Digest Journal of Nanomaterials and Biostructures Vol. 7, No. 4, October-December 2012, p. 1891-1896

POLYMERIC MAGNETIC SILICA MICROSPHERES AS A DRUG LOADER FOR ANTIMICROBIAL DELIVERY SUBSTANCES

A. M. GRUMEZESCU[a*], A. FICAI[a], D. FICAI[a], G. Predan[b], M. C. CHIFIRIUC[b]
[a]*Faculty of Applied Chemistry and Materials Science, Politechnica University of Bucharest, Romania*
[b]*Faculty of Biology, University of Bucharest, Bucharest, Romania*

Na_2SiO_3 was used as surface modification agent of magnetic dextran microspheres. The composition and morphology of treated magnetic polymer microspheres were characterized by Scanning Electronic Microscopy (SEM), Fourier Transform Infrared Spectra (FT-IR) and powder X-ray Diffraction (XRD). The antimicrobial property was evaluated using antibiotic susceptibility on *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 strains. By analyzing the FT-IR spectrum, the stretching band characteristic of the silica pattern is observed. The magnetite was identified in the sample as the only one crystalline phase. Silica could not be identified by the XRD due to its amorphous nature. The SEM micrographs of polymeric magnetic silica microspheres reveal that they are presenting as well shaped spheres with a rather rough surface. The size of the magnetic microspheres ranges between 50 and 80 μm. The *in vitro* assay of the inhibitory activity of different antibiotics on the bacterial growth in the presence of polymeric magnetic silica nanocomposites demonstrated the improvement of the antimicrobial activity of some antibiotics in the presence of composite particles, which could act as a drug delivery system for microbial cells targeting and infectious diseases therapy.

(Received September 11, 2012; Accepted November 28, 2012)

*Keywords***:** magnetic microsphere, dextran, drug delivery, antimicrobial

1. Introduction

Fe_3O_4 are the subject of many recent studies [1,2,3], especially in biological applications such as cell separation and sorting [4],drug delivery [5,6], magnetic resonance imaging [7], stabilization of essential oils [8], and inhibition of microbial biofilm development [9]. Compared with other metallic oxides, superparamagnetic cores exhibit higher biocompatibility and chemical stability [10,11]. Nontoxic materials, such as SiO_2, can be easily grown onto magnetic nanoparticles for further modification with amino or carboxyl groups [12,13]. Subsequently, by virtue of the activated groups on the surface of silica coatings, nanocomposites can be easily functionalized with another functional polymer shell to improve the imaging [14], drug-loading performance [15,16], the biocompatibility [17], and water solubility [18,19]. Magnetic biocompatible composites have attracted intense interest in recent years [20], particularly due to the possibility of external manipulation using magnetic field gradients and/or alternating magnetic fields [21]. Dextran, which consists of a-1,6-linked D-glucopyranose residues, is a bacterial-derived polysaccharide generally produced by enzymes from certain strains of *Leuconostoc* or *Streptococcus* [22]. Dextran hydrogels have received an increased attention due to their variety of biotechnological and biomedical applications [23]. Owing to their low tissue toxicity and high enzymatic degradability at desired sites, dextran hydrogels have been frequently considered as a potential matrix system for controlled release of bioactive agents [24,25]. Recent reported studies

* Corresponding author: grumezescu@yahoo.com

revealing the capability of magnetic polymeric hybrid materials for delivery of antibiotics increased their utility in biomedical applications [26]. Our previously reported results demonstrated that the magnetic dextran microspheres could be used as macromolecular carriers for large-spectrum antibiotics, especially for those with small, polar molecules belonging to penicillins, aminoglycosides, rifampicines and quinolones classes. The obtained results are suggesting that the size and the electric charge of the active drugs are influencing the specific interactions between the drug carrier and the active substance [27]. In this context, the major focus of the present study was the preparation and characterization of a polymeric magnetic silica drug loader as well as the evaluation of the biological activity of the employed chemotherapeutic agents against Gram-positive and Gram-negative reference strains.

2. Experimental

2.1 Preparation of core/shell magnetic microspheres

Magnetic microspheres were prepared according to our previous published papers [28,29]. Briefly, five grams of dextran were solubilized in a known volume of ultrapure water, corresponding to a 1.00% (w/w) solution, under stirring at room temperature. Then, 8 mL of a basic aqueous solution consisting of 28% NH_3 were added to dextran solution. Thereafter, 200 mL aqueous solution of 1,2 grams $FeCl_3$ and 2 grams $FeSO_4$ x $7H_2O$ were dropped under permanent stirring up to pH = 8, leading to the formation of a black precipitate. The product was separated by applying a magnetic field and repeatedly washed with ultrapure water [30]. 100 mL solution of Na_2SiO_3 (2%) was added over the black precipitate and H_2SO_4 (1%) was added drop by drop under vigorous stirring until pH reached 7. After this, the obtained microspheres were separated by applying a magnetic field and washed several times with ultrapure water and methanol.

2.2 Characterization of core/shell magnetic microspheres

X-ray diffraction analysis was performed on a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu Kα radiation from a Cu X-ray tube (run at 15 mA and 30 kV) was used. The samples were scanned in the Bragg angle 2θ range of 10-80.

The magnetic microspheres were assessed by SEM analysis that was performed on a HITACHI S2600N electron microscope, at 15 keV, in primary electrons fascicle, on samples covered with a thin silver layer.

A Nicolet 6700 FT-IR spectrometer (Thermo Nicolet, Madison, WI) connected to the software of the OMNIC operating system (Version 7.0 Thermo Nicolet) was used to obtain FT-IR spectra of hybrid materials. The samples were placed in contact with attenuated total reflectance (ATR) on a multibounce plate of ZnSe crystal at controlled ambient temperature (25^{o}C). FT-IR spectra were collected in the frequency range of 4,000–650 cm^{-1} by co-adding 32 scans and at a resolution of 4 cm^{-1} with strong apodization. All spectra were rationed against a background of an air spectrum. After every scan, a new reference air background spectrum was taken. The plate was carefully cleaned by wiping with hexane twice followed by acetone and dried with soft tissue before filling in with the next sample. The spectra were recorded as absorbance values at each data point in triplicate.

2.3 Biological assay

An adapted diffusion method was used in order to assess the potentiator effect of the magnetic microspheres on the antimicrobial actitivity of VA (vancomycin), DA (clindamycin), AZM (azithromycin), OX (oxacyllin), SXT (trimethoprim/sulfamethoxazole), RA (rifampicin), OFX (ofloxacin), TE (tetracycline), P (penicillin), CIP (ciprofloxacin), GM (gentamycin), TZP (piperacillin/tazobactam), FEP (cefepime), ATM (aztreonam), CAZ (ceftazidim) and PRL (piperacillin) against *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 reference strains. The tested antibiotics have been chosen according to the last edition of CLSI recommendations. Standardized antibiotic discs have been placed on the Mueller Hinton agar medium distributed in

Petri dishes previously seeded with a bacterial inoculum with a density corresponding to the 0.5 McFarland standard. Five μL of the stock solutions of the dispersed magnetic micropsheres were spoted over the antibiotic disks. The plates were incubated for 24h at 37°C, and the inhibition zones diameters for each antibiotic, after the addition of the tested microsphere suspensions were quantified and compared with the growth inhibition zones obtained for the respective antibiotic disks.

2. Results and discussion

There is a considerable interest in the preparation of functional magnetite due to its strong magnetic properties, which were firstly used in biology and then in medicine [31].

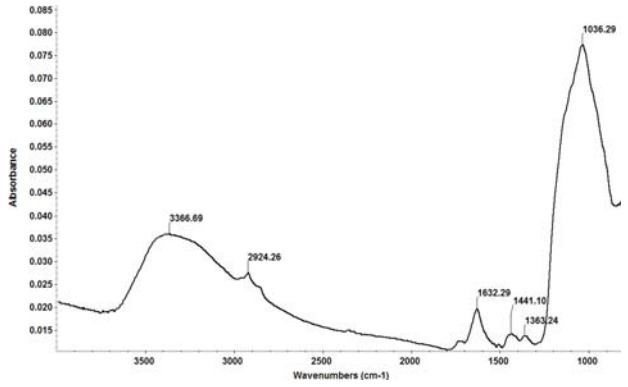

Fig. 1. FT-IR spectrum of polymeric magnetic silica microspheres

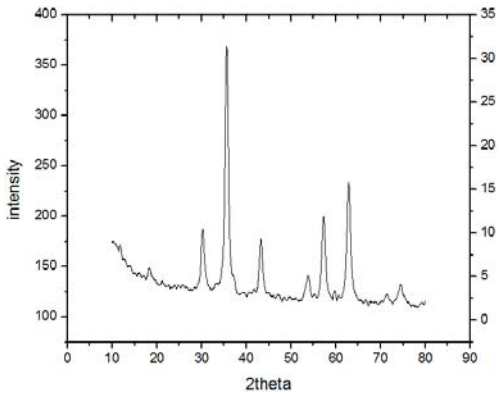

Fig. 2. XRD pattern of polymeric magnetic silica microspheres

In this paper we have investigated the potential of polymeric magnetic silica microspheres to improve the antimicrobial properties of currently used antibiotics. In order to interpret the obtained results, the spectrum of the polymeric magnetic silica microspheres was recorded and is presented in figure 1. By analyzing the FT-IR spectrum, the stretching bands characteristic of the silica pattern is observed. The absorption band centered at 1036 cm^{-1} is ascribed to the Si–O–Si. The band in the region of 2924cm^{-1} was due to C–H vibration and the peak at 1632 cm^{-1} was characteristic to δ(HOH) that indicates the existence of water molecules in a complex structure [32,33]. The bands appearance at about 1363 cm^{-1} from δ(CH) vibrations and the band about 1441 cm^{-1} from δ(OH) vibrations is characteristic for one of more possible positions of the CH_2-OH group [34].

The X-ray diffraction pattern of the polymeric magnetic silica microspheres is shown in Figure 2. The magnetite peaks were identified in the sample as the only one crystalline phase (the main peaks of magnetite are centered at 2θ = 30.31, 35.71, 43.31, 57.61 and 62.81). Based on the intensity of the characteristic peaks of magnetite no preferential directions of crystallization were identified [35]. Silica could not be identified by the XRD due to its amorphous nature.

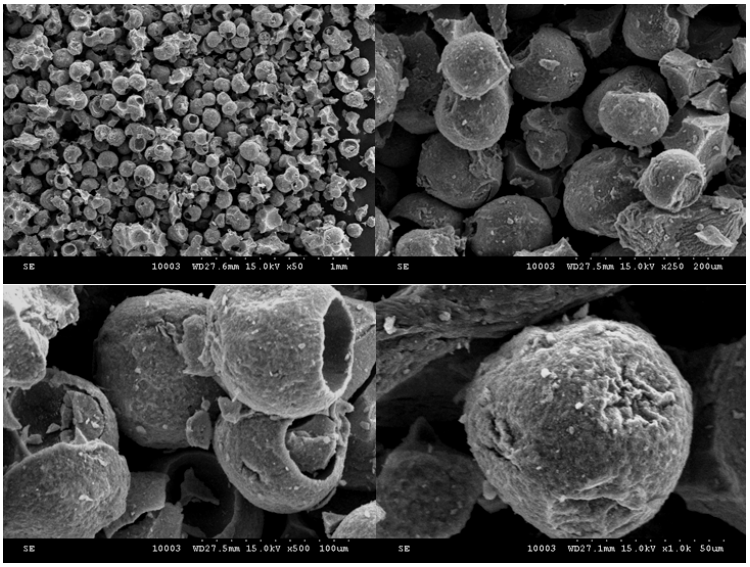

Fig. 3. SEM images of polymeric magnetic silica microspheres

Figure 3 shows the SEM micrographs of polymeric magnetic silica microspheres reveals that are well shaped spheres with a rather rough surface. The size of the magnetic microspheres ranges between 50 and 80 µm.

In order to evaluate the biological potential of the obtained systems, the antimicrobial susceptibility profiles of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 strains were evaluated versus VA (vancomycin), DA (clindamycin), AZM (azithromycin), OX (oxacyllin), SXT (trimethoprim/many antibiotics.

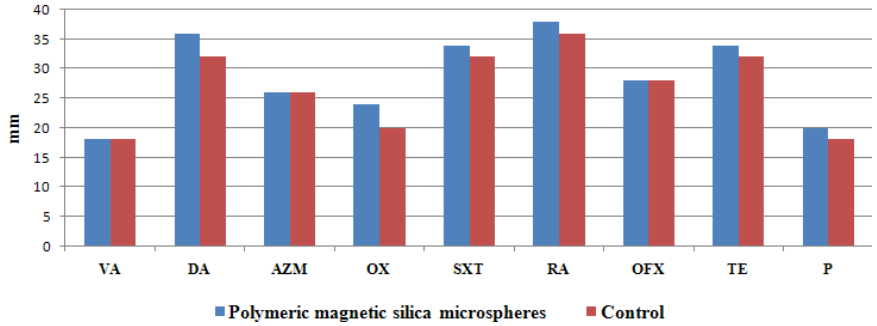

Fig. 4. The growth inhibition zone diameters (mm) obtained for the tested antibiotics in the presence of polymeric magnetic silica drug loader on the S. aureus ATCC 25923 strain (M- standard antibiotic disk)

In case of *S. aureus*, excepting for vancomycin, aztreonam and ofloxacin whose antimicrobial activity was not influenced), the tested system exhibited a potentiator effect on the antimicrobial activity of all the other anti-staphylococcal agents, as revealed by the increase of the growth inhibition zone (fig. 4).

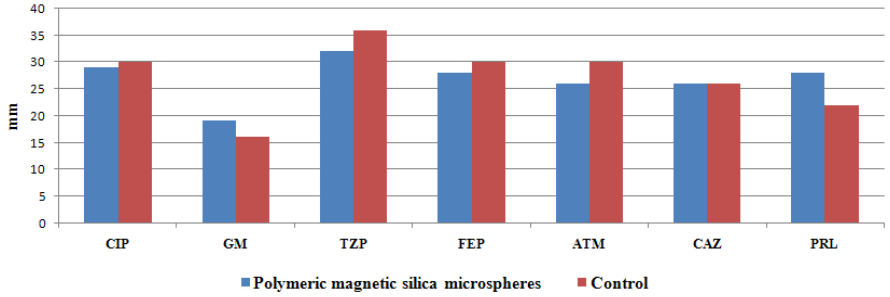

Fig. 5. The growth inhibition zone diameters (mm) obtained for the tested antibiotics in the presence of polymeric magnetic silica drug loader on the P. aeruginosa ATCC 27853 strain

In case of *P. aeruginosa,* the tested systems decreased the efficiency of ciprofloxacin, piperacillin plus tazobactam, cefepime, aztreonam, probably by interfering with the antibiotic diffusion from the paper disk (exhibiting a physical and/or chemical capture of these antibiotics) and did not influence the ceftazidime activity. The gentamycin and piperacillin have been potentiated in the presence of the polymeric magnetic silica drug loader. The antimicrobial activity of the tested antibiotics was differently influenced by the drug loader system, either depending on the tested antibiotic, or the tested microbial strains, signifying that there are probably specific interactions between the tested antibiotic and the carrier system, interfering with the diffusion rate of the antibiotic from the polymeric magnetic silica microspheres. The potentiated antibiotics are belonging to different classes, exhibiting diverse chemical structures and mechanisms of action, including the nucleic acid transcription (rifampicin), protein and pholates synthesis (gentamycin, doxicyline, tetracycline and trimetoprim-suphametoxazole), demonstrating that the composite particles charged with antibiotics probably penetrate the bacterial cell wall and deliver the antibiotic in active forms to the intracellular targets.

3. Conclusions

Biocompatible magnetic microspheres with inorganic shells structure were prepared, successfully modified with Na_2SiO_3 and tested for the potential use as antimicrobial drugs carriers. The *in vitro* assay of the inhibitory activity of different antibiotics on the bacterial growth in the presence of polymeric magnetic silica composites demonstrated the improvement of the antimicrobial activity of some antibiotics in the presence of composite particles, which could act as a drug carrier for microbial cells targeting and infectious diseases therapy.

Acknowledgment

The authors acknowledge the financial support offered by the Romanian National Research projects Human Resources 135 (Contract No. 76/2010) and Ideas (Contract no. 154/2011).

References

[1] D. Manzu, A. Ficai, G. Voicu, B. S.Vasile, C. Guran, E. Andronescu, Mat. Plast. **47**, 24 (2010).
[2] M. Pilloni, J. Nicolas, Vé. Marsaud, K. Bouchemal, F. Frongia, A. Scano, G. Ennas, C. Dubernet, Int. J. Pharm. **401**, 103 (2010).
[3] D. Ficai, A. Ficai, B. S. Vasile, M.Ficai, O. Oprea, C. Guran, E. Andronescu, Digest J. Nanomat. Biostr. **6**, 943 (2011).
[4] J. Kundu, Y.Chung, Y.H. Kim, G. Tae, S.C. Kundu, Int. J. Pharm. **388**, 242 (2010)
[5] A.M. Grumezescu, E. Andronescu, A. Ficai, C. Saviuc, D. Mihaiescu, C. M. Chifiriuc, Rom. J.Mater. **41**, 383 (2011).
[6] D.E. Mihaiescu, M. Horja, I. Gheorghe, A. Ficai, A.M. Grumezescu, C. Bleotu, M.C. Chifiriuc, Lett. Appl. NanoBioSci. **1**, 45 (2012).
[7] M.D. Mantle, Int. J. Pharm. **417**, 173 (2011).
[8] M. C. Chifiriuc, V. Grumezescu, A. M. Grumezescu, C. M. Saviuc, V. Lazar, E. Andronescu, Nanoscale Res. Lett. **7**, 209 (2012)
[9] C. Saviuc, A. M. Grumezescu, M. C. Chifiriuc, C. Bleotu, G. Stanciu, R. Hristu, D. Mihaiescu, V. Lazăr, Biointerface Res. Appl. Chem. **1**, 31 (2011).
[10] S. Yu, G. M. Chowa, J. Mater. Chem. **14**, 2781 (2004).
[11] O. Schneeweiss, R. Zboril, N. Pizurova1, M. Mashlan, E. Petrovsky, J. Tucek, Nanotechnology **17**, 607 (2006).
[12] C. Ren, J. Li, X. Chen, Z. Hu, D. Xue, Nanotechnology **18**, 345604 (2007).

[13] D. Ficai, A. Ficai, M. Alexie, M. Maganu, C. Guran, E. Andronescu, Rev. Chim. (Bucahrest) **62**, 622 (2011).
[14] Y. Liu, Y. Mi, J. Zhao, S.S. Feng, Int. J. Pharm. **421**, 370 (2011).
[15] H.Tang, J. Guo, Y.Sun, B. Chang, Q. Ren, W. Yang, Int. J.Pharm. **421**, 388 (2011).
[16] J. L. Zhang, R. S. Srivastava, R. D. K. Misra, Langmuir **23**, 6342 (2007).
[17] W. W. Yu, E. Chang, J. C. Falkner, J. Zhang, A. M. Al-Somali, C. M. Sayes, J. Johns, R. Drezek and V. L. Colvin, J. Am. Chem. Soc. **129**, 2871 (2007).
[18] O. Planinšek, B. Kovačič, F. Vrečer, Int. J. Pharm. **406**, 41 (2011).
[19] D. Chen, M. Jiang, N. Li, H. Gu, Q. Xu, J. Ge, X. Xiaa, J. Lu, J. Mater. Chem. **20**, 6422 (2010)
[20] S.F. Medeiros, A.M. Santos, H. Fessi, A. Elaissari, Int. J. Pharm. **403** 139 (2011).
[21] P. C. Morais, V. K. Garg, A. C. Oliveira, L. B. Silveira, J. G. Santos, M. M. A. Rodrigues, A. C. Tedesco, Hyperfine Interact **190**, 87 (2009).
[22] S. G. Levesque, M. S. Shoichet, Biomaterials **27,** 5277 (2006).
[23] A. Wieber, T. Selzer, J. Kreuter, Int. J. Pharm. **421**, 151 (2011).
[24] R. Cassano, S. Trombino, R. Muzzalupo, L. Tavano, N. Picci, Eur. J. of Pharm. Biopharm. **72**, 232 (2009).
[25] J. Parmentier, M. M.M. Becker, U. Heintz, G. Fricker, Int. J. Pharm. **405**, 210 (2011).
[26] C. M. Chifiriuc, A. M. Grumezescu, C. Saviuc, C. Croitoru, D. E. Mihaiescu, V. Lazar, Int.l J. Pharm., doi: 10.1016/j.ijpharm.2012.06.031 (2012).
[27] E. Andronescu, A. M. Grumezescu, A. Ficai, I. Gheorghe, M. Chifiriuc, D. E. Mihaiescu, V. Lazar, Biointerface Res. Appl. Chem. **2**, 332 (2012).
[28] A. M. Grumezescu, C. Saviuc, A. Holban, R. Hristu, G. Stanciu, C. Chifiriuc, D. Mihaiescu, P. Balaure, V. Lazar, Biointerface Res. Appl. Chem. **1**, 160 (2011).
[29] C. Saviuc, A. M. Grumezescu, A. Holban, C. Bleotu, C. Chifiriuc, P. Balaure, V. Lazar, Biointerface Res. Appl. Chem. **1**, 111 (2011).
[30] A.M. Grumezescu, E. Andronescu, A. Ficai, D. E. Mihaiescu, B. S. Vasile, C. Bleotu, Lett. Appl. NanoBioSci. **1**, 31 (2012).
[31] C. Fan, W. Gao, Z. Chen, H. Fan, M. Li, F. Deng, Z. Chen, Int. J. Pharm. **404**, 180 (2011).
[32] W. Cao, X.Q. Li, L. Liu, T.H. Yang, C. Li, H.T. Fan, Carboh. Polym. **66**, 149 (2006).
[33] G. S. Nikoli, M. Caki, Z. Mitic, B. Ilic, P. Premovic, Rus. J. Phys. Chem. A. **83**, 1520 (2009).
[34] M.D. Cakin, G. S. Nikolic, L.A. Ilie, Bull. Chem. Tech. Macedonia. **21**, 135 (2002).
[35] D. Ficai, A. Ficai, B.S. Vasile, M. Ficai, O. Oprea, C. Guran, E. Andronescu, Digest J. Nanomat. Biostruct. **6**, 943 (2011).