STUDIA UBB CHEMIA, LVI, 3, 2011 (p. 239 – 246)

ADSORPTION AND RELEASE STUDIES OF TETRACYCLINE FROM A BIOACTIVE GLASS

EMILIA VANEA[a], SIMONA CAVALU[b], FLORIN BĂNICĂ[b], ZSOLT BENYEY[a], GÜLTEKIN GÖLLER[c],VIORICA SIMON[a,*]

ABSTRACT. This study aims to characterize a bioactive glass and to evaluate its ability to adsorb and release antimicrobial agents like tetracycline. Tetracycline adsorption and release were investigated using Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy and scanning electron microscopy. The amounts of withdrawn solution, after drug release, were analyzed by differential pulsed voltammetry and UV-Vis spectroscopy. The investigated glass system promotes an efficient response concerning the tetracycline loading and release in terms of drug delivery system for biomedical applications.

Keywords: *tetracycline, XPS, SEM, FTIR, UV-vis, bioactive glass*

INTRODUCTION

Bioactive glasses have achieved great application success in orthopaedic surgery and dentistry [1]. One major problem frequently associated with implants is the incidence of the osteomielitis that may lead to implant failure. Because of the poor blood circulation in osseous defect sites, antimicrobial drugs must be supplied directly to the affected regions [2], in this way the adverse effects of the systemic administration are avoided and a higher concentration of antimicrobial agent reaches the affected site [1]. In order to diminish the bacteriological risk associated with the implant materials, the addition of an antimicrobial drug such as tetracycline can be considered due to its broad antibiotic spectrum together with bone resorption inhibition and anti-inflammatory action [3-5]. This antibiotic can thus be used to improve bone growth and regeneration in the treatment of bone defects.

The objective of this study was to characterize a bioactive glass and to evaluate its ability as a controlled release system of tetracycline. For this

** Corresponding author: viosimon@phys.ubbcluj.ro*
[a] *Babes-Bolyai University, Faculty of Physics & Institute of Interdisciplinary Research in Bio-Nano-Sciences, Kogalniceanu 1, 400084 Cluj-Napoca, Romania*
[b] *University of Oradea, Faculty of Medicine and Pharmacy, 1 Decembrie 10, 410073 Oradea, Romania*
[c] *Istanbul Technical University, Metallurgical and Materials Engineering Department, 34469 Istanbul, Turkey*

purpose we used different spectroscopic methods like Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, UV-Vis spectroscopy, as well as scanning electron microscopy, and differential pulse voltammetry.

RESULTS AND DISCUSSION

SEM images of tetracycline free sample and after drug uptake (Figure 1) show that the surface gets a fuzzy appearance associated to tetracycline adsorption [2].

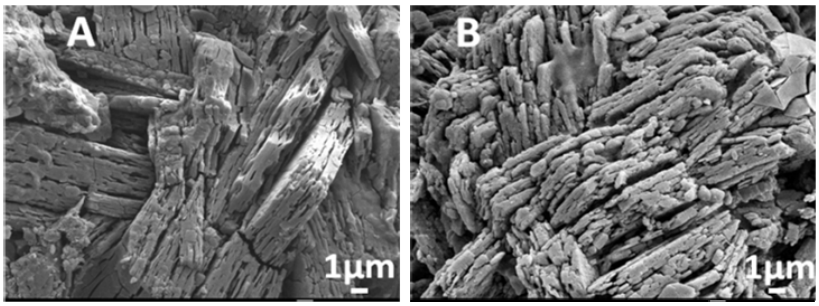

Figure 1. SEM images of bioactive glass before (A) and after (B) tetracycline loading.

XPS spectra (Figure 2) recorded for pure tetracycline, for free tetracycline and tetracycline loaded samples both before and after immersion in SBF aimed to determine the composition of the outermost layer of the investigated bioactive glass, based on the fact that XPS is a highly surface-specific *technique* with a typical analysis depth of 5—10 nm [8]**.** The results are presented in Table 1.

Both carbon and nitrogen can be used as markers of tetracycline adsorption [9] because the two elements are not present in glass, even though carbon contamination is unavoidable and unpredictable in practice. Table 1 shows that the level of nitrogen was almost zero for unloaded sample and increased significantly for the tetracycline incubated one. The elemental analysis also indicates a remarkable increase of carbon content after incubation, content close to the value obtained for tetracycline itself, which proves tetracycline adsorption. The level of the elements entering the glass composition, i.e. Si, Ca, P, Na and O, decreases after immersion of the bioactive glass in both SBF and tetracycline solution, but more drastically in tetracycline solution, which also denotes an advanced coverage of the glass surface with tetracycline.

The N 1s, C1s and O 1s high resolution XPS spectra of (Figure 3) strengthen these results. One observes the high intensity of the nitrogen and carbon spectra (Figs. 3Ad and 3Bd) recorded from the tetracycline loaded glass, according to the tetracycline corresponding ones (Figs. 3Ac and 3Bc). The O 1s high resolution spectra (Figure 3C) show that before immersion

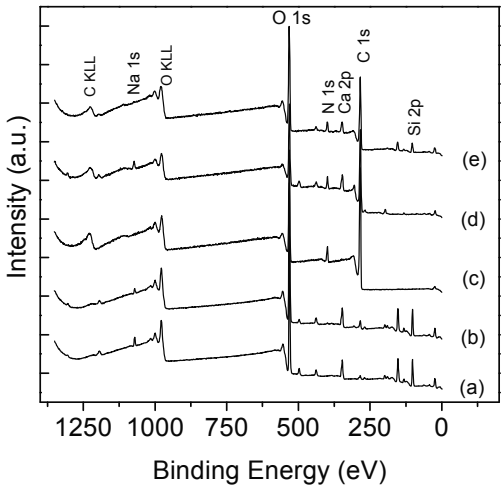

Figure 2. XPS survey spectra of glass (a), glass immersed in SBF (b), tetracycline (c), and glass with tetracycline (d) and of glass after 96 hr immersion in SBF for tetracycline release (e).

Table 1. Surface chemical composition before and after immersion of the bioactive glass (BG) in tetracycline (TCL) solution and after 96 hr immersion in SBF.

Sample	Elemental composition (at %)						
	C	N	O	Si	Ca	P	Na
BG	5.6	-	53.1	32.6	5	2.3	1.4
BG/SBF	8.8	0.5	52.3	30.8	4.7	2.1	0.8
TCL	72.9	5.4	21.7	-	-	-	-
BG/TCL	65.7	5.3	23.6	-	3.2	1	1.2
BG after TCL release	54.4	4.2	30.1	8.3	2.1	0.9	-

in tetracycline solution the O 1s photoelectron peak consists of a component at 532.4 eV, related to the bridging oxygen atoms implied in the structural units of the glass [10] but they could be assigned also to hydroxyl groups present on the glass surface due to the chemisorbed water molecules from environmental moisture [11]. After immersion in tetracycline solution, the O 1s peak decreases in intensity and shifts to lower binding energy and the amount recorded is comparable to the value recorded for pure tetracycline which is less rich in oxygen atoms.

After the release process the amounts of C, N slightly decrease while O increases together with silica which is revealed at the surface of the sample again. This means the tetracycline was released in great amount, but significant quantity is still detected at the surface of the glass, which can be considered irreversibly bound.

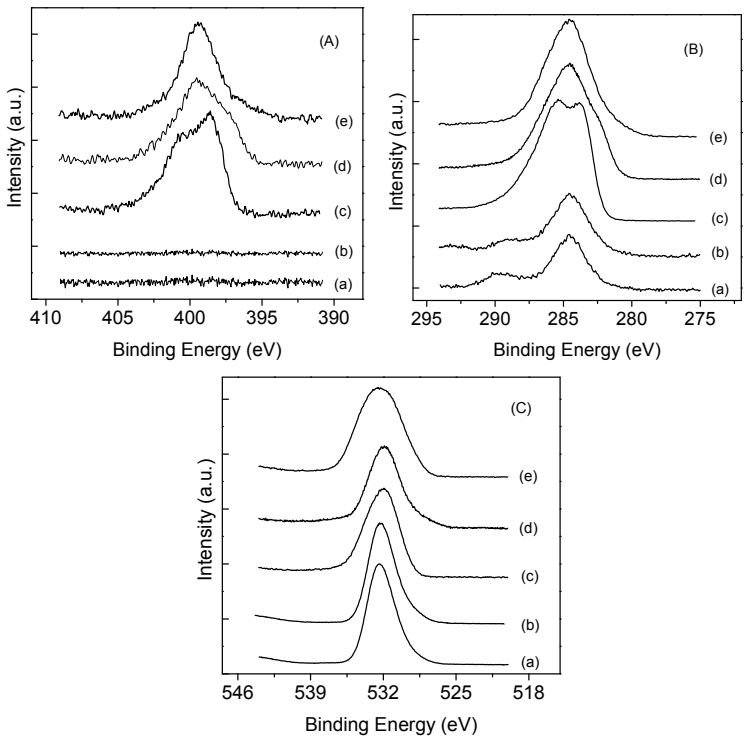

Figure 3. XPS high-resolution N 1s (A), C 1s (B), O 1s (C) spectra of glass (a), glass immersed in SBF (b), tetracycline (c), glass with tetracycline (d), and of glass after 96 hours immersion in SBF for tetracycline release (e).

The analysis of FTIR spectra (Figure 4) recorded from glass and tetracycline, as well as from glass immersed in SBF, tetracycline loaded glass and after 7 days glass immersion in SBF for tetracycline release brings additional information on the glass/tetracycline interaction. The absorption bands at 1418 and 1470 cm^{-1} can be attributed to carbonate impurities present in the samples and peaks at 1200, 1090, 800, 466 cm^{-1} are associated to the vibration of Si-O-Si bonds in the glass network [2]. The absorption band around 960 cm^{-1} is due to the Si-OH vibrations [12]. The doublet at 606 and 564 cm^{-1} is assigned to bending vibrations of PO_4^{3-} ions [13]. The FTIR spectra of tetracycline and tetracycline-loaded glass are very similar showing the same amide I band around 1600 cm^{-1} [14, 15], a proof of tetracycline adsorption in great amount. The release of tetracycline after 96 hours immersion in SBF solution under dynamic condition is evidenced by reappearance of absorption bands specific to glass network like the one present at 1090 cm^{-1}. The carbonate specific peak is also well evidenced. The great intensity of the peak at 1600 cm^{-1} is explained by the presence of water in great amount considering the sample cannot be dried at high temperature to not affect the tetracycline structure.

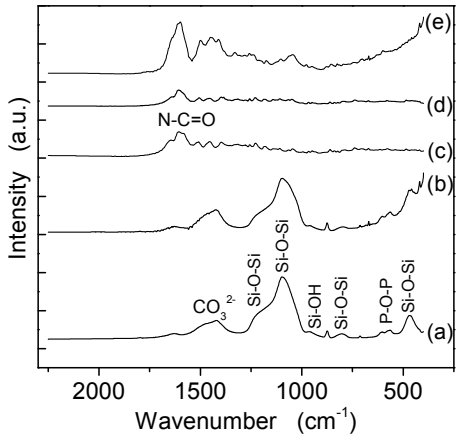

Figure 4. FT-IR spectra of glass (a), glass immersed in SBF (b), tetracycline (c), glass with tetracycline (d) and of glass after 96 hours immersion in SBF for tetracycline release (e).

Ultraviolet-visible absorption spectroscopy (UV-vis) was used to estimate the amount of tetracycline released following the evolution of the absorption peak at 269 nm [1]. The drug release is found to be fast for the initial 2 hours, gets slower for the next 5 and 24 and finally the release becomes constant for longer periods of time (Figure 5).

The tetracycline release profile in SBF was also obtained by determining the tetracycline concentrations (Table 2) using differential pulsed voltammetry (DVP) curves (Figure 6).

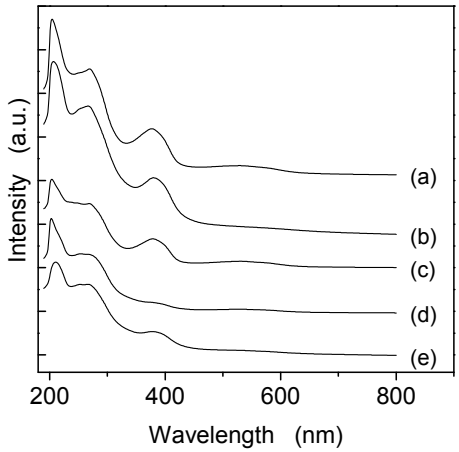

Figure 5. UV-vis spectra recorded after 2 (a), 5 (b), 24 (c), 48 (d) and 96 (e) hours of tetracycline release from glass sample.

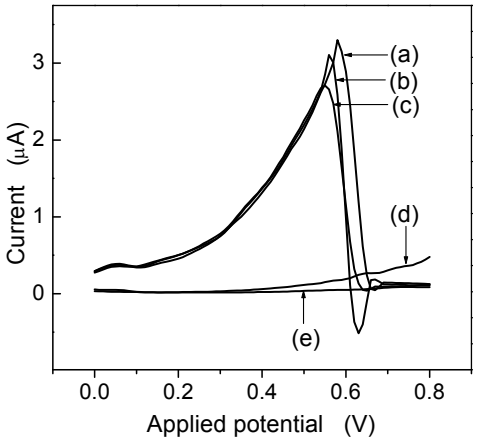

Figure 6. DPV curves obtained after 2 (a), 5 (b), 24 (c), 48 (d) and 96 (e) hours of tetracycline release from glass sample.

Table 2. Tetracycline concentration obtained by pulsed voltammetry.

Time (hr)	2	5	24	48	96
Current intensity (μA)	3.28	3.11	2.73	0.25	0.15
Concentration (μM)	0.71	0.61	0.37	0.04	0.02

The data obtained by this technique show a constant decrease in the tetracycline concentration in the first 24 hours, followed by very low release values in the next periods. Because the concentrations of tetracycline released from a controlled delivery system [16] are reported to have reached steady-state levels within 2-3 days, the low values determined after 2 and 4 days (Table 2) are most likely steady-state concentrations.

CONCLUSIONS

The results obtained in the present study show that the investigated bioactive glass can be loaded with tetracycline that is released to the highest degree in the first 24 hours. Even though the drug concentration found in the solutions after 96 hours is highly diminished, a great amount of tetracycline is still detected on the sample surface, suggesting that a part of the drug is strongly attached to the bioactive glass and could act for longer time as antimicrobial agent.

MATERIALS AND METHODS

The bioactive glass with the composition $45SiO_2 \cdot 24.5CaO \cdot 24.5Na_2O \cdot 6P_2O_5$ (mol %) was synthesized by means of sol-gel method using as starting materials tetraetoxisilan (TEOS), $Ca(NO_3)_2 \cdot 4H_2O$, $(NH_4)_2 \cdot HPO_4$ and

Na_2CO_3 as SiO, CaO, P_2O_5 and Na_2O precursors, respectively. After maturation at room temperature for 30 days, the sample was heat treated at 310°C for 1 hour.

Tetracycline adsorption was carried out by incubating the sample in phosphate buffered saline (PBS) enriched with tetracycline (7 mg/ml). The sample was analysed after immersion at 37°C for about 6 hours in order to establish the amount of adsorbed tetracycline.

For delivery assays, 35 mg tetracycline loaded glass was individually immersed in 10 ml simulated body fluid (SBF) prepared according to Kokubo protocol [6], pH 7.4, and kept at 37°C for 2, 5, 24, 48 and 96 hours in Falcon tubes, with continuous agitation. After each period, the SBF solution was completely removed from the tube for analysis and replaced by another freshly prepared solution. The same amount of glass without tetracycline was immersed in SBF for a total time of 96 hr and the SBF was replaced according to the immersion periods of time previously mentioned. The amounts of withdrawn solution were analyzed by UV-vis Spectroscopy and Differential Pulsed Voltammetry (DPV). The concentration of the released tetracycline was monitored in a three electrode configuration TRACELAB 150, using a hanging mercury drop electrode [7].

Sample characterization was made using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopic (FTIR) and X-ray Photoelectron Spectroscopy (XPS).

SEM images before and after tetracycline loading were recorded using a JEOL JSM 7000F Scanning Electron Microscope. XPS measurements were performed using a SPECS PHOIBOS 150 MCD system equipped with monochromatic Al-K_α source (250 W, hv=1486.6 eV), hemispherical analyser and multichannel detector. The vacuum in the analysis chamber during the measurements was in the range of 10^{-9}-10^{-10} mbar. The binding energy scale was charge referenced to the C 1s at 284.6 eV. The FTIR spectra were recorded in reflection configuration by a Jasco IRT-5000 FT-IR spectrometer in the range 4000-650 cm^{-1} with a resolution of 4 cm^{-1}.

ACKNOWLEDGMENTS

Theisresearch was accomplished in the framework of PNII Idei PCCE-248 /2008 project granted by the Romanian National University Research Council. E.V. and Z. B acknowledge financial support from a program co-financed by the European Social Fund through the Sectoral Operational Program for Human Resources Development, Contract POSDRU/89/1.5/S/60189 - „Innovative Postdoctoral Programs for Sustainable Development in a Knowledge Based Society” and respectively POSDRU/107/ 1.5/S/76841 with the title „Modern Doctoral Studies: Internationalization and Interdisciplinary”.

REFERENCES

1. A.L. Andrade, D.M. Souza, W.A. Vasconcellos, R.V. Ferreira, R.Z. Domingues, *Journal of Non-Crystalline Solids*, **2009**, *355*, 811.
2. A.L Andrade, D. Manzi, R.Z. Domingues, *Journal of Non-Crystalline Solids*, **2006**, *352*, 3502.
3. Z.R. Domingues, M.E. Cortes, T.A. Gomesa, H.F. Diniz, C.S. Freitas, J.B. Gomes, A.M.C. Fariac, R.D Sinisterra, *Biomaterials,* **2004***, 25*, 327.
4. U.M.E. Wikesjo, P.J. Baker, L.A. Christersson, R.J. Genco, R.M. Lyall, S. Hic, R.M. Diflorio, V.P. Terranova, *Journal of Periodontal Research*, **1986**, *21*, 322.
5. B.N.A. Vandekerckhove, M. Quirynen, D. van Steenberghe, *Journal of Periodontology*, **1997**, *68*, 353.
6. T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi, T. Yamamuro, *Journal of Biomedical Materials Research*, **1990**, *24*, 721.
7. Y. Ni, S. Li, S. Kokot, *Food Chemistry*, **2011**, *124*, 1157.
8. M.I. Goller, C. Barthet, G.P. McCarthy, R. Corradi, B.P. Newby, S.A. Wilson, S.P. Armes, S.Y. Luk, *Colloid and Polymer Science*, **1998**, *276*, 1010.
9. E. Vanea, V. Simon, *Applied Surface Science*, **2011**, *257*, 2346.
10. S. Simon, R.V.F. Turcu, T. Radu, M. Moldovan, V. Simon, *Journal of Optoelectronics and Advanced Materials*, **2009**, *11*, 1660.
11. G. Polzonetti, M.V. Russo, A. Furlani, G. Iucci, *Chemical Physics Letters*, **1991**, *185*, 105.
12. H. Yoshino, K. Kamiya, H. Nasu, *Journal of Non-Crystalline Solids*, **1990**, *126*, 68.
13. I. Rehman, W. Bonfield, *Journal of Materials Science: Materials in Medicine*, **1997**, *8*, 1.
14. G. Reiter, N. Hassler, V. Weber, D. Falkenhagen, U.P. Fringeli, *Biochimica et Biophysica Acta*, **2004**, *1699*, 253.
15. G. Falini, E. Foresti, I.G. Lesci, B. Lunelli, P. Sabatino, N. Roveri, *Chemistry - A European Journal*, **2006**, *12*, 1968.
16. L. Olanoff, J.M. Anderson, *Journal of Pharmaceutical Science*, **1979**, *68*, 1151.