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Antimicrobial Activity of Synthesized Multi-Metallic Nanoparticles using Traditional Indian Siddha Method

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In present work, multi-metallic nanoparticles were synthesized by chemical method in a controlled environment by using silver, lead, mercury, egg shell powder (contains 1% calcium phosphate, 1% magnesium carbonate, 94% calcium carbonate and 4% organic matter), potassium nitrate, potassium alum and extracts of citrus lemon by following the process defined in Traditional Indian Medicine, Siddha System of Medicine. The morphology, compositions and structure of the product were characterized by powder X-ray diffraction (PXRD), Fourier transform infrared (FTIR), scanning electron microscopy (SEM), energy dispersive X-ray (EDX) and transmission electron microscopy (TEM) techniques. Highly uniform spherical multi-metallic nanoparticle was subjected for the antibacterial activities. The particles were agglomerated as observed by SEM micrographs. The particles were homogeneous, spherical in shape and loosely agglomerated as seen by TEM pictures. The antibacterial activity of the synthesized multi-metallic nanoparticles against *B. cereus*, *S. aureus*, *E. coli* and *P. aeuroginosa* was demonstrated using the zone of inhibition technique. The synthesized multi-metallic nanoparticle can find plausible biological applications.

Keywords: Siddha medicine, Multi-metallic nanoparticle, Antimicrobial activity.

INTRODUCTION

Nanotechnology is quickly expanding by developing the nanoproducts and nanoparticles with unique and size-related physico-chemical characteristics, which differ dramatically from bigger materials [1]. Pharmaceuticals, cosmetics, renewable energy, bioremediation and medical gadgets have all benefited from the innovative features of nanoparticles [2,3]. To synthesize various types of nanoparticles, a large number of physical, chemical and green synthesis methods are currently available [4,5]. Though physical and chemical procedures are more commonly used to make nanoparticles, the usage of hazardous substances restricts their utilization. Because of the ease and adaptability of the techniques, the development of safe, environmentally acceptable technologies for biogenetic manufacturing is gaining attention [6]. Biological nanoparticles are finding crucial uses in medicine due to their capacity to perform biological activities [7-9].

The Siddha (Cita) system of medicine is one of the oldest traditional medical systems, having originated in India and been practiced mostly in the country's southern regions for the treatment of numerous ailments, particularly chronic conditions [10]. Siddha's pharmacopeia includes medications made from minerals, metals and animal products, in addition to plants. One of the distinctive aspects of the Siddha system of medicine in the preparation of remedies from metals and minerals [11]. These medications were made into Parpam (Parpam), Chendooram (Senthuram or Canturam), Chunnam (Cunnm), Kattu (Kattu), Kalangu (Kalanku) and other forms.

Metal based nanoparticles are responsible for their extensive interaction with biomolecules both within and outside the cell. Cell permeability is aided by their large surface area. Surface

charges and physico-chemical characteristics of metal nanoparticles are also important [12]. Metal nanoparticles have unique features that make them promising treatments for infectious disorders. In addition, utilizing metal nanoparticles to prevent and treat the microbial infection can result in more effective immune responses.

Higher metabolic stability, increased membrane permeability, greater bioavailability and longer action are all advantages of nano-drug systems. Nanotransporters provide specialized tailored medication delivery of anticancer medicines to the tissue and cellular levels. Size, surface charge and hydrophobicity are the most important characteristics that influence mucosal or transdermal absorption. The size of the particles is important; smaller nanoparticles induce more transcellular uptake than bigger ones. However, intestinal cells were unable to absorb nanoparticles, bigger than 300 nm. Only nanoparticles, smaller than 50 nm can get into the bloodstream [13]. In biomedicine, there are a growing number of nanoparticle based medicines that have been authorized for clinical use, the majority of which employ spherical liposomes. On the other hand, researchers are continuously working on a larger range of nanoparticle designs, including core materials with magnetic, optical and biological capabilities, as well as nanoparticles of various sizes, shapes, hardness, and surface features. Nanoparticles provide effective imaging capabilities in addition to their therapeutic uses [14]. For the green synthesis of nanomaterials, a variety of physical and chemical processes have been used, including the solvothermal method, hydrothermal route, pulsed laser ablation technique, sonochemical procedure and microwave irradiation [15]. Traditional techniques include drawbacks such as cost, poor end products, harmful toxic chemicals, high temperature, *etc*.

Green synthesis, on the other hand, is a cost-effective and environmentally friendly way of synthesizing nanostructural materials with customizable architectures, morphologies and particle size distributions. Green synthesis techniques use organisms such as plants, bacteria, fungus, and other microbes to synthesize nanomaterials and they've sparked a lot of interest in the field because of their less hazardous or non-toxic nature, eco-friendliness and low cost of production. Plant extracts are made from diverse plants' leaves, stems, flowers and seeds. Protein, amino acids, enzymes, vitamins, terpenoids, flavonoids, alkaloids, phenolic acids and other compounds included in the extracts function as capping and reducing agents, reducing metal ions during the bioreduction process to form nanoparticles or nanostructures of various dimensions and morphologies [16].

However, no systematic evidence has been reported for silver, lead, mercury, eggshell powder (consists of 1% calcium phosphate, 1% magnesium carbonate, 94% calcium carbonate, and 4% organic matter), potassium nitrate, potassium alum and citrus lemon extracts using the process defined in the Traditional Indian Medicine (TIM), Siddha System of Medicine (SSM) and compound-based synthesized multi-metallic nanoparticles. In light of this, researchers have turned their attention to the multi-metallic nanoparticles found in the Siddha System of Medicine in order to learn more about their undiscovered phytoconstituents and antibacterial potential against *B. cereus*, *S. aureus*, *E. coli* and *P. aeuroginosa*.

EXPERIMENTAL

The multi-metallic nanoparticle was synthesized using a mixture of grinding, drying, heat treatment, sublimation and grinding for a certain time period by following a standard approach as described in the Traditional Indian Medicine, Siddha System of Medicine (TIM-SSM). Utilizing a combination of grinding and sublimation, a grinding tool made of hard granite and instruments developed and constructed using the clay used for the synthesis of the multi-metallic nanoparticle was created.

Synthesis of multi-metallic nanoparticle: Cleaned eggshell about 50 g was initially well-grounded with citrus lemon juice. Then an equal amount of potassium alum was added to the sample and ground well. This sample was undergone for further grinding with egg albumin, calcined and followed by the addition of 100 g of lead and mercury added each and the product was an amalgam. It further calcination and then added potassium nitrate, grinded and calcined. Finally, 200 g of silver and potassium nitrate was added to the sample. This sample was undergone to calcination process thrice a time.

FT-IR analysis: Nicolet-TM 6700 FT-IR spectrometer from Thermo Scientific was used for the FTIR spectral analysis which ranged from 5000 to 500 cm^{-1}.

SEM-EDAX analysis: The SEM with EDX from Carl Zeiss AG, SUPRA 40 with 1.3 nm@15 kV resolution, 12-900,000X magnification, thermal field emission type emitter with acceleration voltage 0.1-30 kV, propel current 4pA-10nA, high efficiency.

XRD analysis: X-ray diffractometer having multipurpose technology characteristics of D/teX Ultra silicon strip detector, independent θ-θ geometry, 3 kW sealed X-ray tube.

TEM analysis: An FEI Tecnai T20 Ultra Twin transmission electron microscope was used for the TEM microscopic films. This microscope was equipped with a 200 kV thermionic gun compatible with W and LaB6.

Microorganisms: The Vellore Institute of Technology in Vellore, India provided all of the organisms utilized in this study. *Bacillus cereus*, *Enterobacter cloacae*, *Streptococcus aeruginosa* and *Pseudomonas aeruginosa*.

Dilution assays: Broth microdilution tests was applied as per method reported by Wiegand *et al*. [17]. In brief, Hinton-Muller and the 96-well microtiter plate with a cover was also used to test broth for antibacterial screening. Microtiter plates were incubated for 18-20 h at 37 ºC. Following the incubation period, 20 L of Alamar blue was added to each well and incubated for a few minutes to see observe the any colour changes occurred or not.

RESULTS AND DISCUSSION

A "one component at a time" factorial design was used to optimize the nanoparticle production. The experiment was based on changing one component at a time while remaining constant with the others. Silver, lead, mercury, eggshell powder, potassium nitrate, potassium alum, and extracts of citrus lemon,

metal salt concentration and mixing ratio were all taken into account. FT-IR spectrum, SEM with EDAX, TEM and particle size and zeta potential approach was used to investigate the influence of various process factors on the synthesis of multi-metallic nanoparticles.

FT-IR studies: In the FT-IR spectra of a multi-metallic nanoparticle sample exhibit peaks at 3385, 3027, 2970, 2952, 2664, 2401, 2115, 1993, 1862, 1741, 1641, 1367, 1097, 1042, 966, 819, 727, 672 and 589 cm^{-1} (Fig. 1). As a result, this multi-metallic nanoparticle sample mostly comprises potassium and sodium compounds. The majority of the multi-metallic nanoparticles in the sample are alkyne in origin. As a result, it may deduce, which can easily neutralize acids. The fingerprints created by these FT-IR characterization data will be used to standardize this Siddha multi-metallic nanoparticles.

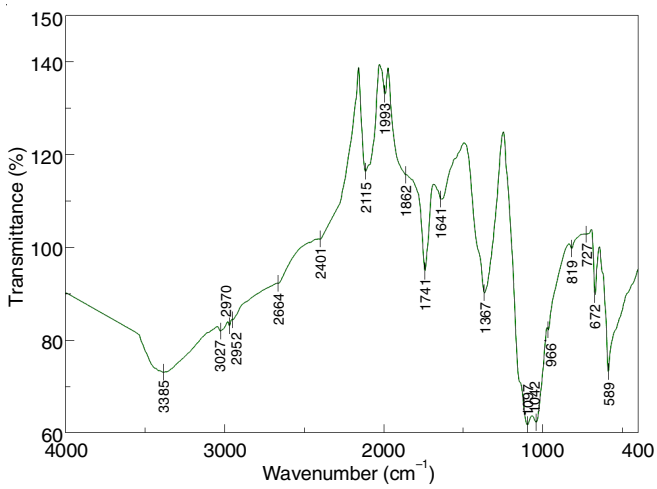

Fig. 1. Functional groups analysis of multi-metallic nanoparticle using FT-IR

SEM studies: The majority of the studies employ extra fuels or binders to synthesize nanocrystalline multi-metallic nanoparticles, however, this breakthrough was achieved without them. The morphological analysis and crystallite size were determined using the SEM technique as shown in Fig. 2. The size of the particle is found in nanometers indicating that it will be simple or rapid to assimilate the medicine, boosting its efficacy.

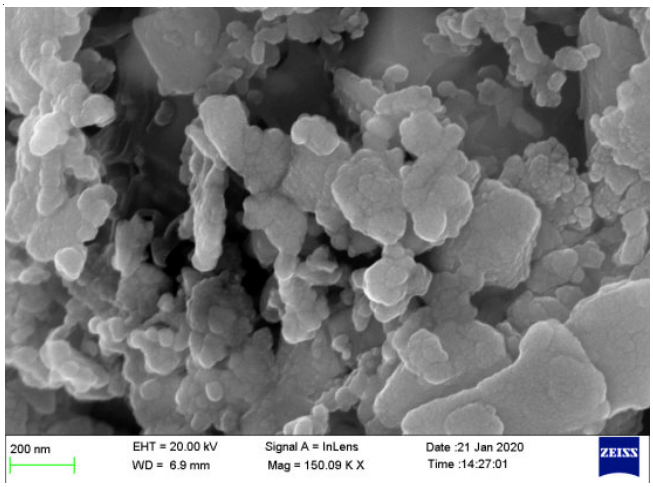

Fig. 2. SEM morphology of nanocrystalline multi-metallic nanoparticle

EDAX studies: The prepared multi-metallic nanoparticles exhibit the presence of several atoms as measured by energy dispersive X-ray analysis (EDAX) (Fig. 3). The atom percentages of the elements based on the spectra were evaluated as C = 3.4%, O = 18.0%, Mg = 0.1%, Al = 2.0%, Si = 0.8%, Mo = 12.3%, Cl = 10.2%, Ag = 20.6%, Ca = 2.2%, Fe = 2.4%, Hg = 22.2% and As = 5.8% (Table-1).

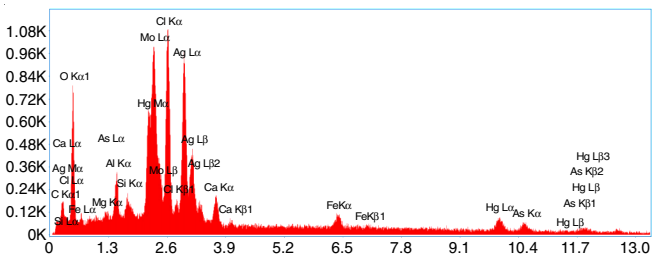

Fig. 3. EDAX of multi-metallic nanoparticle

TABLE-1
CONCENTRATION OF MAIN ELEMENTS IN THE MULTI-METALLIC NANOPARTICLE

Element	Weight (%)	Atomic (%)	Error (%)	K ratio
CK	3.4	11.7	17.8	0.0078
OK	18.0	46.8	11.8	0.0326
MgK	0.1	0.1	91.0	0.0004
AlK	2.0	3.0	11.1	0.0113
SiK	0.8	1.1	17.7	0.0054
MoL	12.3	5.3	4.5	0.1016
ClK	10.2	11.9	6.4	0.0776
AgL	20.6	7.9	5.9	0.1469
CaK	2.2	2.3	14.4	0.0182
FeK	2.4	1.8	13.3	0.0247
HgL	22.2	4.6	20.7	0.1587
AsK	5.8	3.2	16.5	0.0655

XRD studies: The crystalline form of multi-metallic nanoparticles was confirmed by the typical XRD peaks of biogenic Ag and Au nanoparticles corresponding to (111), (200), (220), and (311) planes (Fig. 4). Because of their atomic density, the diffraction planes contain energetically unique places that interact with biological systems. On the (111) plane, the multi-metallic nanoparticles have a high atomic density. The crystallization of bioorganic components present in the citrus lemon aqueous extract on the surface of the multi-metallic nanoparticle results in unassigned Bragg peaks near diffraction planes of multi-metallic nanoparticles.

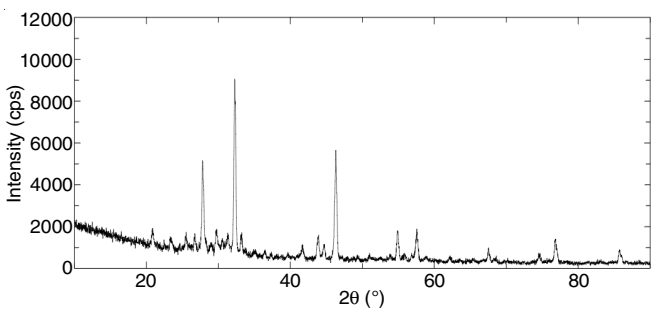

Fig. 4. XRD pattern of multi-metallic nanoparticle

TEM studies: TEM micrographs of the multi-metallic nanoparticle sample's reveal a sponge-like structure with particle sizes in the nano range. It is obvious from the TEM picture that many crystallites have agglomerated on the signal particle, resulting in a microcrystalline structure with grain boundaries lost. The size of the particles is not consistent, as seen by TEM images. Fig. 5 shows a higher number of particles in 43-70 nm region.

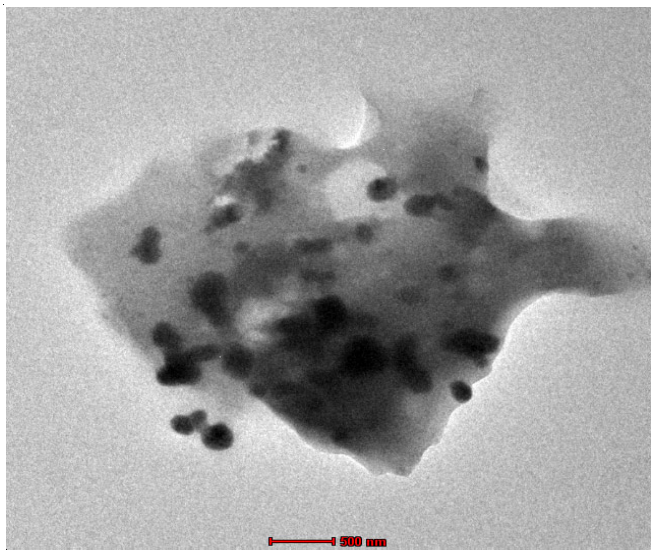

Fig. 5. TEM images of multi-metallic nanoparticle

Particle size: The TEM micrographs of multimetallic nanoparticles revealed the virtually spongy-like shapes. The size of the main particles was less than 13.4 nm. The DLS method was used to determine the secondary particle size. Multi-metallic nanoparticles were found to have hydrodynamic diameters of 53.5-56.2 nm (Fig. 6). Both TEM and DLS investigations revealed distinct particle sizes. The DLS determines the hydrodynamic size of particles in a colloidal solution which is moving in a Brownian motion. The particle sizes evaluated by TEM and DLS were found in between 43 and 123.23 nm, respectively.

Antimicrobial activity: The antimicrobial activity data of the multi-metallic nanoparticles is presented in Table-2. This multicombo nanoparticles exhibited significant antimicrobial activity against all tested bacteria. Considering the *B. cereus*, the growth inhibition emerges at 6 µg/mL. Similarly, *S. aureus* population was restricted even at 4 µg/mL. However, it exhibited low activity against *E. coli*. Earlier report clearly explains that *P. aeuroginosa* is highly resistance. Therefore, we tested with higher concentration than treated with other bacteria. The prepared multimetal nanoparticles showed a good antibacterial against *P. aeuroginosa* at 16 µg/mL.

It is well documented that presence of Al and Fe metal ions react with membrane phospholipids thereby enhance the cytotoxicity of pathogens [17]. It is noteworthy that the relationship between physico-chemical properties and biological activity in multi-metallic nanoparticles difficult to generalize but the effectiveness of clinical response will be important to consider than using single metal particle. It is an advantage that antibacterial efficiency of particular one can be complemented by non-toxicity of other. Similar fashion TiO_2 nanoparticles along with Ag exhibited increased effectiveness against *S. aureus* than TiO_2 alone [18]. Number of studies showed that doping metal with Mg promotes the antibacterial efficacy [19]. Mercury and arsenic can binds SH groups, such as in cysteine, which can directly disrupt the function of folded proteins resulting in the detrimental effects [20]. Hence, it is concluded that the presence of minerals and multi-metallic nanoparticles exhibit the cytotoxicity against tested bacterial pathogens through enhancing the membrane permeable and disrupting intracellular proteins.

Conclusion

The present study reports the facile approach of synthesizing multi-metallic nanoparticle using citrus lemon extract. The intriguing finding is that the shape and size of the multi-metallic nanoparticles produced have a direct and substantial

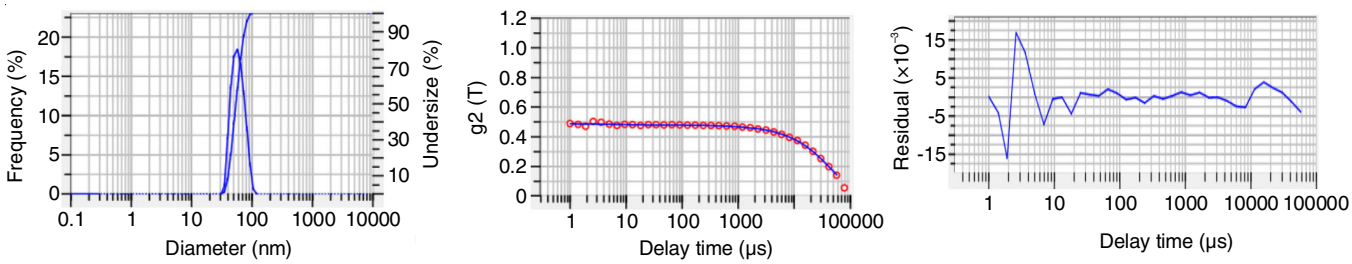

Fig. 6. Confirming the presence of multi-metallic nanoparticle by DLS

TABLE-2
ANTIMICROBIAL ACTIVITY OF PREPARED DRUG

Items	Activity	Pathogen	Various concentration of drug				
			2 µg/mL	4 µg/mL	6 µg/mL	8 µg/mL	10 µg/mL
Kanamycin		*B. cereus*	+++	+++	++	++	++
Broth	–	*E. coli*	+++	+++	+++	+++	+++
Bac.Broth	+++	*S. eureus*	+++	++	++	++	++
Vehicle control	+++	*P. aeruginosa*	8 µg/mL	10 µg/mL	12 µg/mL	16 µg/mL	20 µg/mL
Pos. control	++	*P. aeruginosa*	+++	+	+++	++	++

impact on the process variable employed in the experiment and are dependent on it. The crystalline structure of the green synthesized multi-metallic nanoparticle was characterized by XRD investigation. From FT-IR and XRD analyses, it was observed that citrus lemon extract acted as apparent and showed more activity in the stabilizer for the synthesis of multi-metallic nanoparticles. The present investigation also exerted the antibacterial studies of synthesized multi-metallic nanoparticle were highly effective with different inhibition zone capability against studied bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. Y. Ju-Nam and J.R. Lead, *Sci. Total Environ.*, **6**, 143 (2008); https://doi.org/10.1016/j.scitotenv.2008.06.042
2. G.R. Chaudhuri and S. Paria, *Chem. Rev.*, **112**, 2373 (2012); https://doi.org/10.1021/cr100449n
3. C. Morasso, D. Mehn, R. Vanna, M. Bedoni, E. Forvi, M. Colombo, D. Prosperi and F. Gramatica, *Mater. Chem. Phys.*, **143**, 1215 (2014); https://doi.org/10.1016/j.matchemphys.2013.11.024
4. N.A. Luechinger, R.N. Grass, E.K. Athanassiou and W.J. Stark, *Chem. Mater.*, **22**, 155 (2010); https://doi.org/10.1021/cm902527n
5. J. Liu, S.Z. Qiao, Q.H. Hu and G.Q. Max Lu, *Small*, **7**, 425 (2011); https://doi.org/10.1002/smll.201001402
6. M. Popescu, A. Velea and A. Lorinczi, *Dig. J. Nanomater. Biostruct.*, **5**, 1035 (2010).
7. V. Dushenkov, P.B.A.N. Kumar, H. Motto and I. Raskin, *Environ. Sci. Technol.*, **29**, 1239 (1995); https://doi.org/10.1021/es00005a015
8. Y.V. Anisimova, S.I. Gelperina, C.A. Peloquin and L.B. Heifets, *J. Nanopart. Res.*, **2**, 165 (2000); https://doi.org/10.1023/A:1010061013365
9. S. Suri, H. Fenniri and B. Singh, *J. Occup. Med. Toxicol.*, **2**, 16 (2007); https://doi.org/10.1186/1745-6673-2-16
10. A. Ram, D. Arul Joseph, S. Balachandar and V. Pal Singh, *Int. J. Pharm. Anal.*, **1**, 975 (2009).
11. V.V. Mody, R. Siwale, A. Singh and H.R. Mody, *J. Pharm. Bioallied Sci.*, **2**, 282 (2010); https://doi.org/10.4103/0975-7406.72127
12. W.H. De Jong, W.I. Hagens, P. Krystek, M.C. Burger, A.J.A.M. Sips, and R.E. Geertsma, *Biomaterials*, **29**, 1912 (2008); https://doi.org/10.1016/j.biomaterials.2007.12.037
13. D. Chenthamara, S. Subramaniam, S.G. Ramakrishnan, S. Krishnaswamy, M.M. Essa, F.H. Lin and M.W. Qoronfleh, *Biomater. Res.*, **23**, 20 (2019); https://doi.org/10.1186/s40824-019-0166-x
14. N. Zahin, R. Anwar, D. Tewari, M.T. Kabir, A. Sajid, B. Mathew, M.S. Uddin, L. Aleya and M.M. Abdel-Daim 6, *Environ. Sci. Pollut. Res. Int.*, **27**, 19151 (2019); https://doi.org/10.1007/s11356-019-05211-0
15. T. Satyanarayana and S.S. Reddy, *Int. J. Res. Appl. Sci. Eng. Technol.*, **6**, 2885 (2018); https://doi.org/10.22214/ijraset.2018.1396
16. S.H. Khan, Eds.: M. Naushad and E. Lichtfouse, Green Nanotechnology for the Environment and Sustainable Development, In: Green Materials for Wastewater Treatment, Environmental Chemistry for a Sustainable World, Springer, Cham, vol. 38, pp. 13-46 (2020).
17. I. Wiegand, K. Hilpert and R.E. Hancock, *Nat. Protoc.*, **3**, 163 (2008); https://doi.org/10.1038/nprot.2007.521
18. S.C. Londono, H.E. Hartnett and L.B. Williams, *Environ. Sci. Technol.*, **51**, 2401 (2017); https://doi.org/10.1021/acs.est.6b04670
19. S. Stankic, S. Suman, F. Haque and J. Vidic, *J. Nanobiotechnol.*, **14**, 73 (2016); https://doi.org/10.1186/s12951-016-0225-6
20. Y.N. Slavin, J. Asnis, U.O. Häfeli and H. Bach, *J. Nanobiotechnol.*, **15**, 65 (2017); https://doi.org/10.1186/s12951-017-0308-z