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An analysis of green synthesis of manganese oxide nanoparticles including applications and prospects as anticancer activity (MTT, ROS & MMP)

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Abstract

Nanotechnology is an emerging area of nanoscience that has a lot of potentials. The need for biocompatible materials for numerous applications in an area such as medicine, water treatment, and hygiene and other has bought more attention towards these fields in recent years. The green synthesis of various nanoparticles (NPs) has been extensively researched in recent years. The co-precipitation method was used to quantitatively synthesize cost-effective colloidal MnO_2 nanoparticles. Manganese dioxide nanoparticles with particle size and composition were synthesized with the aid of salts such as sulphates and a pH control solution such as NH_3. Fourier transforms infrared spectroscopy (FTIR), X-ray diffraction (XRD) was used to determine the scale, shape, and morphology of MnO_2 nanoparticles. The XRD analysis was used to measure the total particle size of manganese oxide nanoparticles. MnO nanoparticles had an average particle size of 20 to 65 nm with spherical shapes. To test the cytotoxicity of the NPs we used rat skeletal myoblast L-6 cell lines and extract from *Tinospora Cardifolia* (stems).

Keywords: MnO NPs, Co-precipitation method, characterizations, *Tinospora cardifolia*, anticancer activity

Introduction

Nanotechnology is a field of research, technology, and engineering that deals with measurements of substances on an atomic and molecular scale of less than 100 nm. Nanoparticles have attracted a growing amount of scientific attention over the last three decades. Nanoparticles have a high surface-to-volume ratio due to their small size which gives them distinct characteristics (Mandal *et al.*, 2005) [11]. This is due to nanoparticles specific size-dependent properties, which are also conceived of as a distinct and intermediate state of matter between individual atoms and bulk materials (Kim and Park, 2004) [7]. Nanoparticle synthesis has gained popularity due to its distinct optical, magnetic, electrical, and mechanical properties in comparison to bulk materials. Due to their compact scale and huge surface area, they have rare and unusual properties (Prabhu and Johnson, 2015) [15]. Nano biotechnology and nano biology are concepts used to describe the intersection of nanotechnology and biology (Quester *et al.*, 2013) [16]. Metal oxide nanoparticles are inherently inorganic such as Ni, Fe, Mn, Co, and Zn are widely used in a variety of applications such as recording devices, magnetic fluids, magnetic sensors, telecommunication, and microwave absorbers (Willard *et al.*, 2004) [19]. Manganese is the twelfth most common element on the earth and the third most common transition element after iron and titanium (Veeramani *et al.*, 2016) [18]. One of the most appealing inorganic compounds is Manganese oxide (Moon *et al.*, 2015) [13] and have already been studied extensively, leading to the discovery of their utility in a variety of fields including protein immobilization (Lvov *et al.*, 2000) [10], electrochemical capacitors (Xu and Bao, 2011) [20], metal adsorption (Dinh *et al.*, 2015) [4], and sensitive biosensors (Luo *et al.*, 2004) [9], etc. manganese is an essential micronutrient for fish and aquatic animals prevention and growth (Asaikkutti *et al.*, 2016) [1]. Since manganese oxide nanoparticles have a greater surface region, they interact strongly with negatively charged particles. It is a significant transition metal oxide of P-type semiconducting material among the different metal oxide nanoparticles. It is one of the most important compounds and numerous researchers have focused on its effectiveness as well as the material's electromagnetic properties (Jayandran *et al.*, 2015) [6]. In this study, MnO_2 nanoparticles were synthesized using a co-precipitation method. Due to their chemical and physical properties and wide applications in catalysis, biosensors, ion exchange, and energy storage manganese oxide nanoparticles are among the most fascinating inorganic materials as a critical genetic metal oxide (Kumar *et al.*, 2013) [8]. Two approaches are commonly used to synthesize: bottom-up and top-down (Hussain *et al.*, 2016, Schröfel *et al.*, 2014) [5, 17]. Bulk materials are partitioned into nanomaterials in the top-down approach, while molecules or atoms are assembled to form NPs in the bottom-up approach (Narayanan and Sakthivel, 2011) [14]. For green synthesis and chemical synthesis of NPs, the bottom-up method is typically used. Green nanoscience and technology have advanced significantly in recent years as a result of their widespread application in luminescence tagging (Bakshi *et al.*, 2009) [2], drug distribution, biomedical applications (Mayedwa *et al.*, 2018) [12], and biological marking. The biological activities of MnO_2 NPs made from plant extract as reducing agents are important. This study aimed to make manganese oxide nanoparticles from *Tinospora Cardifolia* (stems), *Tinospora Cardifolia* is among the most common herbaceous plant. It is commonly used in traditional

medicine and ayurvedic medicine in India for its anti-inflammatory, antipyretic, antioxidant, and anti-diabetic properties (Begum *et al*., 2019) [3]. The plant extract of this plant has been used to treat cancer, asthma, diabetes, diarrheal and eye disease, etc.

Materials and methods

a. Chemical used: In the experiment analytic reagent grade chemicals are used. Merck in India manufactured Manganese sulphate. SRL provided us ammonium hydroxide (liquor ammonia). Throughout the trials, we used deionized water.

b. Preparation of the extract: *Tinospora Cardifolia* (stems) were washed thoroughly and dried in the shade. In a grinder dried (stems) are broken into small pieces and powdered. 100 grams of powder was heated for around 3 hours at 100º C in a 1:1 mixture of ethanol and purified water. The extract was filtered using Whatman No.1 filter paper after it had cooled. In a rotary evaporator collect the filtrate and evaporate the solvent.

c. Synthesis of nanoparticles: A manganese sulphate solution was made with deionized water. *Tinospora Cardifolia* has been used at a concentration of 1% in 0.2 M manganese sulphate in 200 mL deionized water. To this solution, 1% of the extract should be added. The extract was mixed and aqueous ammonia was added drop by drop to the solution mixture while stirring continuously until the pH reached 10. The mixture was stirred for 3 hours and the precipitates were then filtered into a Buckner funnel and cleaned several times with purified water. The precipitates were dried for 24 hours at 700º C before being calcined for 5 hours in a muffle furnace at 600º C.

d. Characterization techniques

The functional group and chemical composition of the nanoparticles were analyzed using Fourier transform infrared spectroscopy (Perkin Elmer-spectrum FTIR) in the 400 cm^{-1} to 4000 cm^{-1} wavelength range. Transmission electron microscopy (TEM) was used to examine the morphology of MnO NPs. A drop of nanoparticles was dropped onto a carbon-coated copper grid and allowed to dry at room temperature. A Hitachi (H-7500) with a 120 Kv accelerating voltage and energy-dispersive X-ray spectroscopy (EDS) was used for TEM micrographs and the same instrument was used for elemental mapping. Image J program was used to measure diameters with the TEM. Analytics Xpert Pro measured X-ray diffraction of MnO NPs. Cu K-alpha-1 radiation is used, with nickel-metal acting as a beta shield. The crystallinity and morphology of MnO NPs were studied at a temperature ranging from 10-80º C. Scanning electron microscope (SEM) Model JSM6100 was used to determine the structure and scale of nanoparticles.

e. Anticancer activity

Cell lines and culture: In DMEM medium, a human cancer cell line (L-6) was cultured and maintained. Penicillin G (24mg), Streptomycin (100 mg), Sodium bicarbonate (300 mg), Gentamycin (500 mg), Fetal bovine serum (20 ml), double distilled water (180 ml) were added to the DMEM (2.70 gm) media after the color changed to pink. The CO_2 cell was cultured at 37º C, 90% relative humidity, and 5% CO_2. The cells were tested with extract dissolved in DMSO, while untreated test cultures obtained only DMSO.

MTT assay: The procedure was used to investigate the cytotoxicity of bioinspired MnO NPs against the cancer cell line L-6, which was cultured in supplemented DMEM media. The cultures were incubated in an incubator at 37º C with 5% CO_2. The MTT assay was carried out in 96 well microplates at various concentrations by incubating for 48 hours and then adding 20µL of MTT to each well and keeping them in an incubator for 3 hours. The media was discarded and 100µL of DMSO was inserted for three minutes. A spectrophotometer set to 517 nm has been used to count the number of viable and nonviable cells.

ROS assay: The cells in 96 well plates were able to settle and adhere for 6 hours until being cultured for 24 hours with or without extract. After 12 hours of therapy, the cells were cultured by extracting medium and trypsin cells from floating cells. After the test, the 2,7-dichlorofluorescein diacetate (DCFHDA) was applied. The resulting cell pellet was centrifuged and dissolved in 100µL of cold PBS. The DCFHDA was used to assess the IC_{50} of L-6 cells treated with extractfor 12 hours. The cell was examined at 485 nm (excitation) and 528 nm (emission).

MMP process: The cancer cells are separated from culture flasks with trypsin and their viability is determined with trypan blue dye in this method. In this type of cancer cell, the necessary 96 well-plates at a density of $1*10^5$-$3*10^5$ cells/well in 1 ml/well growth medium have been inserted. The medium was discarded afterthe plate was incubated for 24 hours at 5% CO_2 and 37º C. After adding IC_{50} and IC_{70} of compounds formulated in growth media and incubating for 24 hours, each medium was discarded and a freshly prepared dye (10 gm rhodamine, 123 dye/ml of FBS growth medium) was applied to each well (1ml/well). The medium was gently removed from the cells, and they were washed in 1*sterile PBS before being tested with a 485 nm excitation microplate reader and a 528 emission.

Result and discussion

a. FTIR: The organic and inorganic compounds in the sample are determined using FTIR spectroscopy shown in fig (1). Peaks in the spectrum ranged from 4000-400 cm^{-1}. The peaks between 450-550 cm^{-1} ascribe the bond between Mn-O. For the MnO NPs, the observed peaks for *Tinospora Cardifolia* were 3116, 2102, 2005, 1088, 477 cm^{-1}. –OH stretching is responsible for the peaks at 3116 cm^{-1}, 2102 cm^{-1} ascribe to the terminal alkynes, 2005 cm^{-1} ascribe the =C-H stretching, and 1088 cm^{-1} ascribe the –C-O stretching.

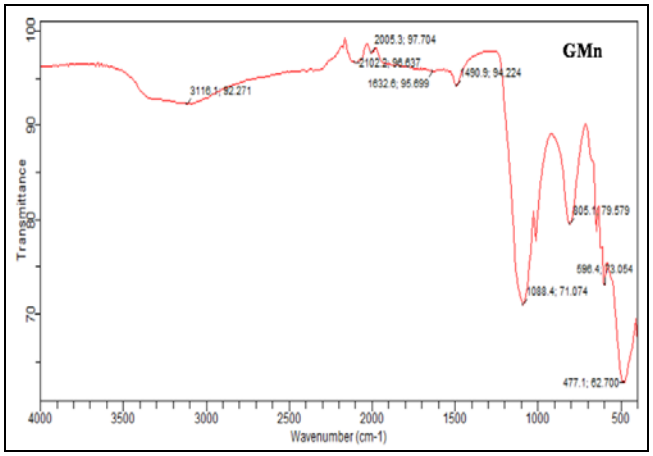

Fig 1: FTIR image of synthesized MnO NPs.

b. SEM: The particle size and morphology of synthesized manganese oxide nanoparticles were determined using a scanning electron microscope (SEM). The particles in the samples were stably organized and nearly spherical as seen in fig (2). Manganese oxide nanoparticles synthesized were found to be 62.59 nm in size.

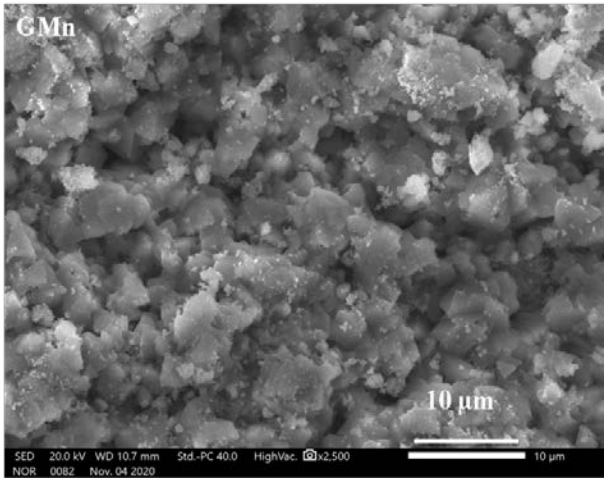

Fig 2: SEM image of green synthesized MnO NPs

c. XRD: Powder X-Ray Diffraction is a quick and versatile technique used for determining phase and unit cell dimensions of nanomaterials. The XRD technique is also used for detection of mineral, sample purity, and the study of crystalline materials. Figure (3) shows X-ray diffraction study of MnO synthesized by co-precipitation method using *Tinospora Cardifolia* (stems) plants extract. The pattern of XRD in the Fig. (3) showed diffraction peaks at 2θ of 23.25º, 33.45º, 38.80º, 56.74º, these peaks corresponded to the miller indices of 110, 101, 111, and 211 respectively. The average size of MnO nanoparticles was quantitatively measured employing Debye-Scherrer equation (1)

$$d = K\lambda/\beta\cos\theta \quad \text{Eq (1)}$$

Where d is the thickness of the crystal
K is the Debye-Scherrer constant (0.89)
λ is the X-ray wavelength (0.154nm)
β is the width of the peak with the maximum intensity in half height
θ is the diffraction angle

The results obtained from the analyses of the XRD pattern displayed that the average size of MnO nanoparticles was about 62.59 nm.

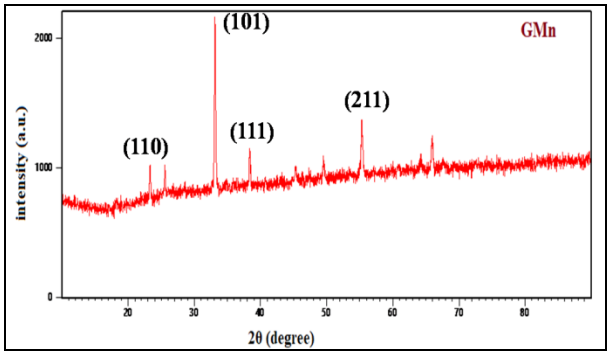

Fig 3: XRD data of green synthesized MnO NPs.

d. TEM

The representation of the plants *Tinospora Cardifolia* (GMn) sample taken with a transmission electron microscope (TEM) is shown in fig (4). The average particle size is 20 to 65 nm, as seen in the TEM images. As seen in fig (4), the Image J program was also used to determine particle size using a histogram. The particle size of MnO NPs in the histogram range from 62.59±2.1.

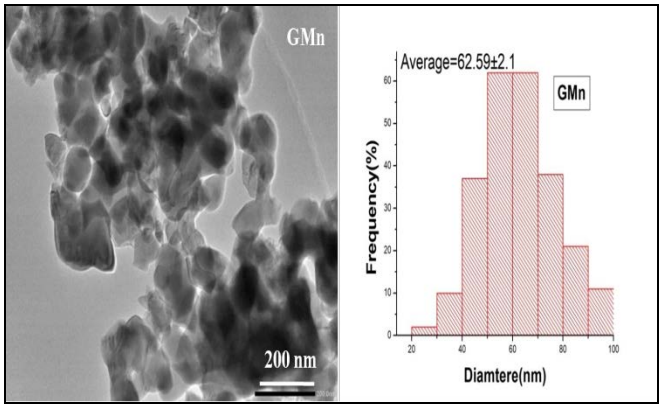

Fig 4: TEM & Histogram image of synthesized MnO NPs

e. MTT ASSAY

Tinospora Cardifolia (GMn) had a strong antiproliferative effect on immortalized rat skeletal myoblast cells. The IC_{50} value was found to be 1.1 mg/ml.

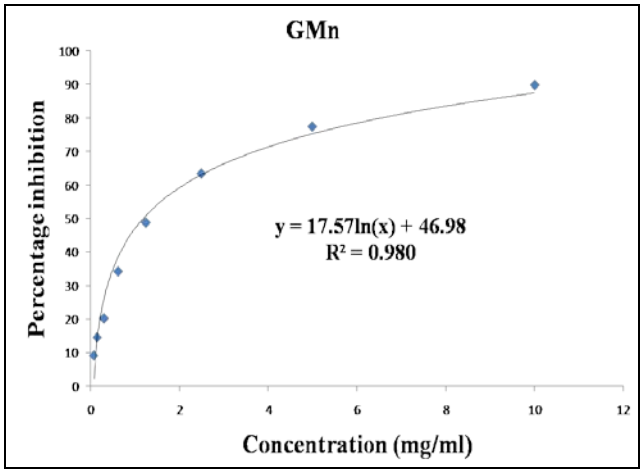

Fig 5: MTT assay of the green synthesized MnO NPs with *Tinospora Cardifolia* plant extract.

f. MMP Assay

The mitochondrial membrane potential (MMP) is produced by the *Tinospora Cardifolia* (GMn) compound. As a consequence cell death cause such as apoptosis move into the cytosol. *Tinospora Cardifolia* (GMn) has the highest efficacy. Treatment with a GMn IC_{50} concentration produces a decrease in MMP, as determined by a decline in mitochondrial intensity from 100% to 23.75%, with a further drop to 21.52% with an IC_{70} concentration.

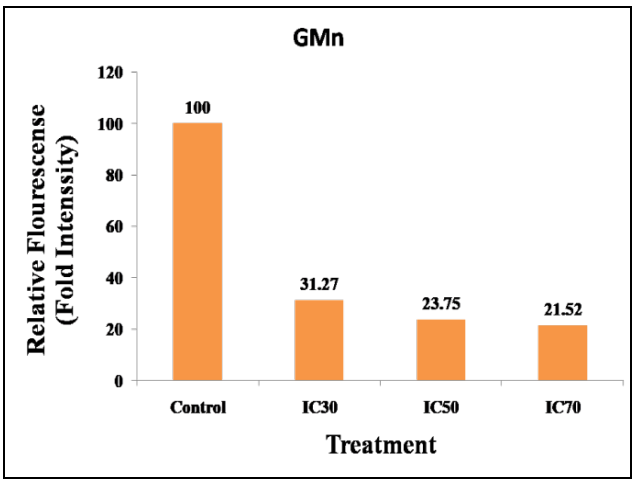

Fig 6: Cytotoxic effect of MMP assay in green synthesized MnO NPs.

g. ROS

A study for reactive oxygen species was used to examine the cause of cellular death. DCFHDA is a non-fluorescent molecule that can jump through easily inside cells. It is converted to DCFH, a non-fluorescent compound by cellular esterase. DCFH has oxidized to DCF a strongly fluorescent compound. The compound *Tinospora Cardifolia* (GMn) caused the greatest rise in ROS levels. In cells, the GMn IC_{50} concentration causes a 1.59 fold increase in ROS levels. After treatment with IC_{70} concentration, the formed ROS was raised to 1.70.

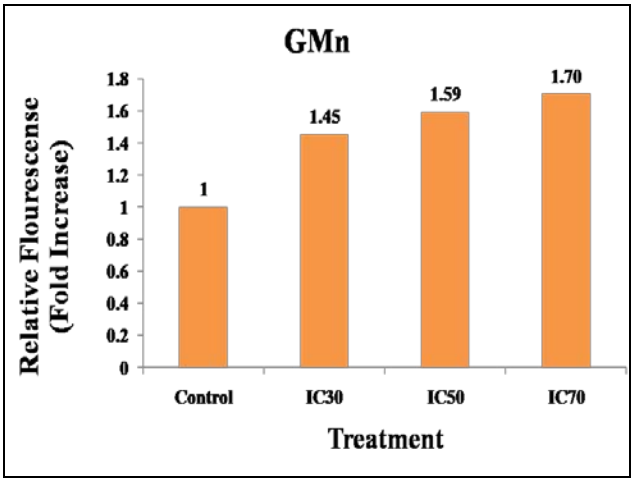

Fig 7: Cytotoxic effect of ROS assay in green synthesized MnO NPs.

Conclusion

To achieve MnO NPs plant extract such as *Tinospora Cardifolia* (stems) were used in a green co-precipitation method synthesis process. FTIR, SEM, XRD, and TEM were used to test the MnO NPs after they were synthesized. The characteristics peaks of Mn-O stretching are shown by FTIR spectral analysis. The average size predicted by XRD spectra is 20-65 nm. The MnO NPs were discovered to be spherical shaped, according to SEM and TEM analysis. The particle size of nanoparticles was found to be uniform at 62.59±2.1 nm using extract from *Tinospora Cardifolia.* The L-6 rat skeleton cell line was used to investigate the cytotoxicity of NPs. According to the MTT assay, MnO NPs reduced cell viability. According to inverted microscopy results *Tinospora Cardifolia* (GMn) has by far the most cytotoxic effect on L-6 cells, with an IC_{50} of 1.1 mg/ml. The application of different extract with ROS and MMP resulted in an IC_{50} of 1.59 and 23.75% respectively.

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Conflict of interest

The author's claim no conflict of interest.

References

1. Asaikkutti A, Bhavan PS, Vimala K, Karthik M, Cheruparambath P. Dietary supplementation of green synthesized manganese-oxide nanoparticles and its effect on growth performance, muscle composition and digestive enzyme activities of the giant freshwater prawn Macrobrachiumrosenbergii. Journal of Trace Elements in Medicine and Biology,2016:35:7-17.
2. Bakshi MS, Jaswal VS, Kaur G, Simpson TW, Banipal PK, Banipal TS *et al*. Biomineralization of BSA-chalcogenide bioconjugatenano-and microcrystals. The Journal of Physical Chemistry C,2009:113(21):9121-9127.
3. Begum HJ, Ramamurthy V, Kumar SS. Study of synthesis and characterization of silver NPs from tinosporaCordifolia. Pharma Innov. J,2019:8(1):612-615.
4. Dinh VP, Le NC, Nguyen NT. Removal of copper (Ii) from aqueous solution by adsorption onto MnO2 nanostructure: Equilibrium and kinetic studies. In *THE 4 th Academic conference on natural science for young scientists, master and PhD. students from asean countries*, 2015, 57
5. Hussain I, Singh NB, Singh A, Singh H, Singh SC. Green synthesis of nanoparticles and its potential application. Biotechnology letters,2016:38(4):545-560.
6. Jayandran M, Haneefa MM, Balasubramanian V. Green synthesis and characterization of Manganese nanoparticles using natural plant extract and its evaluation of antimicrobial activity. Journal of Applied Pharmaceutical Science,2015:5(12):105-110.
7. Kim KY, Park SB. Preparation and property control of nano-sized indium tin oxide particle. Materials Chemistry and Physics,2004:86(1):210-221.
8. Kumar H, Manisha SP, Sangwan P. Synthesis and characterization of MnO2 nanoparticles using co-precipitation technique. Int J Chem Chem Eng,2013:3(3):155-160.
9. Luo XL, Xu JJ, Zhao W, Chen HY. A novel glucose ENFET based on the special reactivity of MnO2 nanoparticles. Biosensors and Bioelectronics, 2004:19(10):1295-1300.

10. Lvov Y, Munge B, Giraldo O, Ichinose I, Suib SL, Rusling JF. Films of manganese oxide nanoparticles with polycations or myoglobin from alternate-layer adsorption. Langmuir,2000:16(23):8850-8857.
11. Mandal M, Kundu S, Ghosh SK, Panigrahi S, Sau TK, Yusuf S M *et al*. Magnetite nanoparticles with tunable gold or silver shell. Journal of colloid and interface science,2005:286(1):187-194.
12. Mayedwa N, Mongwaketsi N, Khamlich S, Kaviyarasu K, Matinise N *et al*. Green synthesis of nickel oxide, palladium and palladium oxide synthesized via Aspalathuslinearisnatural extract: physical properties & mechanism of formation. Applied Surface Science,2018:446:266-272.
13. Moon SA, Salunke BK, Alkotaini B, Sathiyamoorthi E, Kim BS. Biological synthesis of manganese dioxide nanoparticles by Kalopanaxpictus plant extract. IET nanobiotechnology,2015:9(4):220-225.
14. Narayanan KB, Sakthivel N. Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. Advances in colloid and interface science,2014:169(2):59-79.
15. Prabu HJ, Johnson I. Plant-mediated biosynthesis and characterization of silver nanoparticles by leaf extract of Tragiainvolucrata, Cymbopogon citronella, Solanumverbascifolium and Tylophora ovata. Karbala International Journal of Modern Science,2015:1(4):237-246.
16. Quester K, Avalos-Borja M, Castro-Longoria E. Biosynthesis and microscopic study of metallic nanoparticles. Micron,2013:54:1-27.
17. Schröfel A, Kratošová G, Šafařík I, Šafaříková M, Raška I, Shor LM. Applications of biosynthesized metallic nanoparticles–a review. Actabiomaterialia,2014:10(10):4023-4042.
18. Veeramani V, Madhu R, Chen SM, Sivakumar M. Flower-like nickel–cobalt oxide decorated dopamine-derived carbon nanocomposite for high performance supercapacitor applications. ACS Sustainable Chemistry & Engineering,2016:4(9):5013-5020.
19. Willard MA, Kurihara LK, Carpenter EE, Calvin S, Harris VG. Chemically prepared magnetic nanoparticles. International materials reviews,2014:49(3-4):125-170.
20. Xu MW, Bao SJ. Nanostructured MnO2 for electrochemical capacitor. Energy Storage in the Emerging Era of Smart Grids,2011:492:251-278.