Research Article

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COMPARISON OF PHYSICAL AND MORPHOLOGICAL PROPERTIES OF MANDURA BHASMA AND IRON OXIDE NANOPARTICLES

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ABSTRACT

There are several standardization techniques for assuring the quality of Ayurvedic medicines. Bhasmas are nanoparticle medicines, which are used in Ayurveda and quality assurance of these medicines plays an important role in the action of these remedies. As a physiochemical test for this quality assurance, Mandura bhasma was chosen and various physiochemical analysis was done, it was compared with synthesized iron oxide, to get the same physical and chemical parameters. FTIR studies were made for confirming the presence of iron oxide in the sample. XRD was taken on both the samples and the particle size was determined. Morphology of the samples were studied using SEM. Composition was determined using EDAX. The study reveals that the composition of the bhasmas are important in each formulation and it can be studied using EDAX. The impurities can be exactly determined by EDAX, which may be deliberately introduced in the drug for the effectiveness of the Ayurvedic drug. From this study, it is seen that XRD analysis is less important physiochemical analysis than the other methods mentioned in this paper. Further, the morphology from SEM shows that the bhasmas are a combination of various sized nanoparticles which has different spheres of action due to its size, which shows that the same bhasma can be used for different ailments due to its size effect.

Keywords: Mandura bhasma, Nanoparticles, Quality assurance.

INTRODUCTION

Ayurveda is one of the oldest system of medicines and bhasmas play a significant role in therapeutics, which are fast acting and are highly effective in lowest dose[1]. The size of these bhasmas fall in the range of nanometers and so these bhasmas can be called as nanomedicines[2]. Typically, in nanotechnology the size of nanoparticles should be less than or equal to 100 nanometers[3]. However, this definition of size does not impair the functionality of medicinal substances in nanoscale, because it has a large surface area which is the functional part of a nanomedicine. The large surface area is able to bind, adsorb and carry other compounds such as drugs, probes and proteins which explains the effectiveness of these nanomedicines in small quantities. The size of nanoparticles in the context of nanomedicine is considered to be 10 nm to 1000 nm[4,5]. Fabrication of nanoparticles can be broadly be done in two ways one is the top - down approach and the other is the bottom - up approach. Processing bhasmas comes under top-down process and chemical routes are under bottom - up process.

Bhasmas contains metals and its preparation involves calcinations of the metals or minerals treated with specific herbal juices and this process is repeated several times in certain cases. Ayurveda prescribes tests for properly prepared bhasmas, the bhasma should enter the furrows of finger, it should float on the still water surface, the bhasma should float even after rice grains are placed over it on the water surface, it should not emit any fumes when exposed to fire, should not possess any taste, it should not contain any residual shining particles, it should not regain its initial metallic luster and when treated with Silver coin should not increase the weight of Silver coin.

Bhasmas are metal oxides and their preparation methods differ even in classical texts and hence there is a necessity for standardization of these bhasmas[6]. These bhasmas in Ayurveda is not even recognized in biomedical science and hence, there should science based experiments to standardize and link Ayurveda to biomedical science[7]. There are several papers which explains the physiochemical properties of bhasmas[8-13]. In this paper we are going to analyze the physiochemical property of Mandura bhasma with iron oxide, to find which physiochemical analysis is more important in the standardization of bhasmas and also we try to find the mode of action of this bhasma.

MATERIALS AND METHOD

Chemicals used $FeCl_3 \cdot 6H_2O$, $NaAc \cdot 3H_2O$, ethylene glycol, acetyl acetone and Polyvinylpyrrolidone (PVP) were analytical grade, absolute ethanol were chemical grade, procured from Sdfine chemicals.

Synthesis of Iron oxided nanoparticles

The synthesis of Iron oxide has two steps, first is the preparation of Tris (acetylacetonato) iron(III) (Fe(acac)3) and then synthesis of the required nanopowder.

Preparation of Fe (acac)3: 6.75 g $FeCl_3 \cdot 6H_2O$ was dispersed in 25.0 ml deionized water, then the solution was transferred into three necked bottle. 5.00 g acetylacetone and 10.0 ml anhydrous methanol were added into the bottle

under stirring and lasted for 10 min. The solution which contained 6.80 g NaAc · $3H_2O$ and 15.0 ml water was added into the bottle. Red precipitation appeared and it was heated for 10 min, cooled down to room temperature. Air pump filtrated and washed with deionized water, red particles were dried at 100°C for 6 h, after that the product was kept in dryer.

Synthesis procedure: Fe(acac)3 (0.55 g), and PVP (1.00 g) were added to ethylene glycol (25.0 ml) to form mixture under vigorous stirring at room temperature. Then, the mixture was sealed in a Teflon lined stainless steel autoclave of 50 ml capacity. Finally, the autoclave was heated and maintained at 650°C for 12 h, then cooled down to room temperature. The black particles were washed with acetone, absolute ethanol and deionized water, after that black particles were dried at 60°C for 6 h.

Processing of the Mandura bhasma was done according to the process listed in the classical Ayurvedic text Siddha yoga samgraha Mandura bhasma was procured from renowned Ayurvedic manufacturer, for comparison.

Instruments used for analysis

Morphological analysis with Scanning Electron Microscope (SEM) and Elemental Analysis with Energy Dispersive X-Ray Analysis (EDAX)

Both morphological analysis and elemental analysis were done for both the samples with were performed using WEGA3 TESCAN, which is an integrated tool for SEM and EDAX analysis. The samples were spread on carbon foil and kept in vacuum chamber for analysis. Focusing using electron beam was done and the image obtained was recorded. EDAX works on the principle that each atom has a unique atomic structure and it corresponds to unique peaks in X-Ray spectrum. The samples were excited with high energy electron beam, which emits X-Rays, which are detected and a graph is drawn. From this the element present and its percentage could be analyzed.

Size determination and crystalline phase determination using XRD

When monochromatic X-Rays fall on the sample, the interaction between the sample and the X-Rays produce a diffraction pattern and there is a constructive interference if it satisfies Bragg's Law, which is $n\lambda = 2d \sin\theta$. The samples are scanned for 2θ degrees and the diffraction pattern is recorded. n is the order of diffraction which is one and λ is the wavelength of X-Rays. Each element has a unique set of d-spacings, which can be compared with the standard. Copper Cu Kα lines were used as a monochromatic X-Ray source and the samples were scanned from 10^0 to 80^0, 2θ degrees.

Using Debye-Scherrer formula $L = k\lambda/\beta \cos\theta$, the size of the nanoparticle can be determined. Here k is a constant which depends on the shape and is taken as 0.9. β is the peak width at half maximum in radians.

Fourier Transmission Infrared Spectroscopy for confirmation of compounds

The FTIR Spectra were recorded using FTIR spectrometer Perkin Elmer make for vibrational analysis of the samples and to confirm the compounds present in the sample. The range of scanning was from 400 cm^{-1} to 4500 cm^{-1}.

RESULTS

Elemental Analysis with Energy Dispersive X-Ray Analysis (EDAX)

From the elemental analysis of Mandura bhasma, the spectrum shows the presence of Carbon, Oxygen, Sulphur, Calcium, Chromium and Iron, with Iron 47.65% and Oxygen 38.57% by weight percentage and 20.30% and 57.37% by atomic weight percentage respectively. Other elements which are found are Carbon with 10.15% by weight percentage, which is the next major constituent followed by Calcium, Sulphur and Chromium with 2.56%, 0.71% and 0.36% by weight percentage respectively.

The chemically processed Iron Oxide has Iron 65.25%, Oxygen 33.96% and Magnesium 0.79% by weight percentage and by atomic percentage, Iron, Oxygen and Magnesium are 35.16%, 63.86% and 0.98% respectively.

Fourier Transmission Infrared Spectroscopy for confirmation of compounds

In Mandura Bhasma there are two peaks 473 cm^{-1} and 554 cm^{-1} and in Iron Oxide there are two peaks 446 cm^{-1} and 567 cm^{-1} which is associated with Fe-O bending and stretching vibration modes of γ-Fe_2O_3[14]. There has been a shift in the peaks of Iron Oxide from 473 cm^{-1} to 446 cm^{-1} with respect to bending vibration mode and from 554 cm^{-1} to 567 cm^{-1} with respect to stretching vibration mode. This may be due to high temperature annealing in the process of Iron Oxide nanoparticles. Peak at 1590 cm^{-1} in Mandura Bhasma and Peak at 1605 cm^{-1} in Iron Oxide are also attributed to Fe-O Stretching.

From these peaks it is understood that Mandura Bhasma is γ-Fe_2O_3, which is Maghemite which is a member of the family of iron oxides. It has the same structure as magnetite, that is, it is spinel ferrite and is also ferrimagnetic. Maghemite has numerous applications like recording, memory devices, magnetic resonance imaging, drug delivery or cell targeting[15,16].

The peak at 874 cm^{-1} in Mandura Bhasma is assigned to C-H stretching, 1102 cm^{-1} is attributed to C-C stretching vibration and 1366 cm^{-1} is due to C=C stretching vibration. In Iron Oxide, 1090 cm^{-1} peak is attributed to C-C stretching vibration and 1340 cm^{-1} is attributed to C=C stretching vibration.

The peak at 2833 cm^{-1} in Mandura Bhasma and the Peak at 2808 cm^{-1}in Iron Oxide are assigned to -CH_2 vibration. The peak at 3800 cm^{-1} in Mandura Bhasma and the peak at 3750 cm^{-1} are assigned to -OH stretching vibration in these samples.

From the FTIR analysis, it is clear that Mandura Bhasma is γ-Fe_2O_3 and it contains carbon, which is sourced from the organic process, during its manufacture. The FTIR spectrum of synthesized Iron Oxide is also γ-Fe_2O_3 and it also contains carbon, which is from the organic solvents used.

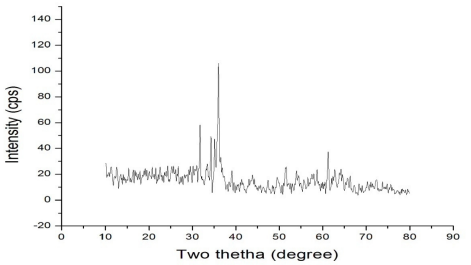
Morphology analysis for the samples using SEM

From the SEM image of Mandura Bhasma, it is clear that Mandura Bhasma is not uniform in size, there is a mixture of different sizes of the particles. The picture view field is 27.7 µm and the scale is 5µm. From this picture itself, it can be inferred that there are several particles which are

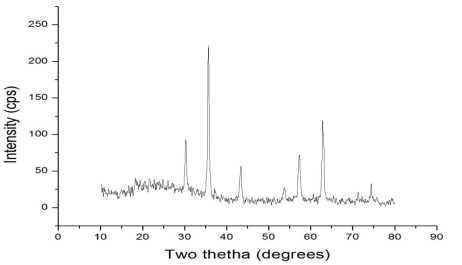
less than 500 nm (the minimum value in the scale).
From the SEM image of Iron Oxide, it is clear that the sample is not uniformly sized but, there are more number of particles below 500 nm, for the picture view field with 22.8 µm. Further, the distribution of the particles is not as random in size as that of Mandura Bhasma.

XRD analysis of the samples

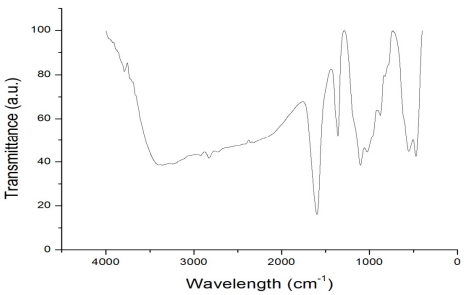
The XRD pattern for Iron Oxide shows peaks at 31.78°, 36.02°, 51.62°, 58.72° and 61.28° which are corresponding to the diffraction peaks of γ-Fe_2O_3 with slight variation from the JCPDS 25-1402, owing to the presence of trace elements and filling up of oxygen in the vacancy positions, indicating that nanoparticles are single phase with tetragonal structure. The particle size of Mandura Bhasma is calculated to be 24 nm, using Debye-Scherrer formula.
The XRD pattern for Iron Oxide shows peaks at 30.28°, 35.66°, 43.42°, 53.89°, 57.34° and 62.88° which are corresponding to the diffraction peaks of γ-Fe_2O_3 (JCPDS 25-1402), indicating that nanoparticles are single phase with tetragonal structure. The average particle size of Iron Oxide nanoparticles are calculated using Debye-Scherrer formula as 18 nm.



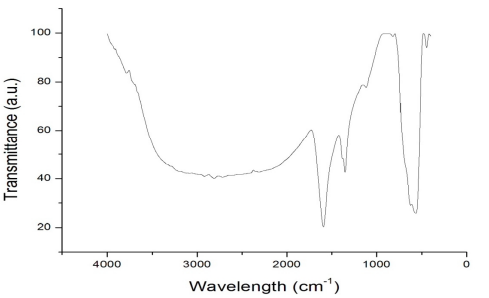
Graph 1: XRD of Mandura bhasma



Graph 2: XRD of Iron Oxide



Graph 3: FTIR Spectrum of Mandura Bhasma



Graph 3: FTIR Spectrum of Iron oxide

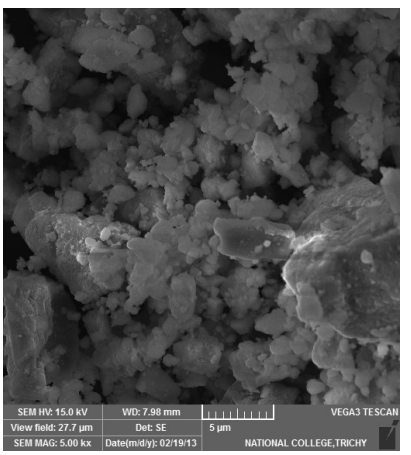


Image 1: SEM image of Mandura Bhasma

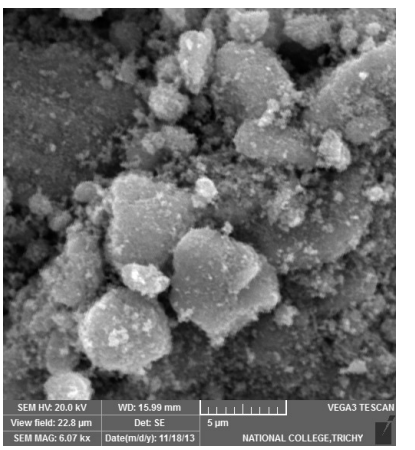


Image 2: SEM image of Iron Oxide

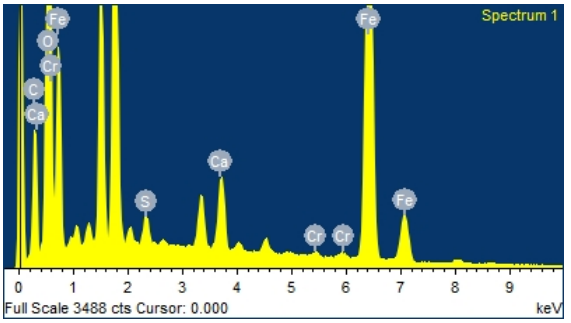


Image 3: EDAX spectrum of Mandura Bhasma

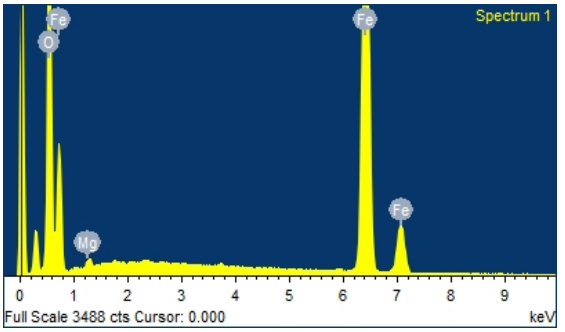


Image 4: EDAX spectrum of Iron Oxide

DISCUSSION

From the elemental analysis from EDAX, it is clear that Mandura Bhasma contains trace elements, which is important for this analysis. It contains several trace elements such as Calcium, Sulphur and Chromium, which are clinically very important. For instance, Chromium which should be present as its oxide is a useful cofactor in the absorption of insulin into the cell. Hence, this may act favorably in the case of a diabetic patient. Thus, these trace elements play an important role in the ingredient of bhasmas. They are not to be viewed as impurities as for as a bhasma is concerned, they may be clinically significant and deliberately added into the system by the way of process, which are described for their preparation classically. These trace elements are not found in the processed Iron Oxide.

FTIR is an important tool for confirmation of the compounds in the sample. In this case, it is proved that Mandura Bhasma is γ-Fe_2O_3, with some trace metal oxides and vibrations of carbon. This confirmation gives some valuable information that there is Carbon-Carbon stretching present in the sample. This is due to the organic residue, present in the system, due to the process of raw mandura in various plant extracts. This makes the difference, from the synthetic process of the same Iron Oxide. Raw Mandura is heated, red hot and quenched in Cow's urine seven times and then it is processed with *Aloe barbadensis* and triphala decoction, In these processes, a considerable quantity of organic material will enter raw mandura and its presence is detected by FTIR, these organic materials remains as processed materials in the bhasma, which should be favorable for its medical action. Though traces of organic material are present in the processed Iron Oxide also.

SEM analysis reveals various important and significant aspects of Mandura Bhasma. It reveals even the mode of action of bhasmas in general. Due to its non-uniform particle size, it is seen from the picture of SEM that there are particles which are around 100 nm and there are particles which are 2000 nm in size. This variation gives us the important clue for the action of bhasma. The cell Nanoparticle interactions are modulated by physiochemical properties of the nanoparticles like size, shape, surface charge and surface chemistry[17]. Size of Iron Oxide nanoparticles plays an important role in cellular uptake of these nanoparticles[18]. Different nanoparticles have different penetration depth in the tissues and organs. Further, nanoparticles with size less than 200 nm penetrates red blood cells (RBC) whereas particles with size more than 1000 nm do not penetrate RBCs. Hence, when the bhasma is administered orally, there is a potential mixture of different particle sizes which enters the system, which has different penetration depth and the time which it circulates in the blood. If the size is less than 100 nm, it penetrates the blood brain barrier and enters the brain[19]. If the particle size is more than 600 nm there is adsorption on the surface of the cells and it induces a strong local membrane deformations and this results in hemolysis[20], this may explain the antimicrobial activity of the nanoparticles. There is a difference in action of the same nanoparticles in living cells because of its size[21]. In this paper it has been proved that bhasmas are a mixture of different sized nanoparticles and due to its size alone, the action will be different and its sphere of action will be more. This is the reason why the same bhasma acts for plenty number of diseased conditions.

From XRD analysis, there is an exact match in the JCPDS data for the synthesized Iron Oxide nanoparticles and for Mandura Bhasma, it does not match exactly but only approximately. This is due to the presence of trace elements and also due to the nanosize of the particles, with filling up of oxygen in the available vacancies. More number of vacant spaces in the crystal structure is also seen from the XRD analysis. The particle size calculation using Debye-Scherrer formula may not be exactly correct in this case as the formula is based on the spherical shape of the particles involved, which is not so as for as Mandura Bhasma is concerned and instrument broadening effect is not taken into consideration. Usually, there is a mixture of different particle sizes are involved in top to bottom approach for the synthesis of nanoparticles. When heat is involved in the synthesis agglomeration of particles takes place, which has happened in this case. Hence, XRD may not be a useful tool in the characterization of Bhasmas. On the other hand, the synthesized Iron Oxide nanoparticles was processed by bottom - top approach, which naturally will give even distribution of the size of particles. In this case, the temperature was raised to 650^{o}C, the same temperature at which the bhasma was synthesized. Further, the time was also increased in that temperature, as done in the case of bhasma. Due to these factors, though there was agglomeration, the size is less than that of Mandura Bhasma. In this case also Debye-Scherrer formula can not be considered as exactly correct.

CONCLUSION

By this comparison, it is found that the SEM, FTIR, EDAX are more important physiochemical method than XRD, since, nanoparticles are involved. It is found that that the action of these bhasmas are due to the presence of different sized nanoparticles, with difference in penetration in cells and its interaction with cells differ due to difference in size of these bhasmas and hence, they are used as drugs which has the capability to address various diseased condition. These analysis should be made for standardization of Ayurvedic Bhasmas. The synthesized Iron Oxide which has almost the same chemical composition and physical parameters is used for comparison with Mandura Bhasma and it has not been tested for its toxicity. Further, the combination with other drugs with Mandura Bhasma called as anupana is not dealt with, which requires further investigation.

REFERENCES

1. Bhatt Gopala Krishna, Rasendra Sara Samgraha, Hindi Commentary by Narendranath Mitra, 4th edition 2007, Adhaya 1/245-246.
2. Chaudhary A. Ayurvedic Bhasma: Nanomedicine of ancient India - Its global contemporary prespective, J. Biomed. Nanotechnol 2011; 7,68-69. http://dx.doi.org/10.1166/jbn.2011.1205
3. Dowling A, Clift R, Grobert N, Hutton D, Oliver R, O'neill O et al. Nanoscience and nanotechnologies : opportunities and uncertainties. London R. Soc. R. Acad. Eng. Rep 2004; 46, 618-618.
4. Baran ET, Ozer N and Hasirci V In vivo half life of nanoencapsulated L-asparaginase. Journal of Materials Science: Materials in Medicine 2002; 13, 1113–1121. http://dx.doi.org/10.1023/A:1021125617828
5. Ferrari M. Cancer nanotechnology: opportunities and challenges. Nat. Rev. Cancer 2005; 5, 161–171. http://dx.doi.org/ 10.1038/nrc1566
6. Kapoor RC. Some observations on the metal-based preparations in the Indian Systems of Medicine 2010; Indian J. Tradit. Knowl 9, 562–575.
7. Bhattacharya B. Elucidating the nanomaterialistic basis for ayurvedic bhasmas using physicochemical experimentation 2011;. J. Biomed. Nanotechnol 7, 66–67.
8. Kumar A, Nair AGC, Reddy AVR and Garg AN. Bhasmas: Unique ayurvedic metallic-herbal preperations, chemical characterization 2006; Biol. Trace Elem. Res. 109, 231-254.
9. Mohaptra S and Jha CB. Physicochemical characterization of Ayurvedic bhasma (Swarna makshika bhasma): An approach to standardization 2010; Int. J. Ayurveda Res. 1, 82–86.
10. Sarkar PK, Choudhary aK, and Prajapati PK. Evaluation of Lauha bhasma on classical analytical parameters - A pilot study 2008; Anc. Sci. Life 27, 24–30.
11. Wadekar MP, Rode CV, Bendale YN, Patil KR and Prabhune AA. Preparation and characterization of a copper based Indian traditional drug: Tamra bhasma 2005. J. Pharm. Biomed. Anal. 39, 951–955.
12. Sachin Mulik B, and Jha CB. Physicochemical characterization of an Iron based Indian traditional medicine: Mandura Bhasma 2011; Anc. Sci. Life 31, 52-57.
13. Sharma KC, Sharma Usha, Kumar Vipin and Yadav Yadavendra. Some observations of the Zinc-metals based preparations and its properties in Ayurveda with regard to stability of medicines in the nano state. Int. J. Res. Ayurveda Pharm. 2015; 6(1): 30-34 http://dx.doi.org/10.7897/2277-4343.0618
14. Kim Il T, Nunnery GA, Jacob K, Schwartz J, Liu X, and Tannenbaum R. Synthesis, characterization, and alignment of magnetic carbon nanotubes tethered with maghemite nanoparticles 2010; J. Phys. Chem. C Vol.114, No.15, pp. 6944–6951.
15. Daou TJ, Greneche J-M, Lee S-J, Lee S, Lefevre C, Sylvie B-C, and Pourroy G. Spin canting of maghemite studied by NMR and In-Field Mossbauer spectrometry 2010; J. Phys. Chem.C Vol. 114, No. 19, pp. 8794-8799.
16. Laurent S, Forge D, Port M, Roch A, Robic C, Elst LV, and Muller RN. Magnetic iron oxide nanoparticles: Synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications 2008; Chem. Rev. Vol. 108, No.6, pp. 2064-2110.
17. Verma A, and Stellacci F. Effect of surface properties on nanoparticle-cell interactions 2010; Small 6, 12–21.
18. Huang J, Bu L, Xie J, Chen K, Cheng Z, Li X, et.al. Effects of nanoparticle size on cellular uptake and liver MRI with polyvinylpyrrolidone-coated iron oxide nanoparticles 2010; ACS Nano 4, 7151–7160.
19. Gao K, and Jiang X. Influence of particle size on transport of methotrexate across blood brain barrier by polysorbate 80-coated polybutylcyanoacrylate nanoparticles 2006; Int. J. Pharm. 310, 213–219.
20. Zhao Y, Sun X, Zhang G, Trewyn BG, Slowing II, and Lin VSY 2011; Interaction of mesoporous silica nanoparticles with human red blood cell membranes: Size and surface effects. ACS Nano. 5(2):1366-1375. http://dx.doi.org/10.1021/nn103077k
21. Shang L, Nienhaus K, and Nienhaus GU. Engineered nanoparticles interacting with cells: size matters 2014; J. Nanobiotechnology 12, 5.

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