasaki, S. 2016. "Laboratory Testing of Airborne Brake Wear Particle Emissions Using a Dynamometer System under Urban City Driving Cycles." *Atmospheric Environment* 131: 269-78.

[46] Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., and Mozafari, M. R. 2018. "Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems." *Pharmaceutics* 57: 1-17.

[47] Limbach, L. K., Li, Y., Grass, R. N., Brunner, T. J., Hintermann, M. A., Muller, M., Gunther, D., and Stark, W. J. 2005. "Oxide Nanoparticle Uptake in Human Lung Fibroblasts: Effects of Particle Size, Agglomeration, and Diffusion at Low Concentrations." *Environmental Science & Technology* 39 (23): 9370-6.

[48] Horie, M., and Fujita, K. 2011. "Chapter Four: Toxicity of Metal Oxides Nanoparticles." *Advances in Molecular Toxicology* 5: 145-78.

[49] Hong, Y., and Brown, D. G. 2006. "Cell Surface Acid-Base Properties of *Escherichia coli* and *Bacillus brevis* and Variation as a Function of Growth Phase, Nitrogen Source and C:N Ratio." *Colloids Surfaces B Biointerfaces* 11: 112-9.

[50] Yoshimura, H., and Nikaido, H. 1982. "Permeability of *Pseudomonas aeruginosa* Outer Membrane to Hydrophilic Solutes." *Journal of Bacteriology* 152: 636-42.

[51] Dhar, S., Kumari, H., Balasubramanian, D., and Mathee, K. 2018. "Cell-Wall Recycling and Synthesis in *Escherichia coli* and *Pseudomonas aeruginosa*—Their Role in the Development of Resistance." *Journal of Medical Microbiology* 67: 1-21.

[52] Cieśla, J., Bieganowski, A., Janczarek, M., and Urbanik-Sypniewska, T. 2011. "Determination of the Electrokinetic Potential of Rhizobium Leguminosarum bv Trifolii Rt24.2 Using Laser Doppler Velocimetry—A Methodological Study." *Journal of Microbiological Methods* 85: 199-205.

[53] Shephard, J., McQuillan, A., J., and Bremer, P., J. 2008. "Mechanisms of Cation Exchange by *Pseudomonas aeruginosa* PAO1 and PAO1 *wbpL*: A Strain with a Truncated Lipopolysaccharide." *Applied and Environmental Microbiology* 22: 6980-6.

[54] Berridge, V. M., Herst, P. M., and Tan, A., S. 2005. "Tetrazolium Dyes as Tools in Cell Biology: New Insights into Their Cellular Reduction." *Biotechnology Annual Review* 11: 127-52.

[55] Stockert, J. C., Blázquez-Castro, A., Canete, M., Horobin, R. W., and Villanueva, Á. 2012. "MTT Assay for Cell Viability: Intracellular Localization of the Formazan Product Is in Lipid Droplets." *Acta Histochemica* 114: 785-96.