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To cite this article: O A Zlygosteva *et al* 2018 *J. Phys.: Conf. Ser.* **1115** 042067

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The use of manganese-doped mesoporous silica nanopowder for targeted drug delivery

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Abstract. The researched manganese-doped mesoporous silica nanopowder (SiO_2-MnO_2 NP) was produced using evaporation caused by a pulsed electron beam in a vacuum. The synthesized material demonstrated high porosity, amorphous structure and magnetic properties increased with the addition of dopant. The evaluation of the sedimentation stability of NP suspensions showed the need for the additional stabilization. It was established that increasing the sonication time, as the way to increase stability, leads to changes in the structure of the NP. PEG stabilized suspensions showed the highest stability. Experimental results indicated that for different drugs individual methods of loading and release are required. Drug loaded NP demonstrated a high drug loading capacity of 0.09 mg Amoxicillin per mg NP, 0.075 mg Doxorubicin per mg NP that is five times higher than loading capacity of chemically synthesized NP.

1. Introduction

Nowadays, the interest in the use of NP for drug delivery has emerged and is significantly increasing.

Conventional drugs frequently exhibit high toxicity in healthy tissues due to their nonspecific distribution and premature degradation. These limitations make it necessary to reduce the injected dose because of increased risk of side effects, which seriously affects drug effectiveness during the therapeutic process. So, perfect drug delivery systems should have the following characteristics: low toxicity to the organism, high biocompatibility, the ability of drug protection against premature degradation, high loading capacity and controlled release, excretion or biodegradation [1].

For the experiments, SiO_2-MnO_2 NP was chosen. Dopant manganese dioxide can act as a contrast agent for MRI that allows creating multi-purpose system for simultaneous visualizing the drug delivery process. For the drug loading and release experiments the antibacterial drug Amoxicillin (Hemofarm koncern AD, Serbia) and the antitumor drug Doxorubicin (Farmfhemi BV, the Netherlands) were chosen.

Notably that NPs with hollow structures such as SiO_2 are of significant interest for drug delivery, because they can efficiently accommodate drugs into not only mesoporous channels but also the hollow interiors [2].

For pharmaceutical applications, NPs are converted into a suspension dosage form to increase the contact area with tissues and, accordingly, the therapeutic effect. However, most NP suspensions do

not exhibit the sedimentation and aggregative stability that can violate the principle of drug dosage uniformity. By dispersing the NP suspensions, the sedimentation stability can be increased. Moreover, sonication of suspensions at the initial stage can facilitate the effective drug loading into the pores of the NP. For the aggregative stability, it is necessary to add surface-active substances that prevent the particles from sticking together and precipitating.

Thus, the main goal of research was to investigate the potential of using SiO_2-MnO_2 NP, obtained by electron beam evaporation, as a drug delivery system. At the same time, we investigated the ways to increase the stability of NP suspensions by selecting the optimal stabilizer and the method of sonication. Considering that the specific surface area (S_{BET}) of NP and porosity affects the effectiveness of drug loading and release, the influence of the sonication time on the texture properties of SiO_2 and SiO_2-MnO_2 NPs was studied. Finally, it was important to develop the effective method for drug loading and release from the SiO_2-MnO_2 NP structure.

2. Experimental

The SiO_2-MnO_2 NPs with the dopant mass concentration of 0.1, 3, 5% were previously obtained using the method of evaporation by a pulsed electron beam in a vacuum (4 Pa) on NANOBIM-2 apparatus [3]. The targets were made of submicron silica powder (AEROSIL 90) and manganese dioxide (GOST 4470-79).

The morphology of NPs was observed by means of the translucent electron microscopy (TEM) on JEM 2100 microscope. Simultaneous thermogravimetry (TG) and differential scanning calorimetry (DSC) measurements were performed on a Demo-STA-409-PC Netzsch thermal analyzer combined with a mass spectrometer QMS-403C at a heating rate of 10 °C/min in air atmosphere. N_2 adsorption–desorption isotherms were obtained on a TriStar 3000 V6.03 apparatus at 77 K under continuous adsorption condition. BET and BJH analyses were used to determine the specific surface area, pore size, and pore volume.

In order to determine the influence of sonication time on suspension stability, samples of NP suspensions (500 µg/ml) were sonicated in an ultrasonic bath PSB-2835-05 100 W for t_s=40, 100 and 150 min. Moreover, stability of NP suspensions (1 mg/ml) was determined by adding sodium citrate (CN) and polyethylene glycol (PEG) in a ratio of 1:1. Then the samples were also sonicated for 40 minutes, and sedimentation curves were plotted, showing the change in the relative optical density D of suspensions with time using PE-5400VI spectrophotometer. The sedimentation rate was determined as the difference between the initial and final optical density D.

In order to evaluate the effect of sonication on NP structure, samples of NP suspensions in the concentration range of 3-6 mg/ml were sonicated for t_s=40 and 100 min. Then samples were centrifuged on Allegra X-30 centrifuge (3000 rpm, 10 min.), separated from the supernatant and dried at 40° C for 72 hours. For these samples, BET and BJH analyses were conducted again.

Samples of SiO_2-5%MnO_2 NP were loaded with drugs by suspending 20±0.5 mg of NP in 10.0 mL of aqueous solutions of Doxorubicin, Amoxicillin (1 mg/mL), further samples Amo-SiO_2-5%MnO_2 and Dox-SiO_2-5%MnO_2, respectively.

For development of the most effective drug loading method, the first part of the samples after suspending was sonicated for 40 minutes and left for 24 hours, while the second part of the samples was kept stirring for 24 hours (280 rmh/min). Then, suspended NPs were separated by centrifugation (4000 rpm, 10 min) and washed with distilled water.

The loading of drugs into NPs was determined by spectrophotometric method [4]. The collected supernatants were analyzed using Thermo Scientific Helios Alpha UV-V is spectrophotometer (λ=490 nm Doxorubicin, λ=270 nm for Amoxicillin corresponding to the maximum absorption of drugs). The concentration of drug in the supernatant C_x (mg/ml) was determined according to the comparative method of quantitative analysis of the standard sample of drug solutions C_C (mg/ml).

Simultaneously, to evaluate the drug interaction process with the NP, a thermal analysis (TA) of a sample Amo-SiO_2-5%MnO_2 NP was conducted. DSC-TG curves and mass spectra of H_2O, CO_2, NO were measured for the prepared sample (chemical formula of amoxicillin – $C_{16}H_{19}N_3O_5S$, melting

temperature 194°C) in following conditions: heating up to 200°C, heating rate of 10 °C/min in air atmosphere.

The release of Amoxicillin, Doxorubicin from NP was determined by resuspending dried NP in 10 ml of distilled water and stirring. After samples were centrifuged, the supernatants were analyzed by spectrophotometric method. Then, the mass of the released drug m_r (mg) and the loading capacity LC of NP (mg drug/mg NP) was calculated according to the equation (1) [5]:

$$LC = \frac{m_{orig} - m_{super}}{m_{NP}} \qquad (1)$$

where m_{orig} is the mass of drug in the original suspension, m_{super} is the mass of drug remaining in the supernatant, m_{NP} is the mass of NP used.

3. Results and discussion

3.1. Characterization of SiO₂-MnO₂ NPs

The samples of NPs were characterized by amorphous agglomerates with unordered interparticle porosity as determined from TEM images (figure 1). The shape of particles is far from spherical. The XRD data confirmed that the SiO_2-MnO_2 NPs with different dopant concentrations are amorphous; the manganese dioxides phases were not observed [6]. The DSC-TG analysis demonstrated a mass gain up to 50% in the temperature range from 40 to 1400°C that may be associated with the oxidation of silica recovered during evaporation (figure 2). According to magnetic measurements, it is established that SiO_2-MnO_2 NPs showed ferromagnetic properties [6]. The increasing of ferromagnetic response, which could be caused by structural defectiveness, was observed with the increasing of dopant concentration.
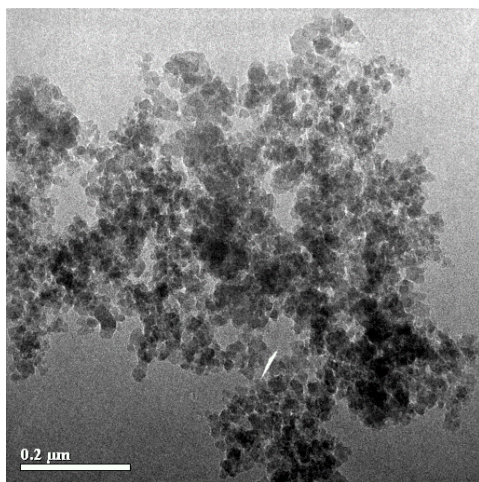

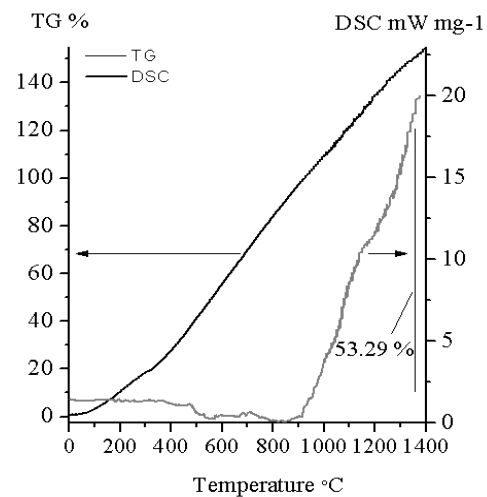**Figure 1.** TEM picture of SiO_2-MnO_2 NP.   **Figure 2.** Heating curves of DSC-TG of NP.

According to the BET-analysis (table 1), the specific surface area S_{BET} increased with the increasing of dopant concentration. For comparison, the table gives the characterization of NP obtained by the chemical method using a template.

The method of evaporation by a pulsed electron beam makes it possible to obtain NPs with a large size and pore volume, in spite of the fact that NPs produced by the chemical method have a substantially larger S_{BET}.

Table 1. Characterization of NP samples.

Sample	Pore size, nm	S_{BET}, m^2/g	Pore volume, cm^3/g	t_s, min
SiO$_2$-0.1%MnO$_2$	20.6	75.78	0.36	0
SiO$_2$-3%MnO$_2$	26.4	134.18	0.88	0
SiO$_2$-5%MnO$_2$	20.8	176.35	0.52	0
Mn–M48SNs [7]	2.9	1365	0.67	-
MCM-41[5]	3.16	1016	0.35	-
SiO$_2$-3%MnO$_2$	13.7	53.37	0.20	40
SiO$_2$	18.6	125.40	0.36	0
SiO$_2$	16.5	88.20	0.28	40
SiO$_2$	16.0	55.52	0.20	100

3.2. Determination of SiO$_2$-MnO$_2$ NPs suspensions

Sedimentation curves demonstrate a nonlinear dependence of the suspension stability on the sonication time. Samples of suspensions sonicated for t_s=40 and 100 minutes showed a 22% and 27% sedimentation rate, respectively, while the lowest stability was demonstrated by a sample sonicated for t_S =150 min.

The results of BET and BJH analyses to investigate the S_{BET} and porosity of NP after sonication are given in table 1. It can be seen that S_{BET} and porosity of samples decreased monotonically as the sonication time increased. Therefore, a longer sonication is only possible at the final stage of creating a stable therapeutic suspension for injection.

The lowest sedimentation rate was shown by PEG stabilized suspension (8% in 15 min.). Suspension stabilized by CN showed sedimentation rate approximately at the level of unstabilized suspensions. Thus, it was established that adding PEG increases the stability of SiO$_2$-MnO$_2$ NP suspensions to an average of 15-20%.

3.3. Drug loading and release experiments

The number and composition of samples, the methods for sample processing are listed in table 2.

The concentration of drugs C_x in the supernatant was estimated by a comparative method of quantitative analysis, m_r and *LC* was determined by the equation (1) (table 2).

An increase of relative optical density D~0.3 was observed after sonication of samples No. 1 and No. 2 in conditions of equal drug concentrations. For samples No. 5 and No. 6, the effect of the loading method on *D* was not revealed.

Table 2. Drug loading and release experiments.

No.	Sample	Loading method	C_c drug mg/ml	C_x, mg/ml	m_r, mg	*LC*, mg drug/mg NP
1	Control Amo	Sonication (40 min)	1.00±0.05	-		-
2	Amo-SiO$_2$-5%MnO$_2$	Sonication (40 min)	1.15±0.05	0.0058	0.029	0.0029
3	Control Amo	Stirring (24 h)	1.24±0.05	-		-
4	Amo-SiO$_2$-5%MnO$_2$	Stirring (24 h)	1.27±0.05	0.18	0.9	0.09
5	Control Dox	Sonication (40 min)	1.00±0.05	-		-
6	Dox-SiO$_2$-5%MnO$_2$	Sonication (40 min)	1.00±0.05	0.15	0.75	0.075
7	Control Dox	Stirring (24 h)	1.00±0.05	-		-
8	Dox-SiO$_2$-5%MnO$_2$	Stirring (24 h)	1.00±0.05	0.14	0.7	0.07

According to the absorption spectra (figure 3ab), relative optical density D of some sample supernatants increased by approximately 2-2.2, which may be due to the presence of NP after centrifugation. Hereby, the mass evaluation of the loaded drug by spectrophotometric analysis of

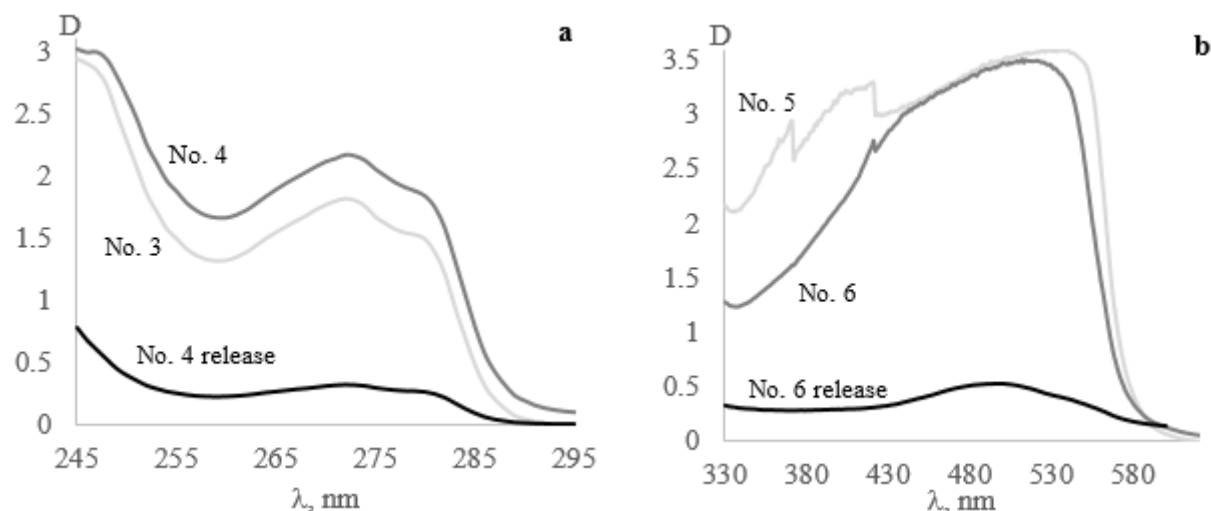supernatants was not sufficiently informative and requires further development for SiO_2-MnO_2 NP suspensions.

Figure 3. Absorption spectra of Amo-SiO_2-MnO_2 NP (stirring) (a), Dox-SiO_2-MnO_2 NP (sonication) (b) (Curve numeration according to sample numeration in table 2).

TA of Amo-SiO_2-5%MnO_2 NP (figure 4) showed that the sample contains molecules of H_2O, CO_2, which further confirms that the drugs have been loaded into the NP. In the region of 100°C, removal of adsorbed water and loss of the sample mass were observed. The decomposition of the organic substances (Amoxicillin) was observed in the DSC curve at further increase in temperature. The concentration of CO_2 increased. The decrease on the TG curve in the temperature range of 130-200°C showed that the weight loss of drug was about 3.3 % of the sample weight (6.3 mg), which corresponds to 0.2±0.05 mg of the loaded drug.
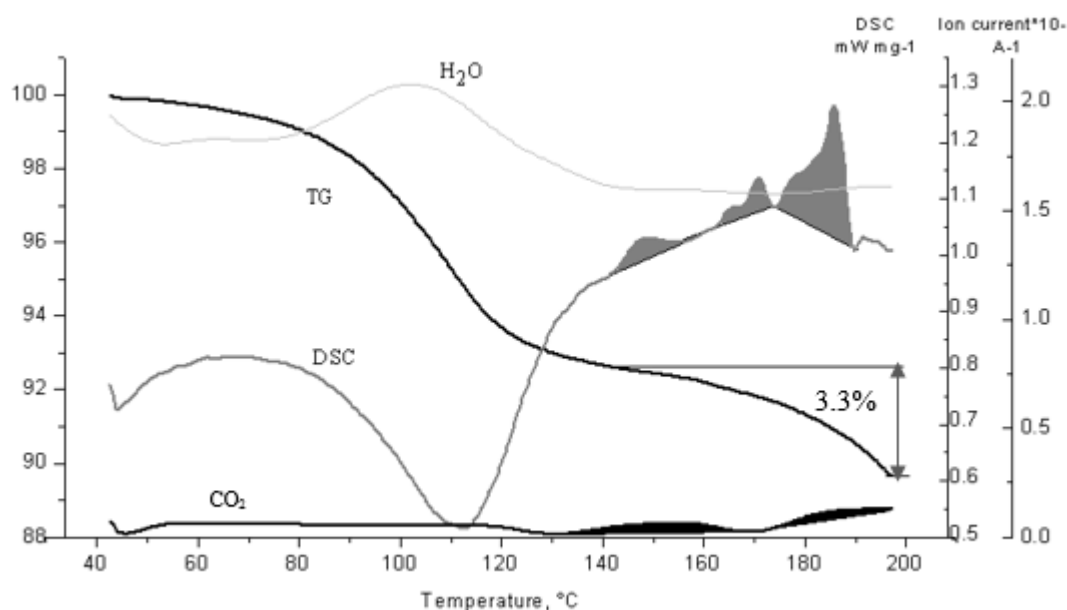

Figure 4. Heating curves of DSC-TG and H_2O, CO_2 mass spectrums of Amo-SiO_2-MnO_2 NP.

The release of drugs from NP was determined by spectrophotometric analysis of resuspended NP supernatants. The lowest concentration of drug C_x in the supernatant accounted for 0.0058 mg/ml and loading capacity LC 0.0029 mg drug/mg NP was obtained for the sample No. 2 (table 2). This may be associated with the decrease in porosity during the sonication at the loading stage or with the need for additional external stimuli during release stage. This conclusion is also confirmed by comparing the TG-analysis data and the spectrophotometric analysis of the sonicated samples.

Sample No. 4 had a higher value of loading capacity LC 0.09 mg drug/mg NP. This result may be explained by stirring loading method, in which the porous structure of the NP should not be disturbed.

The NPs from samples No. 6 and No. 8 after the loading and the washing process had a different structure: the sonicated NPs were adhered and formed separated, dense agglomerates, while the NPs, after stirring, retained a porous, cotton-like structure. The spectra of the Doxorubicin control samples have two peaks in the range of 325-425 nm, as well as a shift in the main absorption peak, which may be due to the superposition of the absorption spectra of the auxiliary substances. According to the spectrophotometric analysis of samples No. 6 and No. 8, a low concentration of released drug was observed, however, the concentration of the released drug in the supernatant increased with the increasing of stirring intensity during release stage (table 2).

Comparing loading capacity LC 0.075 mg drug/mg NP of sample No. 6 with loading capacity LC 0.014 mg drug/mg NP of MCM-41 sample [5], it is evident that the researched NP has a higher LC. The obtained results allow to make a conclusion about the effect of porosity on the loading capacity. Despite the high S_{BET} of MCM-41, which is significantly higher than the S_{BET} of the test sample (table 1), low porosity determines the low loading capacity.

4. Conclusion

Thus, the researched SiO_2-MnO_2 NP obtained by the method of evaporation by a pulsed electron beam demonstrated the potential for its use as a drug delivery system with the following conclusions:

1. The SiO_2-MnO_2 NP has a high porosity in comparison with the MCM-41 NP produced by the chemical method with a significantly higher specific surface area.

2. Stabilization of NP suspensions is necessary to enable in vivo studies and for their subsequent injection to the organism. PEG stabilized suspensions showed the greatest stability.

3. The method of drug loading has a significant influence, and the highest results are obtained with stirring of suspensions during process of loading, because sonication affects the structural properties of NP and, possibly, reduces the drug interaction with the surface of the carrier.

4. The data of the TG analysis of the loaded NP showed that the drugs have been loaded into the NP, however, spectrophotometric analysis showed that additional stimuli as repeated sonication or intensive stirring is necessary to release the drug from the structure. For samples with Doxorubicin, the loading capacity does not depend on the loading method.

7. The researched NP has a higher loading capacity LC 0.075 mg drug/mg NP in comparison with the sample of MCM-41 LC 0.014 mg drug/mg NP that demonstrates the effect of porosity on loading capacity.

Acknowledgements

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